MICRO BIOTEC 23

CONGRESS OF **MICROBIOLOGY**
AND **BIOTECHNOLOGY 2023**

BOOK OF ABSTRACTS

DECEMBER
7TH - 9TH

UNIVERSIDADE DA **BEIRA INTERIOR**
Covilhã

MICRO BIOTEC 23

Congress of Microbiology and Biotechnology 2023

UNIVERSIDADE BEIRA INTERIOR

SPM Sociedade Portuguesa de Microbiologia

spbt sociedade portuguesa de biotecnologia

microbiotec23.organideia.com

UNIVERSIDADE DA **BEIRA INTERIOR**

Covilhã

Welcome to MICROBIOTEC 23

We would like to welcome you to MicroBiotec ´23, which is held in the beautiful city of Covilhã.

Our congress venue, located at the Health Sciences Faculty of the University of Beira Interior, offers a prime setting at the heart of this inviting city. Covilhã, nestled amidst the stunning landscapes of Serra da Estrela and Serra da Gardunha, along with its historic villages, provides a splendid framework for our meeting. We are excited to gather in this vibrant city known for its warm hospitality, and we eagerly await the invaluable discussions and insights that will unfold during our congress.

Our scientific program comprises a wide range of presentations, including 5 plenary lectures, 15 keynote talks, 54 oral presentations, 1 round table, and 325 posters. These discussions delve into the latest advancements in Microbiology and Biotechnology. Over recent years, the world has faced an unparalleled wave of challenges, sparking remarkable progress within Microbiology and Biotechnology. These steps will be highlighted throughout the conference sessions, showcasing the profound influence these challenges have had on these fields. We are honored to have an impressive lineup of invited speakers who will share their expertise and perspectives with us.

Besides our scientific program, we feature a sponsors' exposition and talks that offer valuable chances for interactions with companies. We want to express deep appreciation to our sponsors and partners whose support is vital in enabling this congress and recognize the dedicated work of our PhD candidates and of the organizing and scientific committees. Additionally, a special acknowledgment goes out to our session chairs and committee awards whose contributions are invaluable for the success of our sessions.

As you immerse yourselves in the scientific content, discussions, and networking opportunities, we hope you may find solutions to your microbiology and biotechnology challenges and leave with relevant insights that will drive your research forward.

Enjoy your participation in MicroBiotec´23 and a memorable time visiting Covilhã.

Warm regards,

Ana Cristina Dias Cabral

Susana Ferreira

We thank our sponsors

PLATINUM SPONSOR

Federation of European Microbiological Societies

GOLD SPONSOR

communities

INVITEK diagnostics

Quilaban
uma fórmula de vida

STAB vida
Your easy genetics laboratory

SILVER SPONSOR

concessus
concessus.sa

Dias de Sousa
INSTRUMENTAÇÃO ANALÍTICA E CIENTÍFICA, S.A.

ULTRAGENE®
WWW.ULTRAGENE.PT

NORLEQ

SPECANALÍTICA
Equipamentos Científicos

SARSTEDT

BRONZE SPONSOR

alfagene

LABOR
SPIRIT, Lda.

soquímica

pathogens
an Open Access Journal by MDPI

OTHER PARTNERS

Centro de Investigação em Ciências da Saúde
CICS-UBI

frontiers | Frontiers in Sustainable Food Systems

Museu de Lanifícios
Universidade da Beira Interior

QUEIJOS BRAZ
COVA DA BEIRA · PERAMOA · COVILHÃ · PENALVA

VISIT COVILHÃ

COVILHÃ
MUNICÍPIO
A TECER O FUTURO

NEW HAND LAB
Associação Cultural
Covilhã

Quinta dos Termos
Est. 1945

SerraMel
PRODUTOS NATURAIS
DAS ABELHAS
NATURAL BEE PRODUCTS

INDEX

MICRO BIOTEC 23

Committees

Organizing Committee

Universidade da Beira Interior
Ana Cristina Dias-Cabral **(Chair)**
Ana Palmeira de Oliveira
Ângelo Luís
Carla Cruz
Fani Sousa
Fernanda Domingues
Ilídio Correia
Joana Rolo
Luís Passarinha
Susana Ferreira **(Chair)**

Scientific Committee

Ana Azevedo (IST, University of Lisbon)
Ana Cristina Dias Cabral (University of Beira Interior)
Ana Teresa Caldeira (HERCULES, University of Évora)
Ângela Novais (FF/University of Porto)
Artur Alves (CESAM, University of Aveiro)
Cecília Leão (ECS, University of Minho)
Cecília Roque (Nova University of Lisbon)
Célia Manaia (ESB, Católica Portuguesa University)
Célia Silva (University of Azores)
Eugénio Ferreira (CEB, University of Minho)
Gabriel Monteiro (IST, University of Lisbon)
Helena Santos (ITQB, Nova University of Lisbon)
Hermínia Lencastre (ITQB, Nova University of Lisbon)
Isabel Sá Correia (IST, University of Lisbon)
Jaime Mota (UCIBIO/FCT/Nova University of Lisbon)
João Mano (CICECO, University of Aveiro)
João Queiroz (University of Beira Interior)
Joaquim Cabral (IST, University of Lisbon)
Jorge Pedrosa (ICVS, University of Minho)
Jorge Pereira (DEQ, University of Coimbra)
Jorge Rocha (DEQ, University of Coimbra)
José Teixeira (CEB, University of Minho)

Lucília Domingues (CEB, University of Minho)
Luísa Peixe (FF, University of Porto)
Madalena Pimentel (FF, University of Lisbon)
Manuela Pintado (ESB, Católica Portuguesa University)
Mara Freire (CICECO, University of Aveiro)
Margarida Casal (CBMA, University of Minho)
Maria Ascensão Reis (UCIBIO/FCT/Nova University of Lisbon)
Maria João Amorim (Institute Gulbenkian of Science)
Maria José Saavedra (CITAB, University of Trás os Montes e Alto Douro)
Maria Leonor Faleiro (University of Algarve)
Miguel Teixeira (IST, University of Lisbon)
Miguel Viveiros (IHMT, Nova University of Lisbon)
Mónica Vieira Cunha (FC, University of Lisbon)
Nelson Lima (CEB, University of Minho)
Nelson Simões (CBA, University of Azores)
Nuno Cerca (CEB, University of Minho)
Paula Castro (ESB, Católica Portuguesa University)
Paula Morais (CEMMPRE, University of Coimbra)
Pedro Barros Fernandes (Lusófona University)
Raquel Aires Barros (IST, University of Lisboa)
Teresa Crespo (ITQB/iBET, Nova University of Lisbon)
Teresa Gonçalves (CNC/FM, University of Coimbra)
Vitor Vasconcelos (CIIMAR, University of Porto)

MICRO BIOTEC 23

Conference Programme

Thursday, December 7

11:00h Registration

14:00h

Room 1 – Welcome Session

Magnificent Rector Prof. Mário Raposo; President of the Faculty of Health Sciences Prof. Miguel Castelo-Branco; President of the Faculty of Sciences Prof. Paulo Almeida; President of the Portuguese Society of Microbiology Prof. Jorge Pedrosa; President of the Portuguese Society of Biotechnology Profª. Raquel Aires-Barros; Conference Chairs Prof. Cristina Dias-Cabral and Prof. Susana Ferreira

14:30h

Room 1 – Plenary Lecture #1 (Chairperson: Teresa Crespo & Vitor Vasconcelos)

Maria Barbosa – Wageningen University, Netherlands

PL1 – "Hypes, Hopes and the way forward for Microalgal Biotechnology"

15:30h

Room 1 – Topic #1 – Environmental Microbiology & Biotechnology

(Chairperson: Teresa Crespo & Vitor Vasconcelos)

Keynote lecture – Vitor Vasconcelos, CIIMAR – Universidade do Porto, Portugal

KL1 – "Cyanobacteria and their metabolites: implications for human health and the environment"

Room 2 – Topic #7 – New and Emerging Technologies

(Chairperson: Isabel Sá – Correia & José Teixeira)

Keynote lecture – **Simão Soares**, SilicoLife – Braga, Portugal

KL2 – "AI + Biology for sustainable ingredients"

Room 3 – Topic #5 – Molecular Microbiology and Microbial Physiology

(Chairperson: Arsénio Fialho & Nuno Cerca)

Keynote lecture – **Nuno Cerca**, CEB – Universidade do Minho, Portugal

KL3 – "Probing bacterial interactions in polymicrobial biofilms to better understand bacterial vaginosis"

16:00h

Coffee Break and Poster Session #1

17:00h

Room 1 – Topic #1 – Environmental Microbiology & Biotechnology

(Chairperson: Mónica Cunha & Paula Morais)

Keynote lecture - **Paula Morais**, CEMMPRE – Universidade de Coimbra, Portugal

KL4 – "Microbial technology for sustainable development: exploring bacterial strategies for metal interaction"

Oral presentations

OP1.1 "Mucus of the photosynthetic sea slug *Elysia crispata*: proteome and anti-bacterial activity against *Pseudomonas aeruginosa*"
Diana Lopes, Susana S. Aveiro, Eva Cunha, Sónia Cruz, Manuela Oliveira, Pedro Domingues, Paulo Cartaxana

OP1.2 "The Microbiome of Octocorals: From Community Structure, Function and Metabolic Interaction to Blue Biotech Opportunities"
Tina Keller-Costa, Sandra G. Silva, João Almeida, Daniela Silva, Ângela Taipa, Jorge Gonçalves, Asunción Lago-Lestón, Nikos C. Kyrpides, Ulisses Nunes da Rocha, Rodrigo Costa

OP1.3 "Antibiotic-resistant bacteria hitchhiking microplastics in a river: detailed analysis of carbapenemase-producing Enterobacterales in the Plastisphere"
Isabel Silva, Marta Tacão, Isabel Henriques

OP1.4 "Peptides from entomopathogens as a source of new bioinsecticides"
Duarte Toubarro, Jorge Frias, Tiago Paiva, Nelson Simões

OP1.5 "Screening for plastic-degrading potential in marine bacteria associated with net biofilms and hydrocarbon degradation"
Rafaela Perdigão, Maria Fátima Carvalho, Catarina Magalhães, Sandra Ramos, Cristina Marisa Almeida, Ana Paula Mucha

Room 2 – Topic #6 – Bioprocess Engineering

(Chairperson: Cristina Dias-Cabral & Gabriel Monteiro)

Keynote lecture - Duarte Miguel Prazeres, IST – Universidade de Lisboa, Portugal

KL5 – "Vibrio natriegens as a new host for plasmid DNA production"

Oral presentations

OP6.1 "Continuous production of influenza VLPs as vaccine candidates: a multi-stage bioreactor approach"
Ricardo Correia, Taja Zotler, Miguel Graça, Bárbara Fernandes, Gorben Pijlman, Paula M. Alves, António Roldão

OP6.2 "Assessment of microalgae side streams potential for VFA production in batch fermentation"
Claudia Duarte, Marisa Cardoso, Mariana Matos, Joana Fradinho, Bruno Ferreira, Jorge Pereira, Maria Reis

OP6.3 "Monitoring productivity and predicting crash of *Phaedactylum tricornutum* cultures using spectroscopy and machine learning"
Pedro Brandão, Constança Bertrand, Rodrigo Martins, Francisco Nunes, Claúdia Galinha, Francisco Nascimento

OP6.4 "New shuttle vector-based expression system to improve the biosynthesis and purification of membrane bound catechol-O-methyltransferase"
Ana Margarida Gonçalves, Márcia Correia, Claúdio Jorge Maia, João Queiroz, Maria João Romão, Luís António Passarinha

OP6.5 "A molecular modeling perspective on the development of synthetic affinity ligands"
Carlos Costa, Carolina Natal, Jéssica Rodrigues, Ana Margarida Dias, Ana Cecília Roque, Arménio J. M. Barbosa

Room 3 – Topic #5 – Molecular Microbiology and Microbial Physiology

(Chairperson: Arsénio Fialho & Nuno Cerca)

Keynote lecture - Arsénio Fialho, IST – Universidade de Lisboa, Portugal

KL6 – "Adhesins in opportunistic respiratory pathogens: from seeing to determining their biological function"

Oral presentations

OP5.1 "The unique ability of *Staphylococcus epidermidis* phage SEP1 activating dormant cells"
Maria Daniela Silva, Graça Pinto, Ângela França, Joana Azeredo, Luís Melo

OP5.2 *"Unravelling Mechanisms of Pneumococcal Intra-Species Interactions"*
Carina Valente, Ana Raquel Cruz, Adriano Oliveira Henriques, Raquel Sá-Leão

OP5.3 "Genome mining and biochemical characterization of polyhydroxyalkanoates produced by bacterial isolates from UCCCB"
Paula V. Morais, Diogo Vicente, Diogo Neves Proença

OP5.4 "Exploring the functional and structural aspects of the citrate transporter CexA from *Aspergillus niger"*
João Alves, Maria Sousa-Silva, Pedro Soares, Michael Sauer, Margarida Casal, Isabel Soares-Silva

OP5.5 "Exploring Genomic Plasticity in *Candida glabrata* and its Impact on Biofilm Evolution in Clinical Environments"
Inês Vieira Costa, Maria Zolotareva, Mafalda Cavalheiro, Mónica Galocha, Miguel Cacho Teixeira

19:30h

Beira Interior of Honor

Faculty of Engineering, Universidade da Beira Interior (40°16'42.5" 7°30'42.2")

Friday, December 8

08:30h Registration

9:00h

Room 1 – Plenary Lecture #2 (Chairperson: Cecília Roque & Fani Sousa)

Sophia Hober, KTH – Stockholm, Sweden

PL2 – "Affinity proteins for biotechnological and medical purposes"

10:00h

Conference Photo

10:10h

Coffee Break and Poster Session #1

11:00h (Room 1) / 11:15h (Rooms 2 and 3)

Room 1 – Topic #4 – Health Microbiology

(Chairperson: Madalena Pimentel & Maria Leonor Faleiro)

Keynote lecture – Maria Leonor Faleiro, Universidade do Algarve

KL7 – "Disclosing gut bacterial signatures in elderly from Algarve"

Oral presentations

OP4.1 "Controlling diarrheagenic *E. coli* with bacteriophages: facts and challenges"
Francisca Rodrigues, Isidro García-Meniño, Azucena Mora, Luís Daniel Melo, Ana Oliveira

OP4.2 "A portrait of the interaction established between vaginal colonizing *Lactobacillus* species and the pathogenic yeasts *Candida albicans* and *Candida glabrata"*
Nuno A. Pedro, Gabriela Fontebasso, Sandra N. Pinto, Dalila Mil-Homens, Arsénio Fialho, Nuno Pereira Mira

OP4.3 "Probe-based Metagenomics: an added value for clinical decision?"
Luís Coelho, Rita Ferreira, Daniel Sobral, João Dourado, Joana Isidro, Verónica Mixão, Miguel Pinto, Alexandra Nunes, Sílvia Duarte, Luís Vieira, Vítor Borges, João Paulo Gomes

OP4.4 "How do metalloenzymes help in the fight against *Clostridioides difficile*'s infection?"
Filipe Folgosa, Maria C. Martins, Nicolas Kint, Carolina A. Feliciano, Claire Morvan, Susana F. Fernandes, Bruno Dupuy, Isabelle Martin-Verstraete, Miguel Teixeira

Room 2 – Topic #3 – Health Biotechnology

(Chairperson: Ângela Sousa & Jorge Pereira)

Keynote lecture – Jorge Pereira, CIEPQPF – Universidade de Coimbra, Portugal

KL8 – "Ionic liquids as additives for stabilization and formulation of protein-based products"

Oral presentations

OP3.1 "Avian Antibody Production for SARS-CoV-2 Spike protein: Epitope-Specific Insights"
Tiago Ochôa-Pires, Rafaela Seabra, Beatriz, Ana Marques, Guilherme Gabriel, Rui Jorge Nobre, Luís Pereira de Almeida, Ricardo S. Vieira-Pires

OP3.2 "Keratin-based materials for biomedical applications"
Cariny Polesca, Bruno Neves, Jason Hallett, João Coutinho, Helena Passos, Mara Freire

OP3.3 "A Novel Bacteriophage Receptor Binding Protein for Improved *Salmonella* Detection"
Ana Brandão, Sílvio Santos

OP3.4 "Cytoprotective Guardians: Quantifying the cytoprotective efficiency of extracellular vesicles (EVs) against *Staphylococcus aureus* Alpha Hemolysin (Hlα)"
Diogo Gonçalves, Ricardo Silva, Beatriz Avó, Nuno Bernardes, Ana M. Azevedo, Sandra N. Pinto, Fábio Fernandes

Room 3 – Topic #2 – Food Microbiology & Biotechnology

(Chairperson: Célia Silva & Manuela Pintado)

Keynote lecture – Manuela Pintado, Universidade Católica Portuguesa, Portugal

KL9 – "Enzymatic hydrolysis: A tool to produce functional and sustainable food ingredients"

Oral presentations

OP2.1 "Traditional cheeses as bio-factories for probiotic bacteria"
Susana Serrano, Maria V. Ferreira, Cinthia Alves-Barroco, Maria Teresa Barreto-Crespo, Teresa Semedo-Lemsaddek

OP2.2 "Genotypic diversity and off-flavors production in *Dekkera bruxellensis* as drivers for selection of biocontrol agentes"
João Sousa, Emanuel Peixoto, Rogério Tenreiro, Nuno Mira, Ana Mendes-Ferreira

OP2.3 "Characterization of foodborne pathogenic *Vibrio* spp. in environmental and seafood samples collected from Angola"
Inês C. Leal, Babak Najafpour, Eunice Cassoma, Isaac Bumba, Carmen dos Santos, Adelino V. M. Canário, Deborah M. Power, João C. R. Cardoso

OP2.4 "Fire4CAST – An integrative strategy for accurate prediction of fire blight disease outbreaks in portuguese orchards"
Daniel McGuire, Francisco Pinto, Telma Costa, Joana Cruz, Rui Sousa, Miguel Leão de Sousa, Carmo Martins, Ana Tenreiro, Rogério Tenreiro, Margarida Gama-Carvalho, Leonor Cruz

12:30h (Room 1) / 12:45h (Rooms 2 and 3)

Lunch

13:00h

Room 3 – Lunch Time Sponsor Talk:

QUILABAN

Bruno Taveira - Country Lead Portugal

"A mudança é possível: Dos diagnósticos convencionais à operacionalidade do BD MAX™"

13:30h

Room 3 – Lunch Time Sponsor Talk:

STABVIDA

Francisco Sousa - STAB VIDA Lda, Lisbon, Portugal

"Doctor Vida Pocket PCR – A true lab-on-phone"

14:15h

Room 1 – Plenary #3 (Chairperson: Maria José Saavedra & Susana Ferreira)

Avelino Álvarez-Ordóñez, University of León, Spain

PL3 – "Mapping food microbiomes to identify sources and patterns of succession linked with quality and safety traits"

15:15h

Room 1 – Topic #7 – New and Emerging Technologies

(Chairperson: Ângela Novais &Jorge Pedrosa)

Keynote lecture – Cecília Roque, FCT – Universidade Nova de Lisboa

KL10 – "Nature inspired solutions in Health Biotechnology"

Oral presentations

OP7.1 "Commensalism vs pathogenicity in *Staphylococcus epidermidis* - uncovered by an integrated omics approach"
Luís G. Gonçalves, Elisabete Morais, Filipe Magalhães, Susana Santos, Laidson P. Gomes, Jean Armengaud, Maria Zimmermann-Kogadeeva, Ana Gil, Maria Miragaia, Ana V. Coelho

OP7.2 "Phenotypic, physiological and genomic portrait of a "human-naïve" *Candida glabrata* strain: a shed of light into the path towards colonization of the human host"
Maria Joana Pinheiro, Dalila Mil-Homens, Ana Mendes-Ferreira, Nuno Pereira Mira

Room 2 – Topic #2 – Food Microbiology & Biotechnology

(Chairperson: Ângelo Luís & Teresa Gonçalves)

Keynote lecture – Sandrina Heleno, Instituto Politécnico de Bragança, Portugal

KL11 – "Natural preservatives for the food and beverage industries"

Oral presentations

OP2.5 "More than coloring agents: natural pigments with antimicrobial activity"
Nuno Ribeiro da Silva, Lina Ballesteros, Carla Fonseca, Bruna Basto, José Teixeira, Sara Silvério

OP2.6 "Sustainable film solutions: Exploring cutin recovery from tomato by-products for hydrophobic applications"
Andreia Simões, Isabel M. Coelhoso, Vítor D. Alves, Carla Brazinha

Room 3 – Topic #9 – Biological Resources centers and Networks

(Chairperson: José Teixeira & Nelson Lima)

Keynote lecture – Nelson Lima, Biological Resources centers and Networks, Portugal

KL12 – "Microbial culture collections: strains, services, projects and consortia to underpin the MicroBiotech innovation"

Oral presentations

OP9.1 "EATRIS | European infrastructure for translational medicine"
Helena Paula Baião

OP9.2 "European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP)" COST action presentation
Maria Amparo Faustino

16:15h

Coffee Break and Poster Session #2

17:15h

Room 1 – Topic #4 – Health Microbiology

(Chairperson: Gabriela Silva & Miguel Teixeira)

Oral presentations

OP4.5 "Unveiling the Protective Mechanisms of Pyruvate Kinase Deficiency against Malaria: Transforming Vulnerability into Resilience"
Ana Balau, Daniel Sobral, Patrícia Abrantes, Maria Carvalho, Inês Morais, Verónica Mixão, Márcia M. Medeiros, Filomena A. de Carvalho, Maria Alexandra Antunes, João Paulo Gomes, Sandra Antunes, Ana Paula Arez

OP4.6 "Characterization of biofilm formation of bacteria associated with the midgut of *Anopheles* mosquito vector of malaria"
Margarida Marques, Sofia Santos Costa, Sandra N. Pinto, Henrique Silveira

OP4.7 "Deciphering host-*Rickettsia* interactions: moonlighting APRc recruits human complement regulator C4BP acting as an evasin"
Ana Luísa Matos, Isaura Simões

Room 2 - Topic #1 – Environmental Microbiology & Biotechnology

(Chairperson: Célia Manaia & Jorge Rocha)

Oral presentations

OP1.6 "Bacteria-Based Biorefinery: Generating Bioactive Oligosaccharides from a Single Fermentation of Seaweed"
Duarte Toubarro, Duarte Seixas, Nelson Simões

OP1.7 "Valorization of *Gelidium corneum* by-product through solid-state fermentation"
Marta Ferreira, José Manuel Salgado, Helena Peres, Isabel Belo

OP1.8 "Exploring the fate of chiral pharmaceuticals in an AGS system under saltwater intrusion phenomena"
Catarina Miranda, Catarina Amorim, Clara Piccirillo, Maria Elizabeth Tiritan, Paula Castro

Room 3 – Topic #1 – Environmental Microbiology & Biotechnology

(Chairperson: Lucília Domingues & Paula Castro)

Oral presentations

OP1.9 "The quest for natural microbiomes: Transforming CO2 into valuable bioproducts through photosynthesis"
André Freches, Inês Cardoso, Joana Fradinho, Maria Reis

OP1.10 "An allosteric redox switch enables oxygen protection of a Formate Dehydrogenase from *Desulfovibrio vulgaris* Hildenborough"
Ana Rita Oliveira, Cristiano Mota, Rita Rebelo Manuel, Guilherme Vilela-Alves, Neide Pedrosa, Vincent Fourmond, Kateryna Klymanska, Christophe Léger, Bruno Guigliarelli, Maria João Romão, Inês Cardoso Pereira

OP1.11 "Harnessing *Saccharomyces cerevisiae* for sustainable FDCA production: optimizing 5-HMF detoxification and derivative synthesis"
Cristiana Martins, Carlos E. Costa, Joana Cunha, Lucília Domingues

18:30h

SPBT (room 3) and SPM (room 2) general assemblies

20:00h

Conference Dinner

Puralã – Wool Valley Hotel & SPA (40°16'02.5"N 7°29'53.8"W)

Saturday, December 9

08:30h Registration

9:00h

Room 1 - Plenary lecture #4 (Chairperson: Luísa Peixe & Miguel Viveiros)

Andre G. Buret, University of Calgary, Canada

PL4 – "Enteric infections disrupt gut microbiota biofilms: From mechanisms to therapy"

10:00h

Coffee Break and Poster Session #2

11:00h (Room 1 and 3) / 11:15h (Rooms 2)

Room 1 – Topic #4 – Health Microbiology

(Chairperson: Luísa Peixe & Miguel Viveiros)

Keynote lecture – Luísa Peixe, Universidade do Porto, Portugal

KL13 – "Unleashing the Power Against Superbugs: Unraveling Global Challenges and Innovations in Antimicrobial Resistance"

Oral presentations

OP4.1 "The small non-coding RNA ncRNA3 is involved in antibiotic resistance in bacteria of the *Burkholderia cepacia* complex"
Gonçalo Matos; Joana Feliciano; Beatriz Jesus; Jorge Leitão

OP4.2 "Methicillin-sensitive *Staphylococcus aureus* (MSSA) nasal screening at ICU admission as a predictive tool for subsequent MSSA respiratory infection"
Teresa Conceição, Mafalda Felgueiras, Silvia Ferreira, Marisa Simões, Daniela Carvalho, Angelina Lameirão, Ana Marques, Dulce Pascoalinho, Maria Paula Gonçalves, Ana Fernandes, Hermínia de Lencastre, Valquíria Alves

OP4.3 "SCC*mec* acquisition is linked to *Staphylococcus epidermidis* bloodstream infection outbreaks in a neonate intensive care unit"
Nuno Alexandre Faria, Roberta Filipini Rampelotto, Rosmari Hörner, Maria Miragaia

OP4.4 "Emerging threat: resistance to ceftazidime-avibactam and other last-line antibiotics amongst carbapenem-resistant *Klebsiella pneumoniae* in Portugal"
Ana Beatriz Gonçalves, Valquíria Alves, Isabel Neves, Maria Antónia Read, Luísa Peixe, Ângela Novais

OP4.5 "Cefotaxime-resistant *Escherichia coli* from urine infections and environmental waters: a comparative analysis"
Joana Abreu Silva, Catarina Ferreira, Célia M. Manaia

Room 2 – Topics #3, #4 – Health Biotechnology and Microbiology

(Chairperson: Joana Rolo & Luís Passarinha)

Keynote lecture – Ângela Sousa, CICS-UBI, Universidade da Beira Interior, Portugal

KL14 – "New generation of DNA vaccines against cancers induced by HPV infection"

Oral presentations

OP3.5 Cat derived antibody fragments as a promising strategy for treatment and prevention of SARS-CoV-2 infection (Isa Moutinho, Mafalda Henriques, Sara Cardoso, Teresa Coutinho, Luís Tavares, Solange Gil, Telmo Nunes, Frederico Aires-da-Silva; FMV-UL; Tópico 4.6)

OP3.6 "New fluoroquinolone-phenothiazine hybrids with antibacterial properties against *Staphylococcus aureus"*
Marina Cassago Posso, João Lourenço Serrano, Paulo Almeida, Fernanda Domingues, Samuel Silvestre, Susana Ferreira

OP4.6 "Breaking the Wall: *Mycobacterium bovis* transmission at the animal-environment interface"
André C. Pereira, Daniela Pinto, Mónica V. Cunha

OP4.7 "High and homogeneous levels of oxacillin resistance in MRSA through the activation of the stringent stress response leads to up-regulation of the capsular polysaccharide"
Catarina Milheiriço, Vítor Borges, João Paulo Gomes, Beatriz Silva, Alexander Tomaz, Hermínia de Lencastre

OP4.8 "Characterization of a novel quorum sensing system in *Bacteroides thetaiotaomicron*"
Carina Ribeiro Galhofa, Kai Papenfort, Karina Xavier

Room 3 – Topic #8 – From Science to Society

(Chairperson: Artur Alves & Raquel Aires-Barros)

Keynote lecture – Paula Lopes, Universidade de Trás-os-Montes e Alto Douro, Portugal

KL15 – "Nucleic Acid Biosensing Platform for SARS-CoV-2 Detection"

Oral presentations

OP8.1 Bioconversion of plastics' building blocks into bacterial cellulose towards circular economy"
Cátia Gil, Maria Reis, Filomena Freitas, Cristiana Torres

OP8.2 "Unravelling the potential of orange juice side streams: hesperidin-rich extraction and electrospraying for a bio-based ingredient development"
Ana A. Vilas-Boas, Cristina Prieto, Débora A. Campos, Marta Correia, Jose Maria Lagaron, Manuela Pintado

Round table on Career Development

- Luuk A.M. van der Wielen, University of Limerick, Ireland
- Andre G. Buret, University of Calgary, Canada
- Raquel Aires – Barros, IST – Universidade de Lisboa, Portugal
- Paula Morais, CEMMPRE – Universidade de Coimbra, Portugal
- Diana Gomes, PhD candidate, Universidade da Beira Interior, Portugal

13:00h (Room 1) / 13:15h (Rooms 2 and 3)

Lunch

13:15h

Room 3 – Lunch Time Sponsor Talk:

COMMUNITIES

André Canilho – Communities – Comunicações, Lda.

"Communities – Comunicações, Lda. – services and company presentation"

13:50h

Room 3 – Lunch Time Sponsor Talk:

INVITEK

Josué Carvalho - Invitek Diagnostics

"Simplify your Workflow with Invitek Diagnostics"

14:30h

Room 1 - Plenary #5 (Chairperson: Jorge Pedrosa & Raquel Aires-Barros)

Luuk A.M. van der Wielen, University of Limerick, Ireland

PL5 – "Bioeconomy and other renewables opportunities for Portugal"

15:30h

Career Award Plenaries

Award Professor Júlio Maggiolly Novais

Laureate: Professor Joaquim Manuel Sampaio Cabral, Instituto Superior Técnico – Universidade de Lisboa

Award Professor Nicolau van Uden

Laureate: Professor Rogério Paulo Andrade Tenreiro, Faculdade de Ciências – Universidade de Lisboa

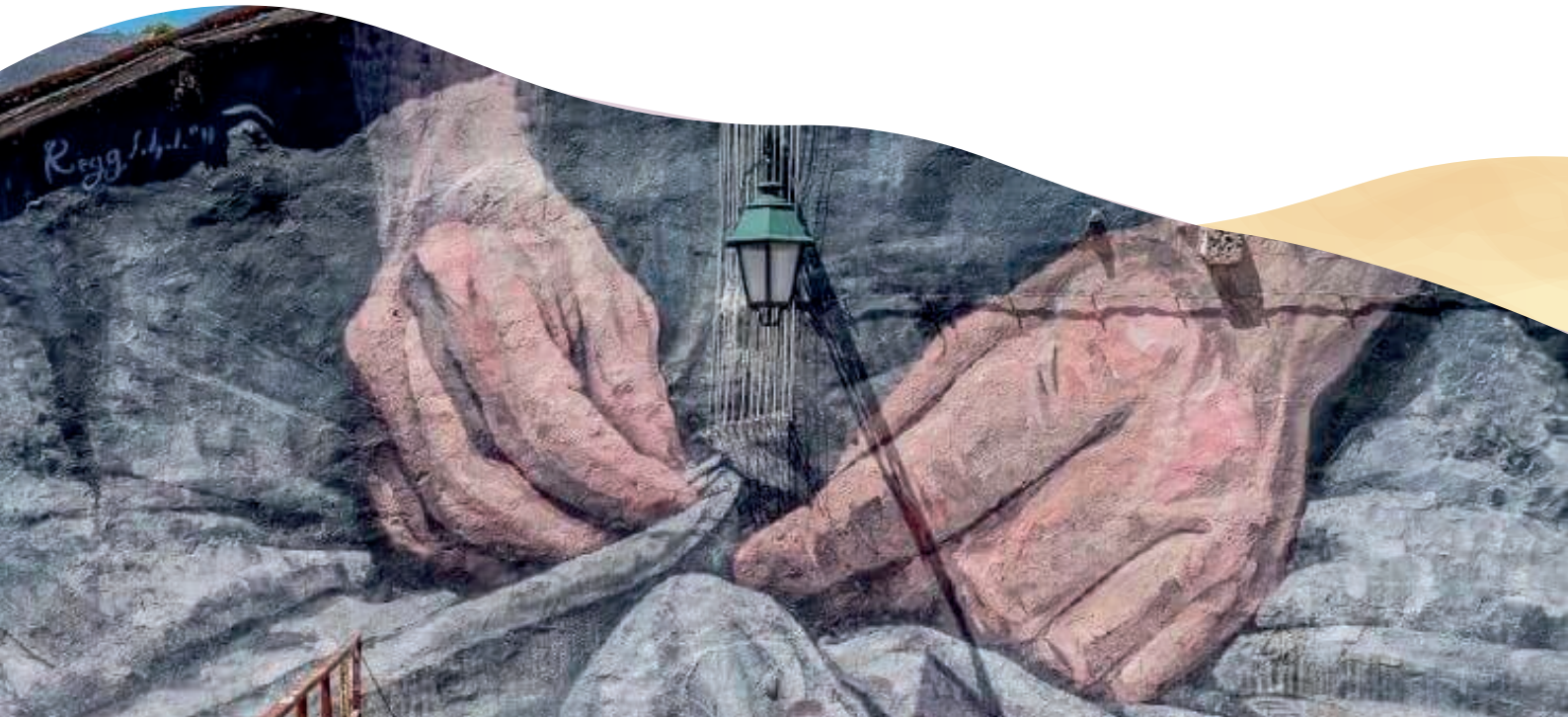
16:30h

Prizes & Closing session

CICS–UBI Scientific Coordinator Prof. Luís Taborda Barata; President of the Portuguese Society of Microbiology Prof. Jorge Pedrosa; President of the Portuguese Society of Biotechnology Profª. Raquel Aires-Barros; Conference Chairs Prof. Cristina Dias-Cabral and Prof. Susana Ferreira

MICRO BIOTEC 23

Carreer Awards



Júlio Maggiolly Novais Award 2023

The Júlio Maggiolly Novais Award was established by the Portuguese Society of Biotechnology (SPBT) in 2021 with the purpose of honoring, every two years, a nationally and internationally renowned Biotechnologist, with an exceptional record of scientific achievements, inspiring generations of professionals in the field of Biotechnology, and making notable contributions to the development of Biotechnology in Portugal. The Biotechnologist Career Award stands as a Homage and Acknowledgment from the SPBT to Professor Júlio Novais, who was a pioneer in the field of Biotechnology education in Portugal and played a crucial role in the development of Portuguese Biotechnology and in shaping the careers of its researchers. Additionally, he was a founding member of the SPBT and served as its first President.

The first Júlio Maggiolly Novais Award was Professor José Manuel Mota, Emeritus Professor at the University of Minho, for his exceptional contributions to the development of Biotechnology in Portugal through his career and the active role on SPBT. He was President of SPBT from 1993 to 2002.

The 2023 Laureate is Professor Joaquim Cabral for his exceptional contributions to the advanced training and scientific leadership in the field of Biotechnology and Bioengineering, both nationally and internationally. He is recognized as one of the most prominent figures among opinion leaders in this field."

Professor Joaquim Cabral is a pivotal figure in the history of the Portuguese Society of Biotechnology and the field of Biotechnology in Portugal, as highlighted in Professor Júlo Maggiolly Novais' article "Contributions to the history of the Portuguese Society of Biotechnology (SPBT)" in the Biotechnology Bulletin No. 2 (Series 2) (https://spbt.com.pt/boletins/), during the celebration of the 30 years of SPBT: "It all began 30 years ago! In 1981, the 2nd European Biotechnology Congress was held, organized by the European Federation of Biotechnology in Eastbourne, U.K. It was surprising to find seven Portuguese present, which was an unusual number for the time. Out of curiosity, here are their names: Maria Teresa Colaço from LNETI, Joaquim Pereira Cardoso, Jorge Bento, and José Pires de Moura from CIPAN, José Cardoso Duarte from QUATRUM, and Joaquim Sampaio Cabral and Júlio Novais from IST." And the History continues: "With the usual gregarious tendency, the Portuguese gathered one night in one of the hotels, and from the conversation, the idea emerged that Portugal should be represented in the European Federation and that, for this purpose, a Portuguese Society of Biotechnology should be formed and that should be a section of the already existing Portuguese Society of Biochemistry (SPB)". Thus, the meeting that took place at IST in 1981 remained as the founding meeting, and Joaquim Cabral was one of the eight initial founding members of SPBT and its President from 1987 to 1992.

The formalization of being member of the European Federation of Biotechnology, one of the initial objectives of SPBT, was accomplished through a request sent in the summer of 1982. To facilitate integration, nine Working Groups were established within SPBT, focusing on domains parallel to the Working Parties of the Federation. Joaquim Cabral served as the coordinator of the "Immobilized Biocatalysts" group. By 1984, SPBT had already participated in the 6th General

Assembly of the European Federation of Biotechnology and Joaquim Cabral played a fundamental role in this integration and in the international promotion of Portuguese Biotechnology.

Professor Joaquim Sampaio Cabral has exerted exceptional influence in the field of Biotechnology and Bioengineering, both nationally and internationally, and is regarded as one of the most prominent figures among opinion-makers in this field. This is evidenced by his presidency or participation in the management or scientific committees of various national and European scientific societies in the areas of Biochemical Engineering, Biotechnology, Applied Biocatalysis, and, more recently, in the field of Stem Cell Engineering and Regenerative Medicine. He has also served as an editor and a member of editorial boards for international journals with an impact in these areas and has been involved in the most prestigious national and international advisory and evaluation panels for research units, projects, scholarships, and other activities in these domains. All his respectability and influence at both the national and international levels have been judiciously leveraged for the advancement and positioning of Portugal in the fields of Biotechnology and Bioengineering.

His activity in terms of advanced training and as a mentor, has also been remarkable not only at IST, where he is a professor, but also throughout the country. He has established a school in the field of Biochemical Engineering Sciences and, more recently, in Stem Cell Engineering, with an impact on the development of these areas that has been notable both nationally and internationally.

And to conclude, we would like to emphasize his inspiring and motivating power to make things happen, as well as his exceptional contribution to the development and international recognition of the Portuguese Biotechnology field. It is inspiring to see that he continues to make things happen just as he did when we first met. Thank you.

Raquel Aires Barros and José Teixeira

JOAQUIM MANUEL SAMPAIO CABRAL
IST Distinguished Full Professor
Director of the Institute for Bioengineering and Biosciences (iBB)
Coodinator of the Associate Laboratory Institute for Health and Bioeconomy (i4HB)
Researcher unique identifier: ORCID 0000-0002-2405-5845
ResearcherID: G-2052-2010
http://scerg.tecnico.ulisboa.pt/jmscabral.html

Biographical Sketch
Joaquim M S Cabral is Distinguished Full Professor of Instituto Superior Técnico (IST), University of Lisbon, Portugal. He was the Founding Head (2011) of Department of Bioengineering, the Founding Director (2013) of the Institute for Bioengineering and Biosciences at IST and the Founding Coordinator (2021) of the Associate Laboratory Institute for Health and Bioeconomy.

UNIVERSITY EDUCATION AND FACULTY POSITIONS: Diploma in Chemical Engineering (1976), PhD (1982) and Habilitation (1988) from IST. Post-doctoral study at Massachusetts Institute of Technology (1983-4); faculty positions: Assistant (1983-6), Associate (1986-91) and Full Professor (1992). He was the first Distinguished Professor (2015) at IST.

RESEARCH INTERESTS AND OUTPUTS:
Joaquim Cabral has authored more than 600 original research articles, review articles, book chapters and books in biotechnology and bioprocess engineering with higher than 14 thousand citations (WoS h index 59). His lab-Stem Cell Engineering Research Group- is focused on cell production platforms for the ex-vivo expansion of stem cells and/or their controlled differentiation into specific cell types and micro-tissues, as well as their integration with bioseparation and high-resolution purification technologies. The goal is to generate large numbers of specific and high quality stem/progenitor cell subsets needed for Regenerative Medicine settings as well as to develop in vitro models for disease modelling and drug testing. His lab has established an international track record in Stem Cell Engineering, as assessed by WTEC, on behalf of NSF, NIST and NIH (The International Assessment of Research in Biological Engineering and Manufacturing 2015; The International Assessment of R&D in Stem Cell Engineering 2013).

LEARNING AND SCIENTIFIC SOCIETIES:

Joaquim Cabral is Member of the Portuguese Academy of Engineering (1999-); Corresponding Member of the Lisbon Academy of Sciences (2015-); a Founding Member of Sociedade Portuguesa de Biotecnologia (1981) and President (1987-92); Founding Member of Sociedade Portuguesa de Células Estaminais e Terapia Celular (2005), Vice-President (2005-2006), President (2009-10); Member of the European Federation of Biotechnology (EFB) Section on Applied Biocatalysis (1985-), Chairman (1990-5) and Honorary Member (2005-); Member of the Executive Committee of the EFB Section on Biochemical Engineering Science (1999-2004) and Chairman of its Task Group on Biotransformation (1996-2004); Member of the Steering Committee of the Process Integration in Biochemical Engineering Programme of the European Science Foundation (1992-6); Member of the Executive Committee of the European Biochemical Engineering Network (1993-6); Founding Member (2013) of the European Society of Biochemical Engineering Science and chair of the Regenerative Medicine Manufacturing Section; Member of the Scientific Board of the Tissue Engineering and Regenerative Medicine International Society Thematic Group on Bioreactor Technologies (2013).

ADVISORY AND EVALUATION COMMITTEES:

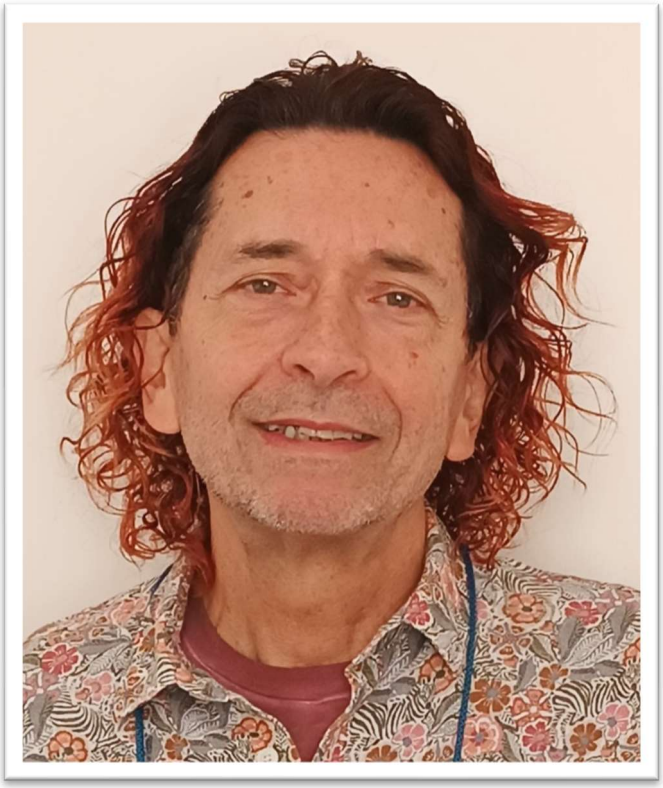
Joaquim Cabral has been serving several scientific committees. European Commission: Portuguese Delegate of the Biotechnology Framework Programme IV (1994-8) and Member of the Product and Process Engineering Evaluation Panel of the European Research Council–Advanced Grants (2009-14); ANR-French National Research Agency; Biotechnology and Biological Sciences, Medical and Engineering and Physical Sciences Research Councils, UK; Member of the Biotechnology Strategy Group of the European Academies' Science Advisory Council, The Royal Society (2002-2006); President of the Scientific Council of Exact and Engineering Sciences of the Portuguese Fundação para a Ciência e a Tecnologia (2010–2013). Coordinator of the Peer Review Committee of the Biotechnology and Biological Engineering Research Grants of the Portuguese Fundação para a Ciência e a Tecnologia (2007-2013); Member (2014-) of the Amgen Bioprocessing Center Advisory Board at Keck Graduate Institute, School of Applied Life Sciences, Claremont, California, USA.

Nicolau van Uden Award 2023

The 2023 van Uden Prize for a "colorful" microbiologist, a singular leader who marked several generations.

The Professor Nicolau van Uden scientific prize was established in 2011 by the Portuguese Society of Microbiology (SPM) to be awarded every two years during the Society's National Congress, to a microbiologist with an international and national reputation, with an exceptional track record of research achievements, mentoring other researchers in the field of microbiology and whose work has had a far-reaching impact on the development of the field of microbiology in Portugal. This Microbiology Career Award also represents a tribute from the SPM to Professor Nicolau van Uden, who is, directly or indirectly, the mentor or scientific inspiration of several generations of yeast biologists and other microbiologists at various universities and research institutes in Portugal.

As president of SPM from 2009 to 2020, Isabel Sá-Correia had the privilege of announcing the first five Professor Nicolau van Uden prizes, awarded to Professors Hermínia de Lencastre (2011), Cecília Leão (12013), Milton da Costa (2015), José Melo Cristino (2017) and Helena Santos (2019). As President of the SPM from 2021, Jorge Pedrosa has announced the award of this prize to Isabel Sá-Correia (2021) and will announce, during MicroBiotec 2023, it's award to Rogério Tenreiro. The 2023 laureate is a "colorful" microbiologist who, in a unique way, has undeniably marked several generations of microbe enthusiasts and scholars, with a recognized impact on society, academia and companies.



As far as SPM is concerned, Rogério Tenreiro is part of its history. He was Secretary General from 2003 to 2008, in the boards chaired by Isabel Spencer-Martins, and from 2009 to 2017, in the boards chaired by Isabel Sá-Correia. In 2017, he felt that it was too long and, as usual, no one stopped him from leaving his place to the younger members of the society. In 2020, in a short farewell message, as president of SPM, Isabel Sá-Correia left the following personal acknowledgement, to an unforgettable Secretary General, who is emeritus in his own right: "I think that all those who worked, without a personal agenda, in favor of building SPM will recognize themselves in the only personal thanks I want to make explicit, to Professor Rogério Tenreiro. Identifying himself, with his well-known sense of humor, as the President's "page", he is responsible for much of the success of SPM's current organization and many other activities, as well as some very well spent SPM moments! To Rogério, we also owe other unsuccessful efforts, that no one else can appreciate..."

Other written testimonies exist and will remain as a proof to the legacy of Rogério Tenreiro, retired Assistant Professor with Habilitation at the Faculty of Sciences of the University of Lisbon (FCUL). They are documented in the book TAXONOMIC ANALYSIS OF Rogerius tenreirus—A POLYPHASICAL PERSPECTIVE, which brings together testimonies and messages from more than a hundred people who shared with him, in different contexts, his path in academia. It only takes a glance at this book, to the contents of which undergraduate, master's or doctoral students have contributed, as well as colleagues from his own and other institutions, in addition to partners from outreach activities in his university and collaborators from companies and other organizations outside academia, to appreciate the reasons why SPM has awarded this prize.

Very briefly, students and colleagues highlighted his excellent, carefully prepared lessons, which aroused students' curiosity and made them want to know more and go further. They considered that he did not just teach classes, he encouraged them to think, to question, to be critical, so he was considered an inspiration more than a teacher. Demanding as a teacher, but also patient and attentive to difficulties. Committed to guiding those who worked with him, with a great ability to lead, suggest new directions, other possible solutions to the impasses in the research being carried out

and demanding in the analysis of experimental results. Bad-tempered against lack of professionalism, pettiness or what he disregarded, he went into battle with courage and determination, ignoring his personal interests. Assertive, forthright, forceful, even caustic, sometimes uncomfortable, but with an immense sensitivity, generosity and solidarity.

In addition to his creativity, sharp critical thinking and rigor (perfectionist), organizational skills and dedication to research and teaching in microbiology, his role in the development and transfer of technology and knowledge should be highlighted. Pragmatic and uncomplicated, those who collaborated with him on projects and scientific development work are unanimous about his leadership qualities, combining vision with enthusiasm and perseverance to achieve goals, an irreproachable professional zeal, in which availability and responsibility were never lacking. In this context, his role in setting up and developing the ICAT/TecLabs - Innovation Center integrated into FCUL, today a startup incubator, linked to scientific projects and a hub for all initiatives related to knowledge transfer and entrepreneurship, should not be forgotten.

Finally, it must be admitted that, although very flattering, the testimonies in the book are short. Missing were many others who know him, consider him, admire him and are grateful to him and adhere to the book's contents. This is the case of the signatories.

Isabel Sá-Correia and Jorge Pedrosa

MICRO BIOTEC 23

Plenary Lecture

Overview

PL1 - HYPES, HOPES, AND THE WAY FORWARD FOR MICROALGAL BIOTECHNOLOGY
Maria J Barbosa

PL2 - AFFINITY PROTEINS FOR BIOTECHNOLOGICAL AND MEDICAL PURPOSES
Sophia Hober

PL3 - MAPPING FOOD MICROBIOMES TO IDENTIFY SOURCES AND PATTERNS OF SUCCESSION LINKED WITH QUALITY AND SAFETY TRAITS
Avelino Alvarez-Ordóñez

PL4 - ENTERIC INFECTIONS DISRUPT GUT MICROBIOTA BIOFILMS: FROM MECHANISMS TO THERAPY
Andre G. Buret

PL5 - BIOECONOMY AND OTHER RENEWABLES OPPORTUNITIES FOR PORTUGAL
Luuk van der Wielen

MICROBIOTEC 23

Keynote Lecture

Overview

KL1 - CYANOBACTERIA AND THEIR METABOLITES: IMPLICATIONS FOR HUMAN HEALTH AND THE ENVIRONMENT
Vitor Vasconcelos

KL2 – AI + BIOLOGY FOR SUSTAINABLE INGREDIENTES
Simão Soares

KL3 - PROBING BACTERIAL INTERACTIONS IN POLYMICROBIAL BIOFILMS TO BETTER UNDERSTAND BACTERIAL VAGINOSIS
Nuno Cerca

KL4 - MICROBIAL TECHNOLOGY FOR SUSTAINABLE DEVELOPMENT: EXPLORING BACTERIAL STRATEGIES FOR METAL INTERACTION
Paula V. Morais

KL5 - *VIBRIO NATRIEGENS* AS A NEW HOST FOR PLASMID DNA PRODUCTION
Duarte Miguel Prazeres, Guilherme F. Mourato, Ana Rita Silva-Santos, Jorge João

KL6 - ADHESINS IN OPPORTUNISTIC RESPIRATORY PATHOGENS: FROM SEEING TO DETERMINING THEIR BIOLOGICAL FUNCTION
Arsénio M. Fialho

KL7 - DISCLOSING GUT BACTERIAL SIGNATURES IN ELDERLY FROM ALGARVE
M. Leonor Faleiro

KL8 - IONIC LIQUIDS AS ADDITIVES FOR STABILIZATION AND FORMULATION OF PROTEIN-BASED PRODUCTS
Nathalia V. Veríssimo, Qi Han, Valéria C. Santos-Ebinuma, Tamar L. Greaves, Jorge F.B. Pereira

KL9 - ENZYMATIC HYDROLYSIS: A TOOL TO PRODUCE FUNCTIONAL AND SUSTAINABLE FOOD INGREDIENTS
Manuela Pintado

KL10 - NATURE INSPIRED SOLUTIONS IN HEALTH BIOTECHNOLOGY
Cecília Roque

KL11 - NATURAL PRESERVATIVES FOR THE FOOD AND BEVERAGE INDUSTRIES
Sandrina A. Heleno, Márcio Carocho

KL12 - MICROBIAL CULTURE COLLECTIONS: STRAINS, SERVICES, PROJECTS AND CONSORTIA TO UNDERPIN THE MICROBIOTECH INNOVATION
Nelson Lima

KL13 - UNLEASHING THE POWER AGAINST SUPERBUGS: UNRAVELING GLOBAL CHALLENGES AND INNOVATIONS IN ANTIMICROBIAL RESISTANCE
Luísa Vieira Peixe

KL14 - NEW GENERATION OF DNA VACCINES AGAINST CANCERS INDUCED BY HPV INFECTION
Ângela Sousa

KL15 - NUCLEIC ACID BIOSENSING PLATFORM FOR SARS-COV-2 DETECTION
Paula Martins-Lopes, Alexandra Lino, Helena Gonçalves

MICROBIOTEC 23

Oral Presentation

Overview

TOPIC 1

OP1.1 - MUCUS OF THE PHOTOSYNTHETIC SEA SLUG *ELYSIA CRISPATA*: PROTEOME AND ANTI-BACTERIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGINOSA*
Diana Lopes, Susana S. Aveiro, Eva Cunha, Sónia Cruz, Manuela Oliveira, Pedro Domingues, Paulo Cartaxana

OP1.2 - THE MICROBIOME OF OCTOCORALS: FROM COMMUNITY STRUCTURE, FUNCTION AND METABOLIC INTERACTION TO BLUE BIOTECH OPPORTUNITIES
Tina Keller-Costa, Sandra G. Silva, João Almeida, Daniela Silva, Ângela Taipa, Jorge Gonçalves, Asunción Lago-Lestón, Nikos C. Kyrpides, Ulisses Nunes da Rocha, Rodrigo Costa

OP1.3 - ANTIBIOTIC-RESISTANT BACTERIA HITCHHIKING MICROPLASTICS IN A RIVER: DETAILED ANALYSIS OF CARBAPENEMASE-PRODUCING ENTEROBACTERALES IN THE PLASTISPHERE
Isabel Silva, Marta Tacão, Isabel Henriques

OP1.4 - PEPTIDES FROM ENTOMOPATHOGENS AS A SOURCE OF NEW BIOINSECTICIDES
Duarte Toubarro, Jorge Frias, Tiago Paiva, Nelson Simões

OP1.5 - SCREENING FOR PLASTIC-DEGRADING POTENTIAL IN MARINE BACTERIA ASSOCIATED WITH NET BIOFILMS AND HYDROCARBON DEGRADATION
Rafaela Perdigão, Maria F. Carvalho, Catarina Magalhães, Sandra Ramos, C. Marisa R. Almeida, Ana P. Mucha

OP.1.6 - BACTERIA-BASED BIOREFINERY: GENERATING BIOACTIVE OLIGOSACCHARIDES FROM A SINGLE FERMENTATION OF SEAWEED
Duarte Toubarro, Duarte Seixas, Nelson Simões

OP1.7 - VALORIZATION OF *GELIDIUM CORNEUM* BY-PRODUCT THROUGH SOLID-STATE FERMENTATION
Marta Ferreira, José Manuel Salgado, Helena Peres, Isabel Belo

OP1.8 - EXPLORING THE FATE OF CHIRAL PHARMACEUTICALS IN AN AGS SYSTEM UNDER SALTWATER INTRUSION PHENOMENA
Catarina Miranda, Catarina Amorim, Clara Piccirillo, Maria Elizabeth Tiritan, Paula Castro

OP1.9 - THE QUEST FOR NATURAL MICROBIOMES: TRANSFORMING CO_2 INTO VALUABLE BIOPRODUCTS THROUGH PHOTOSYNTHESIS
André Freches, Inês Cardoso, Joana Fradinho, Maria Reis,

OP1.10 - AN ALLOSTERIC REDOX SWITCH ENABLES OXYGEN PROTECTION OF A FORMATE DEHYDROGENASE FROM *DESULFOVIBRIO VULGARIS* HILDENBOROUGH
Ana Rita Oliveira, Cristiano Mota, Rita Rebelo Manuel, Guilherme Vilela-Alves, Neide Pedrosa, Vincent Fourmond, Kateryna Klymanska, Christophe Léger, Bruno Guigliarelli , Maria João Romão, Inês Cardoso Pereira

OP1.11 - HARNESSING *SACCHAROMYCES CEREVISIAE* FOR SUSTAINABLE FDCA PRODUCTION: OPTIMIZING 5-HMF DETOXIFICATION AND DERIVATIVE SYNTHESIS
*Cristiana Martins, Carlos E. Costa,, Joana Cunha, Lucília Domingues,**

TOPIC 2

OP2.1 - TRADITIONAL CHEESES AS BIO-FACTORIES FOR PROBIOTIC BACTERIA
Susana Serrano, Maria V. Ferreira, Cinthia Alves-Barroco, Maria Teresa Barreto- Crespo, Teresa Semedo-Lemsaddek

OP2.2 - GENOTYPIC DIVERSITY AND OFF-FLAVORS PRODUCTION IN *DEKKERA BRUXELLENSIS* AS DRIVERS FOR SELECTION OF BIOCONTROL AGENTS
João Sousa, Emanuel Peixoto, Rogério Tenreiro, Nuno Mira, Ana Mendes-Ferreira

OP2.3 - CHARACTERIZATION OF FOODBORNE PATHOGENIC *VIBRIO SPP.* IN ENVIRONMENTAL AND SEAFOOD SAMPLES COLLECTED FROM ANGOLA
Inês C. Leal, Babak Najafpour, Eunice Cassoma, Isaac Bumba, Carmen dos Santos, Adelino V. M. Canário, Deborah M. Power, João C. R. Cardoso

OP2.4 - FIRE4CAST – AN INTEGRATIVE STRATEGY FOR ACCURATE PREDICTION OF FIRE BLIGHT DISEASE OUTBREAKS IN PORTUGUESE ORCHARDS
Daniel McGuire, Francisco Pinto, Telma Costa, Joana Cruz, Rui Sousa, Miguel Leão de Sousa, Carmo Martins, Ana Tenreiro, Rogério Tenreiro, Margarida Gama-Carvalho, Leonor Cruz

OP2.5 - MORE THAN COLORING AGENTS: NATURAL PIGMENTS WITH ANTIMICROBIAL ACTIVITY
Nuno Ribeiro da Silva, Lina F. Ballesteros, Carla Fonseca, Bruna Basto, José A. Teixeira, Sara C. Silvério

OP2.6 - SUSTAINABLE FILM SOLUTIONS: EXPLORING CUTIN RECOVERY FROM TOMATO BY-PRODUCTS FOR HYDROPHOBIC APPLICATIONS
Andreia Simões, Isabel M. Coelhoso, Vítor D. Alves, Carla Brazinha

TOPIC 3

OP3.1 - AVIAN ANTIBODY PRODUCTION FOR SARS-COV-2 SPIKE PROTEIN: EPITOPE-SPECIFIC INSIGHTS
Tiago Ochôa-Pires, Rafaela Seabra, Beatriz Vaz, Ana Beatriz Marques, Guilherme Gabriel, Rui Jorge Nobre, Luís Pereira de Almeida, Ricardo S. Vieira-Pires

OP3.2 - KERATIN-BASED MATERIALS FOR BIOMEDICAL APPLICATIONS
Cariny Polesca, Bruno Neves, Jason Hallett, João Coutinho, Helena Passos , Mara Freire

OP3.3 - A NOVEL BACTERIOPHAGE RECEPTOR BINDING PROTEIN FOR IMPROVED *SALMONELLA* DETECTION
Ana Brandão, Sílvio Santos

OP3.4 - CYTOPROTECTIVE GUARDIANS: QUANTIFYING THE CYTOPROTECTIVE EFFICIENCY OF EXTRACELLULAR VESICLES (EVS) AGAINST *STAPHYLOCOCCUS AUREUS* ALPHA HEMOLYSIN (HLA)
Diogo Gonçalves, Ricardo Silva, Beatriz Avó, Nuno Bernardes, Ana M. Azevedo, Sandra N. Pinto, Fábio Fernandes

OP3.5 - CAT DERIVED ANTIBODY FRAGMENTS AS A PROMISING STRATEGY FOR TREATMENT AND PREVENTION OF SARS-COV-2 INFECTION
Isa Moutinho, Mafalda Henriques, Sara Cardoso, Teresa Coutinho, Luís Tavares, Solange Gil, Telmo Nunes, Frederico Aires-da-Silva

OP3.6 - NEW FLUOROQUINOLONE-PHENOTHIAZINE HYBRIDS WITH ANTIBACTERIAL PROPERTIES AGAINST *STAPHYLOCOCCUS AUREUS*
Marina Cassago Posso, João Lourenço Serrano, Paulo Almeida, Fernanda Domingues, Samuel Silvestre, Susana Ferreira,

TOPIC 4

OP4.1 - CONTROLLING DIARRHEAGENIC *E. COLI* WITH BACTERIOPHAGES: FACTS AND CHALLENGES
Francisca Rodrigues, Isidro García-Meniño, Azucena Mora, Luís Daniel Melo, Ana Oliveira

OP4.2 - A PORTRAIT OF THE INTERACTION ESTABLISHED BETWEEN VAGINAL COLONIZING *LACTOBACILLUS* SPECIES AND THE PATHOGENIC YEASTS *CANDIDA ALBICANS* AND *CANDIDA GLABRATA*
Nuno A Pedro, Gabriela Fontebasso,, Sandra N Pinto,, Dalila Mil-Homens,, Arsénio Fialho,, Nuno Pereira Mira,

OP4.3 - PROBE-BASED METAGENOMICS: AN ADDED VALUE FOR CLINICAL DECISION?
Luís Coelho, Rita Ferreira, Daniel Sobral, João Dourado, Joana Isidro, Verónica Mixão, Miguel Pinto, Alexandra Nunes, Sílvia Duarte, Luís Vieira, Vítor Borges, João Paulo Gomes

OP4.4 - HOW DO METALLOENZYMES HELP IN THE FIGHT AGAINST CLOSTRIDIOIDES DIFFICILE'S INFECTION?
Filipe Folgosa, Maria C. Martins, Nicolas Kint, Carolina A. Feliciano, Claire Morvan, Susana F. Fernandes, Bruno Dupuy, Isabelle Martin-Verstraete, Miguel Teixeira

OP4.5 - UNVEILING THE PROTECTIVE MECHANISMS OF PYRUVATE KINASE DEFICIENCY AGAINST MALARIA: TRANSFORMING VULNERABILITY INTO RESILIENCE
Ana Balau, Daniel Sobral, Patrícia Abrantes, Maria Carvalho, Inês Morais, Verónica Mixão, Márcia M. Medeiros, Filomena A de Carvalho, Maria Alexandra Antunes, João Paulo Gomes, Sandra Antunes, Ana Paula Arez

OP4.6 - CHARACTERIZATION OF BIOFILM FORMATION OF BACTERIA ASSOCIATED WITH THE MIDGUT OF ANOPHELES MOSQUITO VECTOR OF MALARIA
Margarida Marques, Sofia Santos Costa, Sandra N Pinto, Henrique Silveira

OP4.7 - DECIPHERING HOST-RICKETTSIA INTERACTIONS: MOONLIGHTING APRC RECRUITS HUMAN COMPLEMENT REGULATOR C4BP ACTING AS AN EVASIN
Ana Luísa Matos, Isaura Simões

OP4.8 - THE SMALL NON-CODING RNA NCRNA3 IS INVOLVED IN ANTIBIOTIC RESISTANCE IN BACTERIA OF THE BURKHOLDERIA CEPACIA COMPLEX
Gonçalo R. Matos, Joana R. Feliciano, Beatriz Jesus, Jorge H. Leitão

OP4.9 - METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* (MSSA) NASAL SCREENING AT ICU ADMISSION AS A PREDICTIVE TOOL FOR SUBSEQUENT MSSA RESPIRATORY INFECTION
Teresa Conceição, Mafalda Felgueiras, Silvia Ferreira, Marisa Simões, Daniela Carvalho, Angelina Lameirão, Ana Marques, Dulce Pascoalinho, Maria Paula Gonçalves, Ana Fernandes, Hermínia de Lencastre, Valquíria Alves

OP4.10 - SCC*mec* ACQUISITION IS LINKED TO *STAPHYLOCOCCUS EPIDERMIDIS* BLOODSTREAM INFECTION OUTBREAKS IN A NEONATE INTENSIVE CARE UNIT (NICU)
Nuno Alexandre Faria, Roberta Filipini Rampelotto, Rosmari Hörner, Maria Miragaia

OP4.11 - EMERGING THREAT: RESISTANCE TO CEFTAZIDIME-AVIBACTAM AND OTHER LAST-LINE ANTIBIOTICS AMONGST CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* IN PORTUGAL
Ana Beatriz Gonçalves, Valquíria Alves, Isabel Neves, Maria Antónia Read, Luísa Peixe, Ângela Novais

OP4.12 - CEFOTAXIME-RESISTANT *ESCHERICHIA COLI* FROM URINE INFECTIONS AND ENVIRONMENTAL WATERS: A COMPARATIVE ANALYSIS
Joana Abreu Silva, Catarina Ferreira, Célia M. Manaia

OP4.13 - BREAKING THE WALL: MYCOBACTERIUM BOVIS TRANSMISSION AT THE ANIMAL- ENVIRONMENT INTERFACE
André C. Pereira, Daniela Pinto, Mónica V. Cunha,

OP4.14 - UNRAVELLING MECHANISMS OF PNEUMOCOCCAL INTRA-SPECIES INTERACTIONS
Carina Valente, Ana R. Cruz, Adriano O. Henriques, Raquel Sá-Leão

OP4.15 - CHARACTERIZATION OF A NOVEL QUORUM SENSING SYSTEM IN *BACTEROIDES THETAIOTAOMICRON*
Carina R. Galhofa, Kai Papenfort, Karina B. Xavier

TOPIC 5

OP5.1 - THE UNIQUE ABILITY OF *STAPHYLOCOCCUS EPIDERMIDIS* PHAGE SEP1 ACTIVATING DORMANT CELLS
Maria Daniela Silva, Graça Pinto, Ângela França, Joana Azeredo, Luís Melo

OP5.2 - HIGH AND HOMOGENEOUS LEVELS OF OXACILLIN RESISTANCE IN MRSA THROUGH THE ACTIVATION OF THE STRINGENT STRESS RESPONSE LEADS TO UP-REGULATION OF THE CAPSULAR POLYSACCHARIDE
Catarina Milheiriço, Vítor Borges, João Paulo Gomes, Beatriz Silva, Alexander Tomasz, Hermínia de Lencastre

OP5.3 - GENOME MINING AND BIOCHEMICAL CHARACTERIZATION OF POLYHYDROXYALKANOATES PRODUCED BY BACTERIAL ISOLATES FROM UCCCB
Paula V. Morais, Diogo Vicente, Diogo Neves Proença

OP5.4 - EXPLORING THE FUNCTIONAL AND STRUCTURAL ASPECTS OF THE CITRATE TRANSPORTER CEXA FROM *ASPERGILLUS NIGER*
João Alves, Maria Sousa-Silva, Pedro Soares, Michael Sauer, Margarida Casal, Isabel Soares-Silva

OP5.5 - EXPLORING GENOMIC PLASTICITY IN *CANDIDA GLABRATA* AND ITS IMPACT ON BIOFILM EVOLUTION IN CLINICAL ENVIRONMENTS
Inês Vieira Costa, Maria Zolotareva, Mafalda Cavalheiro, Mónica Galocha, Miguel Cacho Teixeira

TOPIC 6

OP6.1 - CONTINUOUS PRODUCTION OF INFLUENZA VLPS AS VACCINE CANDIDATES: A MULTI-STAGE BIOREACTOR APPROACH
Ricardo Correia, Taja Zotler, Miguel Graça, Bárbara Fernandes, Gorben Pijlman, Netherlands Paula M. Alves, António Roldão

OP6.2 - ASSESSMENT OF MICROALGAE SIDE STREAMS POTENTIAL FOR VFA PRODUCTION IN BATCH FERMENTATION
Claudia Duarte, Marisa Cardoso, Mariana Matos, Joana Fradinho, Bruno Ferreira, Jorge Pereira, Maria Reis

OP6.3 - Monitoring productivity and predicting crash of *Phaedactylum tricornutum* cultures using spectroscopy and machine learning
Pedro Brandão, Constança Bertrand, Rodrigo Martins, Francisco Nunes, Claudia Galinha, Francisco Nascimento

OP6.4 - NEW SHUTTLE VECTOR-BASED EXPRESSION SYSTEM TO IMPROVE THE BIOSYNTHESIS AND PURIFICATION OF MEMBRANE BOUND CATECHOL-O-METHYLTRANSFERASE
Ana Margarida Gonçalves, Márcia Correia, Claúdio Jorge Maia, João Queiroz, Maria João Romão, Luís António Passarinha

OP6.5 - A MOLECULAR MODELING PERSPECTIVE ON THE DEVELOPMENT OF SYNTHETIC AFFINITY LIGANDS
Carlos Costa, Carolina Natal, Jéssica Rodrigues, Ana Margarida Dias, Ana Cecília Roque, Arménio J. M. Barbosa

TOPIC 7

OP7.1 - COMMENSALISM VS PATHOGENICITY IN *STAPHYLOCOCCUS EPIDERMIDIS* - UNCOVERED BY AN INTEGRATED OMICS APPROACH
Luís G Gonçalves, Elisabete Morais, Filipe Magalhães, Susana Santos, Laidson P Gomes, Jean Armengaud, Maria Zimmermann-Kogadeeva, Ana Gil, Maria Miragaia, Ana V Coelho

OP7.2 - PHENOTYPIC, PHYSIOLOGICAL AND GENOMIC PORTRAIT OF A "HUMAN-NAÏVE" CANDIDA GLABRATA STRAIN: A SHED OF LIGHT INTO THE PATH TOWARDS COLONIZATION OF THE HUMAN HOST
Maria Joana Pinheiro, Dalila Mil-Homens, Ana Mendes-Ferreira, Nuno Pereira Mira

TOPIC 8

OP8.1 - BIOCONVERSION OF PLASTICS' BUILDING BLOCKS INTO BACTERIAL CELULOSE TOWARDS CIRCULAR ECONOMY
Cátia Gil, Maria A. M. Reis, Filomena Freitas, Cristiana A. V. Torres

OP8.2 - UNRAVELLING THE POTENTIAL OF ORANGE JUICE SIDE STREAMS: HESPERIDIN-RICH EXTRACTION AND ELECTROSPRAYING FOR A BIO-BASED INGREDIENT DEVELOPMENT

Ana A. Vilas-Boas, Cristina Prieto, Débora A. Campos, Marta Correia, Jose Maria Lagaron, Manuela Pintado

TOPIC 9

OP9.1 - EATRIS | EUROPEAN INFRASTRUCTURE FOR TRANSLATIONAL MEDICINE

Helena Paula Baião

OP9.2 - EURESTOP- EUROPEAN NETWORK FOR DIAGNOSIS AND TREATMENT OF ANTIBIOTIC-RESISTANT BACTERIAL INFECTIONS: OPPORTUNITIES AND INITIATIVES COST ACTION [CA21145]

M Amparo F Faustino

MICROBIOTEC 23

Sponsor Talks

A CUIDAR DA SAÚDE E BEM-ESTAR DE PESSOAS COMO NÓS.

Quilaban
uma fórmula de vida

A MUDANÇA É POSSÍVEL: DOS DIAGNÓSTICOS CONVENCIONAIS À OPERACIONALIDADE DO BD MAX™

Bruno Taveira
Country Lead Portugal, Sintra, Portugal

ABSTRACT

Molecular enteric testing is a method of detecting the presence of pathogens that cause gastrointestinal infections, such as bacteria, viruses, and parasites. Molecular enteric testing has several clinical advantages over conventional methods, such as culture, microscopy, and antigen detection. Some of these advantages are:

- Faster and more accurate diagnosis: Molecular enteric testing can provide results in less than 3.5 hours, compared to 2 days or more for culture methods Molecular enteric testing can also detect multiple pathogens simultaneously and identify low-abundance or fastidious organisms that are difficult to grow or observe.
- Improved patient management and infection control: Molecular enteric testing can help clinicians make appropriate treatment decisions, such as prescribing antibiotics or antivirals, or avoiding unnecessary use of antimicrobials that can lead to resistance. Molecular enteric testing can also help prevent the spread of infections by identifying the source and type of pathogens and implementing isolation or hygiene measures.
- Reduced laboratory workload and cost: Molecular enteric testing can simplify the stool bench by eliminating the need for multiple tests and reagents, reducing hands-on time and human error, and increasing throughput and efficiency. Molecular enteric testing can also lower the total cost of ownership by limiting indirect costs, such as hospitalization, complications, and morbidity.

The importance of enteric tests with BD MAX system is that they allow for the rapid and accurate detection of the most common pathogens responsible for infectious diarrhea, such as bacteria, parasites, and viruses. The enteric tests with BD MAX system include the following panels:

- BD MAX™ Enteric Bacterial Panel: detects over 90% of bacteria causing infectious gastroenteritis, including Salmonella spp., Campylobacter spp. (jejuni and coli), Shigella spp./enteroinvasive Escherichia coli (EIEC) and Shiga toxin producing organisms (STEC, Shigella dysenteriae).
- BD MAX™ Enteric Extended Bacterial Panel: detects other bacterial pathogens of lower prevalence, such as Yersinia enterocolitica, E. coli enterotoxigenic, Plesiomonas shigelloides and Vibrio spp.
- BD MAX™ Enteric Parasite Panel: detects Giardia lamblia and Entamoeba histolytica, which are the most common parasites associated with diarrhea.
- BD MAX™ Enteric Viral Panel: detects the main enteric viruses, such as norovirus, rotavirus, adenovirus (40/41), sapovirus and human astrovirus.

The enteric tests with BD MAX system are performed on the BD MAX™ System, which is a molecular platform integrated and automated that can run several BD assays and assays of open system.

SEQUÊNCIA FESTIVA
PREÇOS IMBATÍVEIS EM SEQUENCIAÇÃO DE ADN

ATÉ 31 DE DEZEMBRO

YOU TUBE IT – 3€

Não é truque, é tratamento!
Por causa do Halloween, Sequenciação Sanger de DNA YouTubeIt por 3€ por reacção ao comprar 10 caixas de 50 tubos cada.
Assuste-se com estes preços até final do ano!

SEQUÊNCIA FESTIVA
PREÇOS IMBATÍVEIS EM SEQUENCIAÇÃO DE ADN

ATÉ 31 DE DEZEMBRO

METAGENÓMICA – 65€

Neste Natal contribuímos para que desvendes o mistério das tuas amostras complexas! Serviço premium de metagenómica - Sequenciação de amplicões 16S ou ITS por apenas 65€! Mínimo de 20 amostras para um brinde genómico.

WGS – 130€

Descobre o universo genético dos teus procariotas com o serviço de Sequenciação de Genoma Completo! Na STAB VIDA, quando se aproxima o final do ano, não é apenas o Pai Natal que traz surpresas: os nossos preços vão-te surpreender nesta época festiva: apenas 130 euros por genoma (PE150 e cerca de 1 Gbp de output para as tuas amostras, para qualquer projeto com um mínimo de 10 amostras).

STAB vida
Your easy genetics laboratory

Whatsapp us now!
+351 93 843 77 66
info@stabvida.com

DOCTOR VIDA POCKET PCR – A TRUE LAB-ON-PHONE

Francisco Sousa, Gonçalo Doria, Carla Clemente, Eduardo Coelho, Rui Crespo, Orfeu Flores
STAB VIDA Lda, Lisbon, Portugal

(*) email: ***francisco.sousa@satbvida.com***

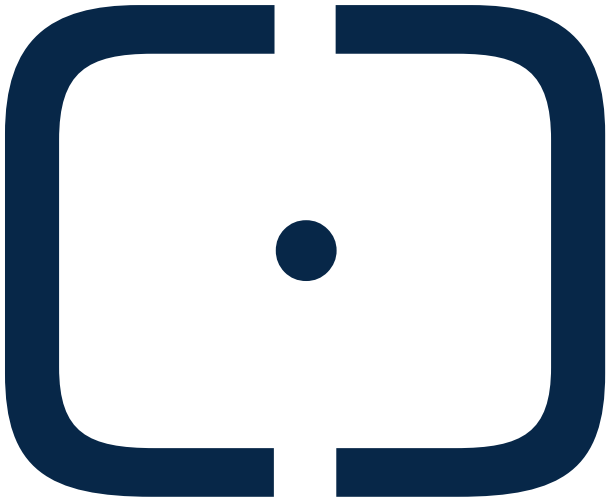
ABSTRACT

The present study highlights the Doctor Vida equipment, developed by STAB VIDA, as an innovative device in the field of genetic biomarker analysis. This portable CE-IVD certified system demonstrates a remarkable ability to amplify a wide range of biomarkers, presenting itself as a valuable tool for healthcare professionals and genetic researchers.

The portability of Doctor Vida, operated by a mobile phone and compatible with power banks, allows its use in various settings, including remote environments and field laboratories. Its exceptional efficiency and accuracy in amplifying genetic biomarkers make sample analysis faster and more precise than conventional methods, showing a detection agreement of up to 98% compared to RT-PCR.

This device offers a practical approach to studying various genetic biomarkers, from SNPs to the detection of associated bacterial agents, viruses, RNA or DNA. With its proven efficiency, Doctor Vida is an ideal partner for driving research, converting bench tests into point-of-care commercial solutions.

This work presents a detailed review of the characteristics and applications of the Doctor Vida equipment, highlighting its fundamental role in conducting advanced studies in the field of genetics and biomarker diagnostics.



COMMUNITIES – COMUNICAÇÕES, LDA. – SERVICES AND COMPANY PRESENTATION

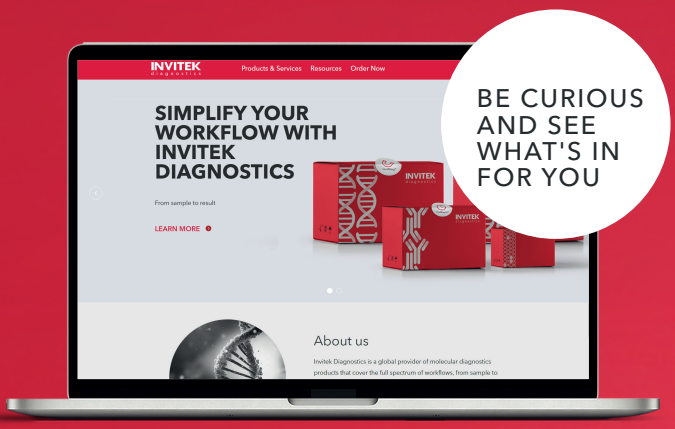
André Canilho
Communities – Comunicações, Lda., Covilhã, Portugal

(*) email: ***andre@communities.pt***

ABSTRACT

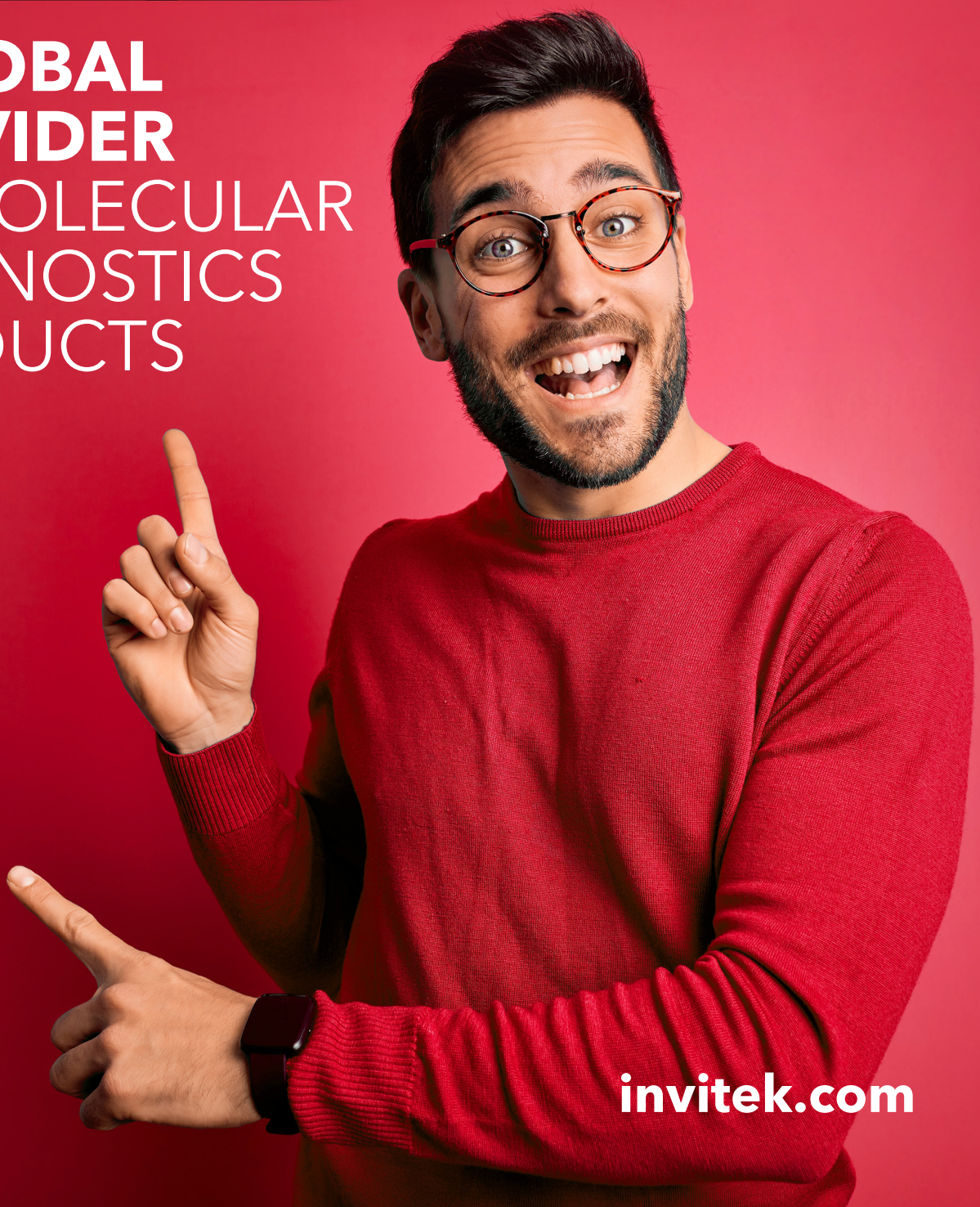
Communities SA was established in 2006, with the aim of developing new communication technologies based on community Internet access and online content.
To date, we have been pioneers in establishing Open-Source business models in Portugal.
In 2008, the society Communities-Comunicações, Lda was born. The start-up was inkubated in Parkurbis Industrial Park of Tortosendo, Covilhã.
Some of our competencies range from:
Data processing activities.
Consultancy in information and computer technologies.
Provision of electronic communications services.
Software editing and development and other activities related to computer information technologies.
Domiciliation of information and related activities such as the management of equipment and telecommunications.
Retail trade and repair of telecommunications equipment, computers, peripheral units, and computer programs, in specialized establishments.
Installation and maintenance of computer networks.

INVITEK
diagnostics



BE CURIOUS AND SEE WHAT'S IN FOR YOU

A GLOBAL PROVIDER OF MOLECULAR DIAGNOSTICS PRODUCTS



FOLLOW US

INVITEK.COM

invitek.com

SIMPLIFY YOUR WORKFLOW WITH INVITEK DIAGNOSTICS

Josué Carvalho
Invitek Diagnostics , Tondela, Portugal

(*) email: ***josue.carvalho@invitek.com***

ABSTRACT

Invitek Diagnostics is a global provider of molecular diagnostics products that offer a comprehensive portfolio of solutions for a wide range of applications. With our modular product approach, ease of use, and superior customer service, we are committed to helping our customers achieve the best possible results. With Invitek Diagnostics, you can streamline your processes and work efficiently from sample collection and nucleic-acid extraction to detection and point-of-use.

Our product portfolio includes a wide range of molecular diagnostics solutions, including sample collection devices, nucleic acid extraction kits and real-time PCR assays for:

- Life Sciences Research: We offer a wide range of products for use in life science research, e.g., cell and tissue analysis or plant sciences. Our products are essential for molecular biology workflows such as DNA sequencing, PCR amplification or gene expression analysis.
- Molecular Diagnostics: Our comprehensive product line includes sample collection and stabilization devices and kits for the extraction of nucleic acids from diverse starting materials, such as stool for microbiome analysis or saliva for genetic testing. Whether for oncology research or detection of infectious diseases in human and veterinary diagnostics, our extraction and detection kits offer versatile solutions.
- Food Testing: Designed for precision and reliability, our kits detect contaminants such as allergens and GMOs as well as pathogens in food, ensuring the highest standards of food quality. From rapid on-site testing to sophisticated laboratory testing, our products cover the diverse needs of the food industry.

In addition, we offer a service for oligonucleotide synthesis. DNA primers and probes with up to 100 bases in length can be flexibly designed with a variety of modifications, purification levels and synthesis scales. Our oligonucleotides are synthesized in ISO13485-certified cleanrooms that offer a high-quality oligonucleotide synthesis service helping our customers achieve their research, development, and diagnostic goals.

MICRO BIOTEC 23

Poster Session

Overview

POSTER SESSION #1 (Thursday / Friday)

TOPIC 1

P1.1 - HETEROLOGOUS EXPRESSION AND CHARACTERISATION OF NOVEL CHITINASES FROM A YET UNCULTURABLE, HIGHLY ABUNDANT BACTERIAL SYMBIONT OF OCTOCORALS
João Almeida, Mafalda Leitão, Nuno Borges, Rodrigo Costa, Ângela Taipa, Tina Keller-Costa

P1.2 - MICROBE-ENHANCED PHYTOREMEDIATION WITH SALICORNIA EUROPAEA IN AQUAPONICS SETUPS
Maria J. Ferreira, Erika Garcia, Isabel N. Sierra-García, Diana C.G.A. Pinto, Javier Cremades, Helena Silva and Ângela Cunha

P1.3 - INSIGHTS INTO THE CULTURABLE BACTERIAL COMMUNITY OF LONG-TERM AQUARIUM TROPICAL OCTOCORALS
Matilde Marques, Elsa Santos, Núria Baylina, Raquel Peixoto, Tina Keller-Costa, Rodrigo Costa

P1.4 - EFFECTS OF DEFORESTATION ON SOILS FROM GUINEA-BISSAU
Rafael António, Ana João Martins, Inês Cordeiro, Eduardo Onofre Feijão, Ana Rita Matos, Filipa Monteiro, Mónica Sebastiana

P1.5 - MINING THE BIOSYNTHETIC POTENTIAL FOR SPECIALIZED METABOLISM OF LEGE CULTURE COLLECTION
Raquel Castelo-Branco, Adriana Rego, João Morais, Vitor Vasconcelos, Pedro N. Leão

P1.6 - EXPLORING THE POTENTIAL OF CO-CULTURES TO ENHANCE SECONDARY METABOLITES PRODUCTION IN THE MARINE BACTERIUM AND ANTIBIOTIC PEPTIDE PRODUCER AQUIMARINA SP. AQ135
Ana Maria Fernandes, Joana F. Couceiro, Tina Keller-Costa, Rodrigo Costa

P1.7 - ENTOMOPATHOGENIC FUNGI AS A POTENTIAL BIOCONTROL AGENT OF SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII* (MATSUMURA), IN BLUEBERRY PRODUCTION
Sara Peixoto, Maria F. Gonçalves, Guilhermina Marques

P1.8 - ELIMINATION OF ANTIBIOTIC RESISTANCE GENES DURING CONVENTIONAL ACTIVATED SLUDGE WASTEWATER TREATMENT: THE EFFECT OF TEMPERATURE
Sara Ribeirinho-Soares, Vasco Braga, Sofia Cabral, Vítor J.P. Vilar, Célia M. Manaia, Olga C. Nunes

P1.9 - APPLICATION OF PLANT GROWTH PROMOTING BACTERIA AS BIOSTIMULANTS IN *OLEA EUROPAEA*
Lara Soares, Pedro Monteiro, Jyoti Chhetri, I. Natalia Sierra-García, Gloria Pinto, Ângela Cunha

P1.10 - PHOTODYNAMIC INACTIVATION OF *OLEA EUROPAEA* PATHOGENS: PROSPECTS ON A NOVEL PHYTOSANITARY APPROACH
Joana Soares, Isabel N. Sierra-Garcia, M. Amparo F. Faustino, Ângela Cunha

P1.11 - FEATHER WASTE RECYCLING: CREATING ECO-FRIENDLY BIO-PESTICIDES
Mário Teixeira, Júlia Gomes, Nelson Simões

P1.12 - EVALUATING OLEA EUROPAEA RHIZOSPHERE COLONIZATION USING GFP-MODIFIED PLANT-GROWTH PROMOTING BACTERIA
Eva Peixoto Martins, Maria Tavares, Jyoti Chhetri, Ângela Cunha, Tânia Caetano, I. Natalia Sierra-Garcia

P1.13 - WIDESPREAD OCCURRENCE OF CHITINASE-ENCODING GENES SUGGESTS THE ENDOZOICOMONADACEAE FAMILY AS A KEY PLAYER IN CHITIN PROCESSING IN THE MARINE BENTHOS
Daniela M. G. da Silva, Filipa R. Pedrosa, M. Ângela Taipa, Rodrigo Costa, Tina Keller-Costa

P1.14 - IMPROVING BIOMETHANE PRODUCTION BY ADDING CONDUCTIVE NANOMATERIALS
Ana Catarina Silva, Cátia S. N. Braga, Inês Oliveira, O. Salomé G. Soares, M. Fernando R. Pereira, Luciana Pereira, Andreia F. Salvador, Gilberto Martins

P1.15 - PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE BIOACTIVE PROPERTIES OF AYAHUASCA BEVERAGES
Joana Gonçalves, Ângelo Luís, Ana Gradillas, Antonia García, José Restolho, Nicolás Fernández, Fernanda Domingues, Eugenia Gallardo, Ana Paula Duarte

P1.16 - *ACACIA LONGIFOLIA* AND THE MICROBIAL CROWD IN ROOT-NODULES: WHAT HAPPENS AFTER A FIRE EVENT?
Joana Jesus, Cristina Máguas, Ricardo Dias, Mónica Nunes, Pedro Pascoal, Marcelo Pereira, Helena Trindade

P1.17 - CHARACTERIZATION OF THE MICROBIOME OF BIOCHAR-SUPPLEMENTED RECYCLED MANURE SOLIDS
Joana Fernandes Guerreiro, Ana Esteves, Ana José Pires, Lélia Chambel, Mónica Nunes, Pedro Pascoal, Marcelo Pereira, David Fangueiro, Luís Tavares, Ricardo Dias, Ricardo Bexiga, Manuela Oliveira

P1.18 - ANTIDERMATOPHYTIC ACTIVITY OF THE AZOREAN BLACK TEA *(CAMELLIA SINENSIS)*
José Sousa-Baptista, Ana Filipa Lenha-Silva, Rita Domingues, Daniela Calheiros, Edmilson Correia, Chantal Fernandes, Teresa Gonçalves

P1.19 - SPENT COFFEE GROUNDS: A POTENTIAL ANTIFUNGAL AGENT?
Daniela Calheiros, Maria Inês Dias, Ricardo C. Calhelha, Lillian Barros, Isabel C. F. R. Ferreira, Chantal Fernandes, Teresa Gonçalves

P1.20 - UNVEILING MICROBIAL ROLE IN STONE PINK DISCOLORATION AT BATALHA MONASTERY
Inês Silva, Cátia Salvador, Ana Z. Miller, António Candeias, Ana Teresa Caldeira

P1.21 - PORTUGUESE MARINE ECOSYSTEMS ARE A SOURCE OF NEW FUNGI
Alberto C. Abreu, Ana Cristina Esteves, Artur Alves

P1.22 - IMPACT OF SALINITY STRESS IN PLANT GROWTH, BIOCHEMISTRY AND MICROBIAL INTERACTIONS IN *OLEA EUROPAEA* CULTIVAR GALEGA VULGAR
Isabel N. Sierra-Garcia, Mónica Marques, Pedro Monteiro, Frederico Leitão, Glória Pinto, Ângela Cunha

P1.23 - INOCULATION OF BACTERIAL CONSORTIA AS A STRATEGY FOR OPTIMISATION OF LEGUME SEED MICROBIOME
Ana Santos, Ricardo Soares, Helena Machado, Ana Barradas, Paula Fareleira, Isabel Videira e Castro

P1.24 - THE CONNECTION BETWEEN ANIMAL ORIGIN OF HISTORICAL PARCHMENT AND MICROBIOME
Maria João Penetra, António Candeias, Catarina Miguel, Ana Teresa Caldeira

P1.25 - RESISTANCE OF *ACETOBACTER ACETI* TO THE MAJOR CHEMICALS FOUND IN WINERY WASTEWATERS
Nuno Ramos, Ana Baía, Alonso Escoto, Maria C. Fernandes, Ana Lopes, Annabel Fernandes

P1.26 - TACKLING CO_2 EXCESS WITH BIOELECTRODES
Nuno Machado, Inês A. C.Pereira, Américo G. Duarte, Felipe Conzuelo

P1.27 - ONE-YEAR SURVEILLANCE OF PATHOGENS IN A FULL-SCALE MUNICIPAL WASTEWATER TREATMENT PLANT
Rafael D. S. Tavares, Elsa T. Rodrigues, Marta Tacão, Isabel Henriques

P1.28 - BIOTECHNOLOGICAL PROSPECTS OF MARINE BACTERIA ISOLATED FROM THE SADO ESTUARY
Rodrigo Martins, Constança Bertrand, Francisco Quintas-Nunes, Pedro Reynolds- Brandão, Teresa Crespo, Francisco X. Nascimento

P1.30 – PROTEIN HYDROLYSATES FROM SALMON HEADS AND CAPE HAKE BY-PRODUCTS: A POTENTIAL SOURCE OF BIOACTIVE PEPTIDES
Matilde Leitão, Amparo Gonçalves, António Marques, Helena Oliveira, Maria Leonor Nunes, Bárbara Teixeira, Rogério Mendes, Romina Gomes, Carla Pires

P1.31 – UNWELCOME ENTHUSIASTS OF ALMADA NEGREIROS MURAL PAINTINGS – A METAGENOMIC APPROACH TO THE MURALS SITUATED IN ALCÂNTARA MARITIME STATION, LISBON (PORTUGAL)
L. Dias, M. Gil, I. Silva, A. Candeias, A.T. Caldeira

P1.32 – FROM EFFLUENTS TO ASSETS: WINERY WASTEWATER UTILIZATION FOR MICROALGAE FARMING
Catarina Dias, Ana Rita Martins, Ana Cláudia Sousa, Ana Gabriela Gomes, Miguel Cachã, Carla Santos

P1.33 – MYCELIA INACTIVATION PROCESSES –MAINTAINING THE FLEXIBILITY AND STRENGTH OF MYCELIUM-BASED BIOCOMPOSITES
Ana T. Oliveira, Miguel A. Ramos, Paula M. L. Castro

P1.34 – SUSTAINABLE PRODUCTION OF YEAST-DERIVED CAROTENOIDS USING RICE BRAN HYDROLYSATES
Thercia Rocha Balbino, Salvador Sánchez-Muñoz, Stephanie Custódio Inácio, Júlio César Santos, Jorge Fernando Brandão Pereira, Silvio Silvério da Silva

P1.35 – ANTI-*CANDIDA* ACTIVITY OF *CYMBOPOGON* SPP. ESSENTIAL OILS: A PROMISING NEW TREATMENT STRATEGY FOR SUPERFICIAL INFECTIONS
Sandra Simões Tomás Daniela Marques Almeida, Ana Sofia Oliveira, Joana Domingues, Rita Palmeira-de-Oliveira, José Martinez-de-Oliveira, Fernanda DelgadoAna Palmeira-de-Oliveira, Joana Rolo

P1.36 – UNLOCKING THE BIOTECHNOLOGICAL POTENTIAL OF MARINE MICROORGANISMS: THE RIVER2OCEAN PROJECT'S CONTRIBUTION TO THE BLUE BIOECONOMY
Ana Rita Bragança, João Gomes, Ana Rita Marques Silva, Tony Collins, Margarida Casal, Isabel Soares-Silva, Raul Machado

P1.37 – WATER DISINFECTION USING UVC DIODES THAT EMIT LIGHT AT DIFFERENT WAVELENGTHS
João Sério, Maria Eduarda Martins, Carolina Santos, Ana Paula Marques, Vânia Pobre, Cecília Arraiano, Mónica Serrano, Adriano Henriques, Maria Teresa Crespo, Vanessa, Jorge Pereira

P1.38 – ANTIPROLIFERATIVE ACTIVITY OF BIOACTIVE COMPOUNDS PRODUCED BY BACTERIAL ISOLATES FROM PRISTINE ENVIRONMENTS.
Patrícia Gatinho, A. Teresa Caldeira, Daniela Grilo, Ana Z. Miller, Amélia M. Silva, Cátia Salvador

P1.39 – TWO NOVEL *FLAVOBACTERIUM* SPECIES ISOLATED FROM ALKALINE MAGNESITE RESIDUES
Leonor Matos, Diogo Neves Proença, Romeu Francisco, Ana Paula Chung, Lorrie Maccario, Søren J Sørensen, Paula V Morais

P1.40 – BACTERIAL INTERACTIONS WITH VANADIUM: INSIGHTS OF TOLERANCE AND RESISTANCE MECHANISMS
Joana Bonito Caldeira, Rita Branco, Paula Vasconcelos Morais

P1.41 – IDENTIFICATION OF POTENTIAL INHIBITION OF CARBONIC ANHYDRASE IN PRESENCE OF AMINO ACID-BASED IONIC LIQUIDS (AA-ILS) USING MOLECULAR DOCKING
Michele Fraga, Mara G. Freire, Matheus M. Pereira

P1.43 – GENOME MINING REVEALS POTENTIAL OF *PSEUDOMONAS SP* STRAINS FOR IMPROVED MICROBIAL-INDUCED CALCIUM PRECIPITATION
Pedro Farias, Amanda Mendonça, Paulo da Venda, Paula V Morais

P1.53 – BIOTECHNOLOGICAL PRODUCTION OF NEW GREEN BIOCIDES FOR APPLICATION IN CULTURAL HERITAGE
Cátia Salvador, Patrícia Gatinho, Inês Caldeira, A. Teresa Caldeira

P1.54 – PLYLOGENOMICS AS BASELINE FOR TAXONOMY DESCRIPTION: *AMPHIBACTER PEREZI* GEN. NOV., SP. NOV.
Sara Costa, Diogo Neves Proença, Isabel Lopes, Paula Vasconcelos Morais

P1.80 - ASSESSING GRAM-NEGATIVE ANTIMICROBIAL RESISTANT BACTERIA IN RIVERS FOR DRINKING WATER: A SEASONAL ANALYSIS
Cátia Matos, António Magalhães, Francisca Pereira, Bárbara Duarte, Margarida Valente, Carolina Tavares, Juliana Rodrigues, Luísa Peixe, Carla Novais, Patrícia Antunes

P1.87 - IS THERE A ROLE FOR TRAMAZEIRA TREE IN OFFERING ALTERNATIVES FOR THE TREATMENT OF INFECTIONS CAUSED BY *CANDIDA* SPECIES?
Joana Valério, Diogo Santos, Maria Joana Pinheiro, Gabriela Vergara, Natália Seixas, Dalila Mil-Homens, Arsénio M. Fialho, Luís Dias, Letícia Estevinho, Nuno P Mira

TOPIC 2

P2.1 - EVALUATION OF PHAGE VIABILITY AT DIFFERENT CONDITIONS FOR PHAGE APPLICATION ON FOOD

Márcia Braz, Carla Pereira, Pedro Costa, Carmen S R Freire, Adelaide Almeida

P2.2 - HYPERBARIC STORAGE AS POSSIBLE SIMULTANEOUS MODERATE PRESSURE PASTEURIZATION METHODOLOGY: IMPACT OF PH AND PRESSURE LEVEL ON MICROBIAL INACTIVATION

Vasco Lima, Jorge Saraiva

P2.3 - CLEAN LABEL ANTIMICROBIAL STRATEGIES FOR FUNGAL SPOILAGE OF PASTRY FILLINGS

Teresa Bento de Carvalho, Miguel Azevedo, Beatriz Silva, Paula Teixeira

P2.4 - PRODUCTION OF EGG WHITE PROTEIN FILMS WITH ANTIMICROBIAL PROPERTIES AS FOOD PACKAGING MATERIALS

Ângelo Luís, Ana Ramos, Fernanda Domingues

P2.5 - CHARACTERIZATION OF FECAL COLIFORM CONTAMINANTS IN SEAWATER AND SEAFOOD SAMPLES COLLECTED FROM NAMIBE IN ANGOLA

Beatriz L Calado, Inês C Leal, Eunice Cassoma, Isaac Bumba, Carmen dos Santos, Adelino VM Canário, Deborah M Power, João CR Cardoso

P2.6 - ARE GUAIACOL AND HALOPHENOLS THE ONLY ONES TO BLAME FOR THE OFF-FLAVOUR SPOILAGE BY *ALICYCLOBACILLUS SPP.*?

Inês Carvalho Leonardo, António Ferreira, Maria do Rosário Bronze, Maria Teresa Barreto Crespo, Frédéric Bustos Gaspar

P2.7 - MICROBIOLOGICAL HAZARDS IN LOCAL PRE-HARVEST PRODUCTION OF VEGETABLES AND FRUITS AND IRRIGATION WATER

Ariana Macieira and Paula Teixeira

P2.8 - PRODUCTION AND QUALITY EVALUATION OF INFANT DIET FORMULATED USING POWDERED PAP AND DIFFERENT NUTS TO ENHANCE FOOD SECURITY

Ayodele Oluwayemisi, Oluwatoyin Modupe, Stephen Olanrewaju, Gbenga Isaac

P2.10 - RHAMNOLIPIDS-BASED NANOEMULSIONS LOADED WITH NATURAL PIGMENTS: EFFECT OF PIGMENT CONCENTRATION AND PARTICLE SIZE ON COLOR AND NANOEMULSIONS STABILITY

Mariana B N. Alves, Miguel A. Cerqueira, Jorge F. B. Pereira

P2.11 - EVALUATE THE EFFECTIVENESS OF ULTRAVIOLET LIGHT-EMITTING DIODES FOR FOOD MICROBIOLOGICAL SAFETY

Ana Paula Marques, Katia Luz, Maria Teresa Barreto Crespo, Vanessa Jorge Pereira

P2.12 - MICROBIAL HYDROLYSATES OF WHEY PROTEIN: GENERATING PROMISING ANTICOAGULANT PEPTIDES

Tiago Paiva, Duarte Toubarro, Nelson Simões

P2.13 - MOLECULAR PROFILING OF HIDDEN INSECT INFESTATION IN STORED RICE GRAINS: A COMPREHENSIVE RT-PCR APPROACH FOR SPECIES DISCRIMINATION AND LIMIT OF DETECTION ASSESSMENT
Ana Campos, Inês Gonçalves de Sousa, Carina Almeida, Carla Brites

P2.14 - CHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF TWO QUINOA (CHENOPODIUM QUINOA) FLOUR VARIETIES FROM PERU
Silvia Melissa García-Torres, José A. Teixeira, Christian René Encina-Zelada, Cristina Luisa Miranda Silva, Ana Maria Gomes

P2.15 - IMPACT OF *CHLORELLA VULGARIS* IN THE SOURDOUGH MICROBIOTA AND CHARACTERISTICS OF SOURDOUGH BREAD
Carla Pestana, Nancy Mahmoud, Cristiana Nunes, Anabela Raymundo, Catarina Prista

P2.16 - DISINFECTION OF IRRIGATION WATER USING ULTRAVIOLET LIGHT EMITTING DIODES
Nicole Ferreira, Ana Paula Marques, Maria Teresa Crespo, Vanessa Pereira

P2.17 - PATHOGENICITY AND VIRULENCE OF *LISTERIA MONOCYOTOGENES* ISOLATES FROM DAIRY FARMS
Ana Rita Barata, Bárbara Nunes, Teresa Nogueira, Maria José Saavedra, Gonçalo Almeida

P2.18 - ENZYMATIC SYNTHESIS OF MONOSACCHARIDE FATTY ACID ESTERS
Bruna Basto, Mara Guadalupe Freire, José António Teixeira, Sara Cruz Silvério

P2.24 - MICROBIOLOGICAL CONTAMINATION OF FRESH CHICKEN MEATS AND OFFALS IN THE RETAIL MARKETS
Sónia Saraiva, Cristina Saraiva, Kamila Soares, Ana Leite, Thomai Lazou

P2.28 - HIGH-PRESSURE HOMOGENIZATION TREATMENT TO OBTAIN CREAMS USING TOMATO WASTE
Helena Ribeiro, Boelo Schuur, Jorge F. B. Pereira

TOPIC 3

P3.1 - CROSS EFFECT OF A POLYCLONAL ANTIBODY AGAINST CYSTIC FIBROSIS BACTERIAL PATHOGENS AS A NEW ALTERNATIVE THERAPY
António M.M. Seixas, Sílvia A. Sousa, Carolina Silva, Sara C. Gomes, Mirela R. Ferreira, Leonilde M. Moreira, Jorge H. Leitão

P3.2 - IMPROVING THE MANUFACTURING OF MRNA NANOMEDICINES USING THERMOREVERSIBLE AQUEOUS BIPHASIC SYSTEMS AND IONIC LIQUIDS
Maria I. Sousa, Luís C. V. Silva, Andreia Correia, Mara G. Freire, Francisca A. e Silva, Augusto Q. Pedro

P3.3 - CREATION OF A FUNGAL LIBRARY AND SCREENING OF ANTIMICROBIAL AND ANTICANCER ACTIVITY
Diogo Ferreira, Lara Areal Hermida, Ana Catarina Rocha, Pilar Baylina, Carmen Sieiro, Rúben Fernandes

P3.4 - MOLECULAR BEACONS FOR PRE-MIRNA SECONDARY STRUCTURE CHARACTERIZATION AND MIRNA QUANTIFICATION
Micaela Riscado, Leonor Mariano, Carla Cruz, Chantal Pichon, Fani Sousa

P3.5 - IONIC LIQUIDS: AN INNOVATIVE CELL DISRUPTION METHOD FOR THE EXTRACTION OF NUCLEIC ACIDS-BASED BIOPHARMACEUTICALS FROM YEAST
Ana S. C. Marques, Augusto Q. Pedro, Cristine Gonçalves, Ana P. M. Tavares, Chantal Pichon, Mara G. Freire

P3.6 - OREGANO ESSENTIAL OIL: AN EFFECTIVE AND NON-TOXIC APPROACH FOR PREVENT OR TREAT RESISTANT CANDIDA SPECIES
Liliana Fernandes, Inês Silva, Elena Blázquez, Ainara Tejada, Artur Ribeiro, Sónia Silva, Nuno Mira, Lorena Cussó, Sofia Costa-de-Oliveira, Maria Elisa Rodrigues, Mariana Henriques

P3.7 - NOVEL PLATFORMS FOR HUMAN SERUM PRETREATMENT AND BREAST CANCER BIOMARKERS EXTRACTION USING IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS
Maria S. M. Mendes, Maria C. Souza, João P. Conde, Mara G. Freire, Francisca A. e Silva

P3.8 - DEVELOPMENT OF A POSTBIOTIC-BASED ORODISPERSIBLE FILM TO PREVENT DYSBIOSIS OF *STREPTOCOCCUS MUTANS* IN THE ORAL CAVITY
Mariana Rebelo, Cláudia Oliveira, Freni Tavaria

P3.9 - NOVEL COMPOUNDS RESCUE P53 AND INDUCE APOPTOSIS IN HPV- POSITIVE CELLS: PROSPECTS FOR ANTI-HPV DRUG DEVELOPMENT
Diana Gomes, Carolina Ribeiro, Vanessa Brito, Samuel Silvestre, Luís Passarinha, Ângela Sousa

P3.10 - A POTENTIAL EFFECT OF CIRCADIAN RHYTHM IN THE DELIVERY/THERAPEUTIC PERFORMANCE OF PACLITAXEL-DENDRIMER NANOSYSTEMS
Tânia Albuquerque, Ana Raquel Neves, Milan Paul, Swati Biswas, Elena Vuelta, Ignacio García-Tuñón, Manuel Sánchez-Martin, Telma Quintela, Diana Costa

P3.11 - SYNTHESIS AND CHARACTERIZATION OF GALLIC ACID-TRIETHYLENE GLYCOL APTADENDRIMERS
André Miranda, Roi Lopez-Blanco, Jéssica Lopes-Nunes, Ana M. Melo, Maria P. C. Campello, António Paulo, Maria Cristina Oliveira, Jean-Louis Mergny, Paula A. Oliveira, Eduardo Fernandez-Megia, Carla Cruz

P3.12 - UREA DETECTION BASED ON A PROTOTYPE OF A MINIATURED NEAR-INFRA-RED SPECTROMETER TO MONITOR IN REAL TIME AND IN SITU KIDNEY FUNCTION
Paulo Zoio, Diogo Barradas, Luís P. Fonseca, Bernardo B. Carvalho, Cecília R.C. Calado

P3.13 - IONIC LIQUIDS AS ADDITIVES FOR STABILIZATION AND FORMULATION OF PROTEIN- BASED PRODUCTS
Nathalia V. Veríssimo, Qi Han, Valéria C. Santos-Ebinuma, Tamar L. Greaves, Jorge F.B. Pereira

P3.14 - PREVENTING *CLOSTRIDIODES DIFFICILE* INFECTION BY USING AN IMMOBILIZED BILE SALT HYDROLASE TO INTERFERE WITH SPORE GERMINATION
Daniela Silva, Mónica Serrano, Adriano O. Henriques

P3.15 - APTAMERS FOR BLOCKING ENTEROTOXIGENIC *ESCHERICHIA COLI*
Maria Margarida Barros, Ricardo Oliveira, Joana Castro, Daniela Araújo, Sónia Silva, Ana Campos, Divanildo Outor-Monteiro, Carina Almeida

P3.16 - OPTIMIZATION OF THE PRODUCTION AND PURIFICATION OF NON-VIRAL VECTORS FOR GENE THERAPY APPLICATIONS
Ana I. Valente, Leonor S. Castro, Inês D. Rios, Augusto Q. Pedro, Ana P. M. Tavares, Fani Sousa, Mara G. Freire

P3.17 - ON THE USE OF ISOTHERMAL AMPLIFICATION WITH A NUCLEIC ACID LATERAL FLOW ASSAY READ-OUT FOR THE DIAGNOSTIC OF RNA VIRUSES
João M. Vindeirinho Eva Pinho, Raquel Guiomar, Nuno F. Azevedo, Ahmed El Abd Wahed, Carina Almeida

P3.18 - INTERACTION OF NUCLEOLIN AND RNA G-QUADRUPLEX MOTIF AND PRELIMINARY CLINICAL EVALUATION IN LUNG CANCER
Joana Figueiredo, André Miranda, Jéssica Lopes-Nunes, Daniela Alexandre, Salete Valente, Jean-Louis Mergny Carla Cruz

P3.20 - SCREEN-PRINTED ELECTROCHEMICAL IMMUNOSENSORS FOR IMPROVING HEART FAILURE PATIENT MANAGEMENT
Bernardo Servolo, Miguel Castelo-Branco, Julia Arcos-Martinez, Ana Cristina Dias-Cabral

P3.22 - PORTABLE COLORIMETRIC SENSORS BASED ON SUPPORTED IONIC LIQUID MATERIALS FOR L-ASPARAGINE DETECTION
João Nunes, Márcia Neves, Mara Freire, Ana Paula Tavares

TOPIC 4

P4.2 - VIRULENCE TRAITS OF CUTIBACTERIUM ACNES: DIFFERENCES AMONG DIFFERENT PHYLOTYPES
Ana Sofia Oliveira, Carlos Gaspar, Joana Rolo, Rita Palmeira-de-Oliveira, João Paulo Teixeira, José Martinez-de-Oliveira, Ana Palmeira-de-Oliveira

P4.3 - FACTORS ASSOCIATED WITH PSEUDOMONAS AERUGINOSA INFECTION IN A UROLOGY AND KIDNEY TRANSPLANTATION UNIT IN CENTRAL PORTUGAL: DETECTION OF ANTIMICROBIAL RESISTANCE
Mariana Duarte Almeida, Belmiro Parada, Sarah M. Johansson, Pedro Valada, João Pereira, Rita Leal, João Frade, Catarina Chaves, Ana Rita Rebelo, Teresa Gonçalves, Rene S. Hendriksen, Célia Nogueira

P4.4 - DECIPHERING THE ANTIBACTERIAL MODE OF ACTION OF CURCUMIN- ANTIBIOTIC COMBINATIONS UNDER PHOTODYNAMIC ACTIVATION AGAINST STAPHYLOCOCCUS AUREUS
Ariana Gonçalves, José Fernandes, Maria José Saavedra, Cristiana Pereira, Manuel Simões, Anabela Borges

P4.5 - EVALUATION OF IN VITRO COMPATIBILITY OF BACTERIAL CANDIDATES AS BIOMODULATORS FOR INTESTINAL WOUND HEALING CONSORTIA
Diana Almeida, Sónia Mendo, José Andrade, Ana Maria Gomes

P4.6 - RHODOTORULA MUCILAGINOSA: A NEW PLAYER IN VULVAR INFECTIONS
Cátia F. Caetano, Mariana Z. Fernandes, Carlos Gaspar, Ana S. Oliveira, Rita Palmeira-de-Oliveira, José Martinez-de-Oliveira, Ana Palmeira-de-Oliveira, Joana Rolo

P4.7 - EVALUATION OF YEAST SURFACE DISPLAY OF THE ENDOLYSIN PLY511 AGAINST LISTERIA MONOCYTOGENES
David Sáez Moreno, Joana Cunha, Luís D.R. Melo, Joana Azeredo, Lucília Domingues

P4.8 - EXPLORING THE ANTIMICROBIAL ACTIVITY OF BLACK SCORPIONFISH (SCORPAENA PORCUS) VENOM
Rita Magalhães, José Neto, Eva Cunha, Joana Robalo, Manuela Oliveira, Frederico Almada

P4.9 - EFFECTS OF BIOCHAR SUPPLEMENTATION ON ANTIMICROBIAL RESISTANCE AND VIRULENCE FACTORS OF ENTEROCOCCI ISOLATED FROM RECYCLED MANURE SOLIDS
Ana José Pires, Catarina Geraldes, Joana Fernandes Guerreiro, Lélia Chambel, David Fangueiro, Ricardo Bexiga, Manuela Oliveira

P4.10 - TOWARDS A SIMPLE AND COST-EFFECTIVE PROTOCOL FOR VIRAL SURVEILLANCE IN WASTEWATERS
Sofia G. Seabra, Mónica Nunes, Victor Pimentel, Aires Moura, Filipe Tomaz, Gonçalo Seixas, Inês M. Alves, Mafalda N. S. Miranda, Daniela Alves, Diocreciano M. Bero, Sádia Pereira, Ana Paula Abílio, Carlos São José, Ana Domingos, Marta Pingarilho, Ana B. Abecasis, Ricardo Parreira

P4.11 - FULL SURVIVAL OF GALLERIA MELLONELLA INFECTED WITH *STAPHYLOCOCCUS AUREUS* AFTER TREATMENT WITH NISIN Z
Isa Serrano, Dalila Mil-Homens, Joana F. Guerreiro, Eva Cunha, Sofia S. Costa, Luís Tavares, Manuela Oliveira

P4.12 - IDENTIFICATION AND GENOMIC ANALYSIS OF THE FIRST *STAPHYLOCOCCUS ARGENTEUS* ISOLATE CAUSING INFECTION IN A COVID-19 PATIENT IN PORTUGAL
Teresa Conceição, Mafalda Felgueiras, Valquíria Alves, Hermínia de Lencastre

P4.13 - TRACKING ANTIMICROBIAL RESISTANCE PATTERNS IN CLINICAL AND HEALTHCARE-ASSOCIATED *ENTEROCOCCUS SPP.*
Joana Monteiro Marques, Mariana Coelho, Teresa Barreto-Crespo, Teresa Semedo- Lemsaddek

P4.14 - COMPARISON OF THE ANTIMICROBIAL ACTIVITY OF INTRACANAL MEDICATION PASTES USING AN *EX VIVO* MODEL OF ENDODONTIC INFECTION BY *ENTEROCOCCUS FAECALIS*
Eva Cunha, Ana Margarida Sousa, Luís Tavares, Manuela Oliveira, António Ginjeira

P4.17 - SHARED EVOLUTIONARY PATH IN SOCIAL MICROBIOMES
Nelson Frazão, Isabel Gordo

P4.18 - VIRULENCE AND ANTIBIOTICS RESISTANCE IN *ENTEROCOCCUS* ISOLATED FROM A VETERINARY BIOLOGIC ISOLATION AND CONTAINMENT UNIT (BICU)
Catarina Geraldes, Mónica Afonso, Eva Cunha, Raquel Abreu, Luís Tavares, Solange Gil, Manuela Oliveira

P4.20 - MACROPHAGE'S GENE EXPRESSION RESPONSE TO OXIDATIVE STRESS IMPOSED BY LYME BORRELIOSIS' SPIROCHETES
Mónica Franco, Maria Luísa Vieira, Ana Armada

P4.21 - FERRITIN RELEASE DURING MYCOBACTERIAL INFECTION – A ROLE FOR EXTRACELLULAR VESICLES?
Leonor T. S. Gomes, Óscar Fonseca, José Pires, Gabriel Oliveira, Ana S. Ramos, Isabel Oliveira, Tânia Silva, Daniela Freitas, M. Salomé Gomes, Ana C. Moreira

P4.22 - PROTEASOME MUTATION PROMOTES RESISTANCE TO ARTEMISININ IN *PLASMODIUM FALCIPARUM*
Adriana Gonçalves; Miguel Teixeira; Ana Rita Pinheiro; Pedro Cravo; Pedro Ferreira

P4.23 - PREVALENCE OF EXTENDED-SPECTRUM B-LACTAMASE (ESBL)-PRODUCING ENTEROBACTERIACEAE IN DOGS OF CAPE VERDE AND SÃO TOMÉ AND PRÍNCIPE
Raquel Abreu, Alice Matos, Luís Capela, Rita Jorge, Eva Cunha, Luís Tavares, Manuela Oliveira

P4.24 - MONITORING OF ANTIBIOTIC RESISTANCE AND CONTAMINANTS OF EMERGING CONCERN IN SMALL-SCALE WETLAND-BASED MUNICIPAL TREATMENT SYSTEMS
Margarida Teixeira, Diana Matos, Lahiruni Halwatura, Diana Aga, Célia M. Manaia

P4.26 - COMPARING SALIVA OF ADULTS AND CHILDREN AS A PROBIOTIC SOURCE
Manuel Cardoso-Baptista, Carla Campos, Teresa Correia-de-Sá, Joana Ferreira- Gomes, Benedita Sampaio-Maia, Carolina Costa

P4.27 - EXPLORING THE ANTIFUNGAL POTENTIAL OF YUCATAN PENINSULA MARINE ORGANISMS AGAINST MEDICALLY RELEVANT CANDIDA SPECIES
Diana Grilo, Dawrin Pech-Puch, Susana Eunice Calva-Pérez, Andreia Pedras, Harold Villegas-Hernández, Sergio Guillén-Hernández, Raúl Díaz-Gamboa, Mateo Forero Tunjano, Jaime Rodríguez, Carlos Jiménez, Oscar A Lenis-Rojas and Catarina Pimentel

P4.28 - ASSESSING THE VIRULENCE POTENTIAL OF DIFFERENT *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES WITH *GALLERIA MELLONELLA* LARVAE MODEL
Mariana Sousa, Sónia Silva, Daniela Araújo, Joana Castro, Gonçalo Almeida, Catarina Ventura, Rui Magalhães, Vânia Ferreira, Paula Teixeira

P4.29 - DEVELOPMENT OF ANTIMICROBIAL COATINGS BASED ON POLYURETHANE DISPERSIONS INCORPORATED WITH UV-LIGHT STABILIZERS AND INVESTIGATION OF THEIR MECHANISM OF ACTION
Tiago Costa, Belém Sampaio-Marques, Nuno M. Neves, Helena Aguilar, Alexandra G. Fraga

P4.30 - EXPLORING GENOMIC SIGNATURES OF *STAPHYLOCOCCUS AUREUS* BEYOND COMPARTMENTS: LINKS AT THE ANIMAL-HUMAN INTERFACE
Beatriz Ramos, Mónica V. Cunha

P4.31 - ANTAGONISTIC ACTIVITY OF *LACTIPLANTIBACILLUS PLANTARUM* AGAINST *ALIARCOBACTER BUTZLERI*
Alexandre Vieira, Cristiana Mateus, Fernanda Domingues, Mónica Oleastro, Susana Ferreira

P4.32 - *N. MENINGITIDIS* CAPSULE MODULATES THE HOST EGR1 SIGNALING AND CONTRIBUTES TO *N. MENINGITIDIS* MEDIATED PATHOGENESIS
Sunil D. Saroj, Riya Joshi

P4.33 - ROLE OF CARBON SOURCES IN THE ANTIMICROBIAL RESISTANCE AND VIRULENCE OF *NEISSERIA MENINGITIDIS*
Poonam Kanojiya, Sunil D. Saroj

P4.34 - THE ROLE OF THE LYME BORRELIOSIS LABORATORY (IHMT) *VS* THE COMMUNITY: A CASUISTIC ASSESSMENT OF THE DISEASE (2019 -023)
Ana Armada, Maria Luísa Vieira

P4.35 - UV-LED INACTIVATION KINETICS OF BACTERIA RESPONSIBLE FOR NOSOCOMIAL INFECTIONS
Beatriz Rito, Leonor Matos, Diogo N. Proença, Paula V. Morais

P4.36 - METAGENOMIC PROFILING OF WASTEWATER DURING THE FIRST WAVES OF COVID-19 HIGHLIGHTS SPECIFIC MICROBIOTA AND RESISTOME SIGNATURES
Beatriz Ramos, Nuno Brôco, Marta Carvalho, Rita Lourinho, João Vilaça, Silvia Monteiro, Ricardo Santos, Mónica V. Cunha

P4.37 - SARS-COV-2 S GENE SEQUENCING IN WASTEWATER SAMPLES ENABLES EARLY LINEAGE DETECTION AND UNCOVERS RARE MUTATIONS
Ana C. Reis, Daniela Pinto, Sílvia Monteiro, Ricardo Santos, João Vieira Martins, Mónica V. Cunha

P4.38 - EFFECTS OF SHORT CHAIN FATTY ACIDS ON *ALIARCOBACTER BUTZLERI*'S VIRULENCE
Inês Fonseca, Cristiana Mateus, Fernanda Domingues, Mónica Oleastro, Susana Ferreira

P4.39 - DIETARY CAROTENOIDS AND THEIR MODULATION OF INTESTINAL MICROBIOTA
Helena R. Rocha, Ana M. Gomes, Manuela E. Pintado, Marta C. Coelho

P4.46 - *GARDNERELLA* PANGENOME ANALYSIS: INSIGHTS INTO THE PHYLOGENY, RESISTOME, VIRULOME AND HOST ADAPTATION
Pedro Teixeira, Márcia Sousa; Filipa Grosso, Teresa Gonçalves Ribeiro, Luísa Peixe

P4.55 - RELIEF OF INFANT COLIC BY FENNEL SEED INFUSION: EFFECTS ON BREAST MILK AND THE BABY'S INTESTINAL MICROBIOTA
Célia C.G. Silva, Beatriz F.M.P. Henriques, Susana C. Ribeiro, Henrique J.D. Rosa

P4.59 - *IN VITRO* SELECTION OF *ALIARCOBACTER BUTZLERI* WITH ERYTHROMYCIN PRODUCE GENETIC CHANGES AND AFFECTS FITNESS AND VIRULENCE
Francisca Couto, Inês Martins, Filipa Vale, Fernanda Domingues, Mónica Oleastro, Susana Ferreira

P4.66 - ST8475: A NEW SEQUENCE TYPE IDENTIFIED IN METHICILLIN-RESISTANT STAPHYLOCOCCI (MRS) IN PORTUGAL
Ricardo Oliveira, Daniela Araújo, Beatriz Loureiro, Joana Castro, Sónia Silva, Carina Almeida

P4.78 - CHARACTERIZATION OF MOLECULAR FEATURES AND VIRULENCE PROFILE OF *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA OXYTOCA* ISOLATES FROM COMPANION ANIMALS IN PORTUGAL
Daniela Araújo, Joana Castro, Ricardo Oliveira, Hugo Oliveira, Liliana Fernandes, Isabel Carvalho, Gyu-Sung Cho, Charles Franz, Carina Almeida, Sónia Silva

P4.79 - GENOMIC AND PHENOTYPIC PROFILING OF MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA VARIICOLA* ISOLATED FROM NORTHERN PORTUGUESE HOSPITALS
Joana Castro, Daniela Araújo, Hugo Oliveira, Liliana Fernandes, Ricardo Oliveira, Gyu-Sung Cho, Charles Franz, Maria José Saavedra, Sónia Silva, Carina Almeida

TOPIC 5

P5.1 - BIOCHEMICAL PORTRAIT OF THE INNER MEMBRANE CYTOCHROME CBCA: INSIGHTS INTO ITS ROLE IN EXTRACELLULAR ELECTRON TRANSFER
Jorge M. A. Antunes, Carlos A. Salgueiro, Leonor Morgado

P5.2 - THE FLAVODIIRON PROTEIN OF *SHARPEA AZABUENSIS* HAS O_2 AND H_2O_2 REDUCTASE ACTIVITY
Diogo Cunha, Susana F. Fernandes, Miguel Teixeira, Filipe Folgosa

P5.3 - *CANDIDA ALBICANS* 5'-3'-ECTONUCLEOTIDASE ACTIVITY: A COMPARATIVE STUDY OF ISOLATES FROM DIFFERENT HUMAN INFECTION NICHES
Mariana Afonso, Bernardo Bandeira, Rodrigo A. Cunha, José Roberto Meyer- Fernandes, Teresa Gonçalves, Lisa Rodrigues

P5.4 - TWO NEW FLAVODIIRON PROTEINS TO FIGTH OXIDATIVE STRESS IN AN ANAEROBIC BACTERIUM?
Maria C. Martins, Filipe Folgosa and Miguel Teixeira

P5.5 - UNCOVERING THE SECRETS OF GEOBACTER URANIIREDUCENS: A STUDY OF KEY PLAYERS IN URANIUM BIOREMEDIATION PATHWAY
Alexandre Almeida, Marta A. Silva and Carlos A. Salgueiro

P5.6 - ARSENATE RESPONSE IN YEAST: EXPLORING NEW PATHWAYS
Teresa Pissarro, Américo G. Duarte, Catarina Amaral, Catarina Pimentel, Sofia M. da Silva

P5.7 - ELUCIDATING THE PHYSIOLOGICAL ROLE OF THE DSRJ CYTOCHROME IN DISSIMILATORY SULFATE METABOLISM
Raquel Mendes Bernardino, Ana Catarina Barbosa, Inês Cardoso Pereira

P5.8 - OPTIMIZING BACTERIAL CELLULOSE PRODUCTION IN *KOMAGATAEIBACTER SPP.* THROUGH METABOLIC ENGINEERING
Pedro Montenegro-Silva, Tom Ellis, Fernando Dourado, Miguel Gama, Lucília Domingues

P5.9 - THE ELECTROGENIC QRCABCD COMPLEX FROM SULFATE-REDUCING PROKARYOTES PLAYS AN IMPORTANT ROLE ON SULFATE RESPIRATION
Gonçalo Manteigas, Américo G. Duarte, Inês A. C. Pereira

P5.10 - THE ROLE OF PNPASE IN THE BIOFILM FORMATION OF *ESCHERICHIA COLI*
Manuel Condinho, Sandra Cristina Pinto, Cecília Maria Arraiano, Vânia Pobre

P5.11 – ROLE OF ENOLASE IN THE FORMATION OF CLOSTRIDIOIDES DIFFICILE BIOFILM
Rute Canhão-Pereira, Isabel Roseiro, Mónica Oleastro, Adriano O. Henriques and Mónica Serrano

P5.12 - PRELIMINARY CHARACTERIZATION OF A HYDROXILAMINE OXIDOREDUCTASE WITH AN NON-CANONICAL N-TERMINAL DOMAIN
Susana F. Fernandes, Catarina M. Alves and Filipe Folgosa

P5.13 - AN9181 MAY REGULATE *ASPERGILLUS NIDULANS* METABOLIC RESPONSE TO DIFFERENT CHEMICAL STRESSES
João Jorge, Celso Martins, Tiago Martins, Diego Hartmann, Gustavo H. Goldman, Cristina Silva Pereira

P5.14 - A DNA-BINDING BACTERIOFERRITIN FROM THE ANAMMOX BACTERIUM *CANDIDATUS BROCADIA PITUITAE*
Catarina M. Alves, Susana F. Fernandes and Filipe Folgosa

P5.15 - MICROBIAL PRODUCTION OF THE BUILDING BLOCK P-COUMARIC ACID: UNLEASHING THE POTENTIAL OF *KLUYVEROMYCES MARXIANUS*
Marlene Baptista, Carlos E. Costa, Lucília Domingues

P5.16 - DISENTANGLING TANGLED CYTOCHROMES: INSIGHTS ON THE FUNCTIONAL MECHANISMS OF A MICROBIAL HEME-TETHERED REDOX STRING
Tomás M. Fernandes, Marta A. Silva, Leonor Morgado, Carlos A. Salgueiro

P5.17 - EXPLORING GEOBACTER QUINONE OXIDOREDUCTASE COMPLEXES: EXPRESSION AND PURIFICATION OF CBC3 AND CBC4 PROTEINS
Mafalda V. Fernandes, Jorge M. A. Antunes, Carlos A. Salgueiro, Leonor Morgado

P5.18 – CELL DIVISION PROTEIN FTSK COORDINATES BACTERIAL CHROMOSOME SEGREGATION AND DAUGHTER CELL SEPARATION IN *STAPHYLOCOCCUS AUREUS*
Helena Veiga, Ambre Jousselin, Simon Schäper, Bruno M. Saraiva, Leonor B. Marques, Patricia Reed, Joana Wilton, Pedro M. Pereira, Sérgio R. Filipe, Mariana G. Pinho

P5.19 - TRACKING STRESS ADAPTATION IN BACTERIA USING A "LIBRARY OF GLOBAL REGULATORS OF GENE EXPRESSION
Ines S. C. Baptista, Suchintak Dash, Andre S. Ribeiro

TOPIC 6

P6.1 - DESIGN OF A SUSTAINABLE TECHNOLOGY FOR ALGAL PROTEIN EXTRACTION: EXPLORATION OF DES-BASED-SALT ATPS FOR C-PHYCOCYANIN PARTITIONING
Bruno Pereira, Carla Brazinha, João G. Crespo, Sónia P. M. Ventura, Cristina T. Matos, Luís Costa

P6.2 - NEW 4-STYRYLCOUMARIN DERIVATIVES AS POTENTIALS FLUORESCENT LABELS FOR BIOMOLECULES: APPLICATION IN RNA-FISH PROBES
Raquel Eustáquio, Ana Teresa Caldeira, Sílvia Arantes, António Candeias, António Pereira

P6.3 - PHYSIOLOGICAL CHARACTERIZATION AND PROMOTER ENGINEERING OF ACETOBACTERIUM WIERINGAE FOR ACETONE PRODUCTION VIA GAS FERMENTATION
João P. C. Moreira, Stefan Pflügl, Joana I. Alves, Lucília Domingues

P6.5 - PHASE SEPARATION HYDRODYNAMICS OF CHOLINIUM CHLORIDE-BASED AQUEOUS BIPHASIC SYSTEMS (ABS) – FINDING THE BEST BIPHASIC REGION OF OPERATION
Alexandre M. S. Jorge, João A. P. Coutinho, Jorge F. B. Pereira

P6.6 - OPTIMIZATION OF ENZYMES PRODUCTION BY *ASPERGILLUS NIGER* IN SOLID STATE FERMENTATION OF AGRO-INDUSTRIAL BY-PRODUCTS
Ana Catarina Mota, Diana Machado, Ana Sofia Pereira, Ana Guimarães, Isabel Belo

P6.7 - EXTRACTIVE FERMENTATION AS A PLATFORM FOR THE PRODUCTION AND PURIFICATION OF LACCASE BY *PICHIA PASTORIS*
Flávia F. Magalhães, Augusto Q. Pedro, Mara G. Freire, Ana Paula Tavares

P6.8 - LIPASE PURIFICATION FROM *YARROWIA LIPOLYTICA* FERMENTATION BROTH USING AN AQUEOUS TWO-PHASE SYSTEM BASED ON QUATERNARY AMMONIUM COMPOUNDS
Filipe Smith Buarque, Adriano Carniel, Bernardo Dias Ribeiro, Maria Alice Zarur Coelho

P6.9 - COMPUTER-ASSISTED IDENTIFICATION OF DEEP EUTECTIC SOLVENTS EFFECT ON *Y. LIPOLYTICA* METABOLIC INHIBITION USING MOLECULAR DOCKING
Filipe S. Buarque, Bernardo D. Ribeiro, Maria A. Z. Coelho, Mara G. Freire, Matheus M. Pereira

P6.10 - OPTIMIZED PRODUCTION OF REFLECTINS AND CHARACTERIZATION OF THEIR REVERSIBLE SELF-ASSEMBLY
Iana Lychko, Cátia Soares, Inês Padrão, Tomás Calmeiro, Arménio Barbosa, Pedro Costa, Margarida Dias, Ana Cecília Roque

TOPIC 7

P7.1 - BIOSYNTHESIS OF SILVER-BASED NANOPARTICLES USING SUPERNATANTS OF MICROBIAL CULTURES FOR CULTURAL HERITAGE PRESERVATION
António Carrapiço, Luís Dias, Elisabete Carreiro, Ana Teresa Caldeira, Maria Rosário Martins

P7.2 - EXPLORING THE POTENTIAL OF *S. CARPOCAPSAE* VENOM PROTEINS IN INNOVATIVE NANO-INSECTICIDES
Jorge Frias, Rui L. Reis, Nelson Simões

P7.3 - ENHANCING ANAEROBIC DIGESTION BY SIMULTANEOUS APPLICATION OF A COMBINED TREATMENT OF NANO-ZERO VALENT IRON AND MAGNETITE NANOPARTICLES
Nicolas Hoffmann, Gonzalo Tortella, Christian Vergara, Gilberto Martins, Olga Rubilar

P7.4 - DEVELOPMENT OF PLASMID DNA AND DRUG LIPID NANOPARTICLES: AN INNOVATIVE ROUTE AGAINST LUNG CANCER
Catarina Diniz, Diana Pedro, Mariana Matias, Adriana O. Santos, Ângela Sousa

P7.5 - FUNGAL LIPIDOMICS OF THE GENUS *LASIODIPLODIA*
Francisca Freitas, Susana Aveiro, Diana Lopes, Artur Alves, Ana Cristina Esteves, Maria do Rosário Domingues

P7.6 - VIRAL DETECTION IN WASTEWATERS: COMPARISON OF BIOINFORMATIC TOOLS FOR VIROME ANALYSIS
André Filipe Santos, Mónica Nunes, Andreia Silva, Teresa Crespo, Victor Pimentel, Marta Pingarilho, Mafalda Miranda, Pieter Libin, Ricardo Parreira, Ana B. Abecasis, Sofia G. Seabra

P7.7 - COMPLETE GENOME OF *PEDOBACTER LUSITANUS* NL19: A COMPREHENSIVE GENOMIC ANALYSIS
Gonçalo Figueiredo, Hugo Osório, Marta V. Mendes, Sónia Mendo

P7.8 - TACKLING NON-HODGKIN LYMPHOMA ON TWO FRONTS: DUAL-TARGETING IMMUNOLIPOSOMES AS A NOVEL DRUG DELIVERY SYSTEM
Ana Leonardo, Ana S. André, Margarida Ferreira-Silva, Sandra I. Aguiar, Sara Nogueira, Marco Cavaco, Lurdes Gano, João D.G. Correia, Miguel Castanho, Manuela Gaspar, Luís Tavares, Frederico Aires-da-Silva, Joana N. R. Dias

P7.9 - AIMING AT THE AUTOMATION OF GENOME-WIDE REGULATORY NETWORK INFERENCE IN *SACCHAROMYCES CEREVISIAE*
Diogo Couceiro, Maria Pereira, Carolina Parada, Romeu Viana, Miguel Cacho Teixeira, Pedro Tiago Monteiro

POSTER SESSION #2 (Friday / Saturday)

TOPIC 1

P1.29 – PHARMACEUTICAL AND BIOTECHNOLOGICAL APPLICATIONS OF THE CORAL MICROBIOME
Joana F. Couceiro, Rodrigo Costa, Tina Keller-Costa

P1.42 – ASSESSMENT OF IONIC LIQUIDS IN MODULATING CARBONIC ANHYDRASE ACTIVITY USING MOLECULAR DOCKING
Michele Fraga, Mara G. Freire, Matheus M. Pereira

P1.44 – WINE LIQUID EFFLUENTS AS NUTRITIOUS CULTIVATION MEDIA TO PRODUCE MICROALGAE BIOMASS
Ana Rita Martins, Catarina Dias, Ana Cláudia Sousa, Ana Gabriela Gomes, Miguel Cachão, Carla Amarelo Santos

P1.45 – ASSESSING THE BIOTECHNOLOGICAL POTENTIAL OF MARINE-DERIVED *TRICHODERMA* ISOLATES
Dmitry Martynov, Alberto Abreu, Carina Félix, Artur Alves, Ana Cristina Esteves

P1.46 – MICROBIOME ANALYSIS AND BACTERIAL CONSORTIUM: KEYS TO HEALTHY AND SUSTAINABLE POTATO SOIL MANAGEMENT
Paula V. Morais, Adriana Mata, Carlos Cruz, Marta Acin-Albiac, Beatriz Garcia-Jimenez, Alberto Acedo, Isabel Conceição, Diogo Neves Proença

P1.47 – TAPPING INTO THE BIOTECHNOLOGICAL POTENTIAL OF ENVIRONMENTAL BIODIVERSITY: THE EXAMPLE OF A PLATINUM HIGH-AFFINITY BIOPOLYMER
Romeu Francisco, Leonor Matos, Diogo Margato, Beatriz Rito, Ana Paula Chung, Ana Paula Piedade, Paula V. Morais

P1.48 – CHEMICAL-MEDIATED INTERACTIONS BETWEEN CYANOBACTERIA AND AMOEBAE
Francisco Camacho, Pedro Leão, Ana Vieira

P1.49 -LEACHING TESTS AS PREDICTION MODELS IN MINE RESIDUE VALORISATION
Rita Branco, Diogo Margato, Joana B. Caldeira, António Correia, Paula V. Morais

P1.50 – DEVELOPMENT OF ANTIGEN-DISPLAYING *BACILLUS* SPORES FOR ORAL VACCINATION AGAINST VIBRIOSIS AND MYCOBACTERIOSIS IN FISH
Gabriela C. Gonçalves, Rafaela A. Santos, José Dias, António P. Carvalho, Patricia Díaz-Rosales, Aires Oliva-Teles, Ana Couto, Cláudia R. Serra

P1.51 – USING AGRO-INDUSTRIAL BYPRODUCTS FOR A MORE SUSTAINABLE PRODUCTION OF NATURAL PIGMENTS
Bruna Basto Nuno Ribeiro da Silva, José António Teixeira, Sara Cruz Silvério

P1.52 – IN-VITRO ASSESSMENT OF BIOCIDES EFFECT ON TWO ACTIVATED-SLUDGE FILAMENTOUS BACTERIAL STRAINS
Vânia Ferreira, Nicolina Dias, Manuel Mota, Olívia Pereira, Ana Nicolau

P1.55 – THE IMPACT OF WILDFIRES ON AMPHIBIAN SKIN MICROBIOME – A CASE STUDY BEFORE AND AFTER HELL
Sara Costa, Lorrie Maccario, Diogo Neves Proença, Søren J Sørensen, Isabel Lopes, Paula V. Morais

P1.56 – NATURAL PRODUCT BIOSYNTHETIC POTENTIAL REFLECTS MACROEVOLUTIONARY DIVERSIFICATION WITHIN A WIDELY DISTRIBUTED BACTERIAL TAXON
Sandra Godinho Silva, Masun Nabhan Homsi, Tina Keller-Costa, Ulisses Nunes da Rocha, Rodrigo Costa

P1.57 – INFLUENCE OF HARVESTING LOCATION AND PERIOD ON BIOACTIVE COMPOUNDS IN PORTUGUESE MACROALGAE: A NUTRITIONAL AND ENVIRONMENTAL PERSPECTIVE
Marta Coelho, Débora Borges, Isabel Costa, Ana M. Gomes, Manuela E. Pintado

P1.58 – *BACILLUS* SPP. NATURAL ANTIMICROBIAL COMPOUNDS ARE PROMISING ANTIMICROBIAL AGENTS FOR AQUACULTURE
Rafaela A. Santos, Mariana Reis, Rodrigo Oliveira, Gabriela C. Gonçalves, Russell Jerusik, Aires Oliva-Teles, Cláudia R. Serra

P1.59 – PREDICTION OF NEUROTOXIC POTENTIAL OF AMINO ACID-DERIVED DBPS USING MOLECULAR DOCKING
Matheus M. Pereira, Rui C. Martins

P1.60 – EXPLORING BIOACTIVE COMPOUNDS: UNVEILING THE HIDDEN TREASURES OF BACTERIAL ISOLATES FROM ALGARVE COAST UNDERSEA CAVES
Cátia Salvador, Patrícia Gatinho, Sílvia Macedo-Arantes, M. Rosário Martins, A. Teresa Caldeira

P1.61 – BIOPOLYMERS PRODUCED BY BACTERIA AS SUSTAINABLE BIOTOOLS FOR METAL REMOVAL
Ana Paula Chung, Paula V.Morais

P1.62 – UNCOVERING THE CULTURABLE ENDOPHYTIC FUNGI FROM HALIMIONE PORTULACOIDES
Cristina Torcato, Sara Alves, Liliana Santos, Cátia Fidalgo, Artur Alves

P1.63 – MICROBIOME RECRUITMENT AND ASSEMBLY DYNAMICS IN *NANNOCHLOROPSIS OCEANICA* CULTIVATIONS
Francisco Nunes, Constança Bertrand, Rodrigo Martins, Pedro Brandão, Teresa Crespo, Francisco Nascimento

P1.64 – TRACE METAL IMPACTS ON N_2O METABOLISM IN DEEP-SEA ISOLATES
Leonor Pizarro, Laurine Mathé, Maria de Fátima Carvalho, C. Marisa R. Almeida, Catarina Magalhães, Miguel Semedo

P1.65 – ADSORPTION OF PERFLUOROOCTANESULFONIC ACID (PFOS) USING A SILICA-BASED AEROGEL
Maria Inês Roque, Vittorina Rocha, Eva Domingues, Rui Martins, Luísa Durães

P1.66 – LOW-COST AND ENVIRONMENTALLY FRIENDLY PURIFICATION OF PHENOLIC COMPOUNDS IN BIOLOGICAL FLUIDS
Cariny Polesca, Helena Passos, Ana Catarina Sousa, Nguyen Tue, João Coutinho, Tatsuya Kunisue, Mara Freire

P1.67 – THE SOIL BACTERIOME OF THREE TRADITIONAL FRUIT TREES IN ALGARVE
Isabel Matos, José Matos, Alcinda Neves, Luís Cabrita, Leonor Faleiro

P1.68 – THE BIOTECHNOLOGICAL POTENTIAL OF MARINE *PENICILLIUM* SPECIES FROM THE PORTUGUESE COAST
Maria Gouveia, Alberto Abreu, Carina Félix, Artur Alves, Ana Cristina Esteves

P1.69 – MICROALGAE ALLOW REDUCING AERATION REQUIREMENTS AND IMPROVE THE EFFICIENCY OF GRANULAR SLUDGE TREATING COASTAL AQUACULTURE STREAMS AIMED AT RECIRCULATION
Ana T. Oliveira, Paula M. L. Castro, Catarina L. Amorim

P1.70 – MACROMOLECULAR CHARACTERIZATION OF DIVERSE MICROALGAE
Ana J. Alves, Decha Kumla, Alcina M.M.B. Morais, Rui M.S.C. Morais

P1.71 – UNLOCKING THE BIOTECHNOLOGICAL POTENTIAL OF AN IRIDESCENT MARINE BACTERIA ASSOCIATED WITH MICROALGAE – *CELLULOPHAGA LYTICA* NFXS1
Constança D. F. Bertrand, Rodrigo Martins, Francisco Quintas-Nunes, Pedro Reynolds- Brandão, Maria Teresa G. Crespo, Francisco X. Nascimento

P1.72 - PATHOGENIC, PHYLOGENETIC, AND ANTIBIOTIC-RESISTANCE PROFILE OF *ESCHERICHIA COLI* IN AQUATIC ENVIRONMENTS
Lúcia Gomes, Ana Machado, Adriano A. Bordalo

P1.73 - WOOL DYEING PERFORMANCE USING CHLOROPHYLL EXTRACTED FROM *CHLORELLA VULGARIS NIVA CHL-108* MICROALGAE
Alexandre M.S. Jorge, Pedro R. M. Pedroso, Jorge F.B. Pereira

P1.74 - FROM LAB TO SEEDS TO SEEDLINGS: ECO-FRIENDLY *PINUS RADIATA* PRODUCTION USING PLANT GROWTH PROMOTING BACTERIAL CONSORTIA
Frederico Leitão, Marta Alves, Glória Pinto, Isabel Henriques

P1.75 - HETEROTROPHIC CULTIVATION AND MONITORIZATION STRATEGIES TO BOOST BIOPROCESSES BASED ON THE PORTUGUESE GREEN MICROALGAE *CHLORELLA* SP. NFX-ARG AND *DYDIMOGENES* SP. NFX-PL1
João Batista, Pedro Brandão, Francisco Nascimento

P1.76 - PROSPECTION OF BIOACTIVE COMPOUNDS PRODUCED BY BACTERIAL ISOLATES FROM CAVES: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES.
Patrícia Gatinho, Cátia Salvador, Silvia Macedo Arantes, M. Rosário Martins, Ana Z. Miller, Amélia M. Silva, A. Teresa Caldeira

P1.77 - HARNESSING THE QUEST FOR ECO-FRIENDLY ALTERNATIVES TO CHEMICAL SURFACTANTS BY EXPLORING EXTREME SALINITY ENVIRONMENTS
Cátia Santos-Pereira, Joana S. Gomes, Joana Sousa, Marta F. Simões, Ricardo Franco- Duarte, Sara C. Silvério, André Antunes, Lígia R. Rodrigues

P1.78 - POTENTIAL EFFECT OF HALOTOLERANT PLANT GROWTH PROMOTING BACTERIA IN PREVENTING OLIVE KNOT DISEASE UNDER SALINE STRESS
Jyoti Chhetri, Lara Soares, Maria João Ferreira, Pedro Monteiro, I. Natalia Sierra-García, João Tedim, Gloria Pinto, Ângela Cunha

P1.79 - DEVELOPMENT OF A COST-EFFECTIVE MEDIA FOR BIOSURFACTANTS PRODUCTION BY *PSEUDOMONAS AERUGINOSA*
Adelaide Braga, Beatriz Cardoso, Margarida Fernandes, M. Gama, João Rainha, Carla Silva, Lígia Rodrigues

P1.81 - UNCOVERING SEASONAL VARIATIONS IN ANTIMICROBIAL-RESISTANT *ENTEROCOCCUS* DIVERSITY WITHIN PORTO REGION'S SURFACE WATERS
Cátia Matos, António Magalhães, Francisca Pereira, Bárbara Duarte, Ana R. Freitas, Margarida Valente, Carolina Tavares, Juliana Rodrigues, Luísa Peixe, Patrícia Antunes, Carla Novais

P1.82 - COLISTIN RESISTANCE IN PORTUGUESE WILD BOAR
Paulo Gaspar, Diana Dias, Sónia Ferreira, Sónia Mendo, Tânia Caetano

P1.83 - AIR QUALITY IN VETERINARY TEACHING FACILITIES – OCCURRENCE OF MOLDS
Isabel Vaz Carvalho, Kamila Soares, Justina Oliveira, Alexandra Esteves, Patrícia Poeta, Isabel Pires, Filipe Silva, Cristina Saraiva

P1.84 - ANTIBIOTIC RESISTANCE MONITORING USING AMPLICON SEQUENCING, PCR ARRAY AND SELECTED BIOMARKERS: A COMPARATIVE APPROACH
Inês Leão, Jorge T. Antunes, A. Margarida Teixeira, Inês Baptista, Rúben Jorge, Ivone Vaz-Moreira, Célia M. Manaia

P1.85 - MOLECULAR PROFILE, AS OBTAINED BY HIGH-THROUGHPUT FT-MIR SPECTROSCOPY, TO CHARACTERISE AND PREDICT ANTI-BACTERIAL EFFECTS OF BIOACTIVE COMPOUNDS
Igor Leite, Pedro Sampaio, Cecília R.C. Calado

P1.86 - LEVERAGING THE ANTIMICROBIAL POTENTIAL OF BEE APITOXIN TO DEVELOP PROMISING ANTI-*CANDIDA* AGENTS
Carolina Glória, Ana Luísa Oliveira, Dalila Mil-Homens, Arsénio M. Fialho, Nélson Moura, Ana Azevedo, Nuno P Mira

P1.88 - CYANOBACTERIAL DIVERSITY FROM UNDEREXPLORED AREAS AND ENVIRONMENTS ACROSS MOROCCO AND CAPE VERDE
João Morais, Raquel Silva, Flávio Oliveira, Pedro Cruz, Pedro Leão, Vitor Vasconcelos

P1.89 - UNCOVERING BIOPLASTIC BIOSYNTHESIS POTENTIAL IN MARINE HOST-ASSOCIATED BACTERIA USING COMPARATIVE GENOMICS
Ana Maria Fernandes, Maria Teresa Cesário, Rodrigo Costa

TOPIC 2

P2.9 - UNDERSTANDING VIRULENCE VARIABILITY AMONG *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES
Mariana Sousa, Rui Magalhães, Vânia Ferreira, Paula Teixeira

P2.19 - ASSESSING THE PREBIOTIC POTENTIAL OF XYLOOLIGOSACCHARIDES PRODUCED BY ONE-STEP FERMENTATION USING AGRO-RESIDUES
Ana Cordeiro, Andreia Fernandes, Joana Sousa, Beatriz Cardoso, Joana Alves, Sara Silvério, António Peres, Lígia Rodrigues, Cláudia Amorim

P2.20 - HIGH-THROUGHPUT MICRO-PLATE FLUORESCENCE QUENCHING FOR SCREENING THE BINDING OF MYCOTOXINS TO ALBUMINS
Heidar Adalsteinsson, Lavínia Pinto, Diana G. Rodrigues, Lucília Domingues, António A. Vicente, Tatiana Q. Aguiar, Luís Abrunhosa

P2.21 - EFFECT OF *MELISSA OFFICINALIS* ESSENTIAL OIL ON BIOFILM FORMED BY *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM MEAT INDUSTRIES
Alexandra Coimbra, Susana Ferreira, Felice Panebianco

P2.22 - ANTIFUNGAL CAPACITY OF A COMMERCIAL FLAVOURING AGENT AGAINST SPOILAGE YEASTS
Beatriz Nunes Silva, Teresa Bento de Carvalho, Miguel Azevedo, Beatriz Silva, Paula Teixeira

P2.23 - PURIFICATION AND CHARACTERIZATION OF A BACTERIOCIN PRODUCED BY *ENTEROCOCCUS FAECALIS* L3A21K6
Susana C. Ribeiro, Célia C.G. Silva

P2.25 - EXPLORE LIGHT EMITTING DIODES AND PHOTOCATALYTIC SURFACES TO PREVENT BIOFILM FORMATION IN FOOD INDUSTRY
Carolina Santos, Ana Paula Marques, João Sério, Maria Teresa Barreto Crespo, Vanessa Jorge Pereira

P2.26 - MODELING LACTIC ACID PRODUCTION IN *LACHANCEA THERMOTOLERANS*
Marcos Esteves, Véronique Gomes, Rogério Tenreiro, Marco Reis, Nuno P. Mira, Ana Mendes-Ferreira

P2.27 - FRUIT PEELS AS SOLID-STATE FERMENTATION SUBSTRATES FOR THE SUSTAINABLE PRODUCTION OF MICROBIAL PROTEIN
Helena Vilela, Liliana Araújo, Isabel Belo, Rita Pinheiro, Marlene Lopes

P2.29 - CHARACTERIZATION OF *CAMPYLOBACTER* SPP. ISOLATES FROM POULTRY SLAUGHTERED FOR HUMAN CONSUMPTION IN PORTUGAL
Sónia Ramos, Maria-Leonor Lemos, Ludovica Di Bella, Selma Marques, Margarida Penteado, Margarida Alves, Adriana Belas, Isabel Santos, Ana Lima, Laurentina Pedroso, João Paulo Gomes, Alexandra Nunes, Mónica Oleastro

P2.30 - GENOMIC DIVERSITY OF *KLEBSIELLA PNEUMONIAE* IN CHICKEN PRODUCTION CHAIN: ANTIBIOTIC RESISTANCE AND METAL TOLERANCE INTERPLAY
Joana Mourão, Marisa Ribeiro-Almeida, Mafalda Magalhães, Andreia Rebelo, Carla Novais, Luísa Peixe, Ângela Novais, Patrícia Antunes

P2.31 - LONG-TERM PERSISTENCE OF MCR-1 CARRYING *ESCHERICHIA COLI* IN INTENSIVE RABBIT FARMS AFTER COLISTIN BAN
Marisa Ribeiro-Almeida, André Pinto de Carvalho, Joana Mourão, Paulo Martins da Costa, Luísa Peixe, Patrícia Antunes

P2.32 - PHAGE THERAPY IN THE CONTROL OF VIBRIO ALGINOLYTICUS IN THE AQUACULTURE LIVE FOOD ARTEMIA FRANCISCANA NAUPLII
Amanda Tadeu, João Duarte, Davi Trindade, Carla Pereira , Adelaide Almeida

P2.33 - BACTERIOPHAGES TO CONTROL VIBRIO PARAHAEMOLYTICUS: A TECHNOLOGY FOR BIVALVE DEPURATION
João Duarte, Carla Pereira, Ricardo Calado, Adelaide Almeida

P2.34 - EFFECTS OF THE NON-SUGAR SWEETENERS XYLITOL AND SUCRALOSE ON *SACCHAROMYCES CEREVISIAE* CHRONOLOGICAL LIFESPAN
Conceição Gonçalves, Sara Dourado, Beatriz Ferreira, Maxime Gomez, Belém Sampaio- Marques, Joris Winderickx, Paula Ludovico

P2.35 - THE ACTION OF THE SWEETENERS CYCLAMATE AND SACCHARIN ON THE CHRONOLOGICAL AGING OF *SACCHAROMYCES CEREVISIAE*
Sara Dourado, Conceição Gonçalves, Beatriz Ferreira, Maxime Gomez, Belém Sampaio- Marques, Joris Winderickx, Paula Ludovico

P2.36 - SEA BASS EMBRYONIC CELL CULTURE USING MICROCARRIERS FOR CULTIVATED SEAFOOD PRODUCTION
Laura Alves, Diana M. C. Marques, Diogo E.S. Nogueira, Catarina Toste, William, O.S. Salvador, Paola Sanjuan-Alberte, Joaquim M.S. Cabral, Frederico C. Ferreira, Alexandrea Duscher, Carlos A.V. Rodrigues

TOPIC 3

P3.19 - LEPT-IFA: AN INDIRECT IMMUNOFLUORESCENCE TEST FOR THE DIAGNOSIS OF LEPTOSPIROSIS
Ana Armada, Wilmer Fernandes, Maria Luísa Vieira

P3.21 - RESCUE OF *MYCOBACTERIUM BOVIS* DNA OBTAINED FROM CULTURED STRAINS DURING OFFICIAL SURVEILLANCE OF ANIMAL TUBERCULOSIS
Daniela Pinto, Gonçalo Themudo, André C. Pereira, Ana Botelho, Mónica V. Cunha

P3.23 - COMPUTER-AIDED DESIGN OF AFFINITY LIGANDS FOR RNA PURIFICATION USING MOLECULAR DOCKING
Rita Carapito, Paulo Almeida, Matheus M. Pereira, Mara G. Freire, Fani Sousa

P3.24 - NIPHARMINS: NOVEL LOW RESISTANCE-INDUCING ANTIMICROBIALS WITH ANTIBIOFILM ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*
João Pedro Carrilho, Nuno Candeias, Alexandra Nunes

P3.25 - COPING WITH ANTIMICROBIAL RESISTANCE SCENARIO RESORTING TO AVIAN IMMUNOGLOBULIN Y (IGY) ANTIBODIES
Catarina Almeida, Débora Sousa, Márcia Neves, Mara Freire

P3.26 - PH-RESPONSIVE NANOPARTICLES FOR EFFICIENT RNA DELIVERY IN CANCER CELLS
Bruno Baptista, Andreia S. R. Oliveira, Patrícia V. Mendonça, Arménio C. Serra, Jorge F. J. Coelho, Fani Sousa

P3.27 - SYNTHESIS AND CYTOTOXICITY EVALUATION OF SUPPORTED IONIC LIQUIDS FOR THE PURIFICATION OF P53-MINICIRCLE DNA BIOPHARMACEUTICALS
João Vasco Valente, Ana I. Valente, Augusto Q. Pedro, Mara G. Freire

P3.28 - PH RESPONSIVE TAXIFOLIN-LOADED DELIVERY SYSTEMS FOR CERVICAL CANCER THERAPY
Miguel Neto, Miguel Ferreira, Diana Gomes, Luís Passarinha, Diana Costa, Ângela Sousa

P3.29 - RECOVERY OF PROTEIN-BASED BIOPHARMACEUTICALS FROM CULTURE MEDIA USING POLYMER-BASED AQUEOUS BIPHASIC SYSTEMS
Leonor S. Castro, Augusto Q. Pedro, Mara G. Freire

P3.30 - TARGETED DELIVERY OF MINICIRCLE DNA VACCINE AGAINST COVID-19 TO ANTIGEN-PRESENTING CELLS USING MANNOSYLATED POLYETHYLENIMINE-CHOLESTEROL-BASED NANOPARTICLES
Dalinda Eusébio, Milan Paul, Swati Biswas, Zhengrong Cui, Diana Costa, Ângela Sousa

P3.31 - MANNOSYLATED CHITOSAN-OCTA-ARGININE-BASED NANOPARTICLES FOR TARGETED DELIVERY OF MINICIRCLE DNA VACCINE AGAINST COVID-19 TO ANTIGEN-PRESENTING CELLS
Dalinda Eusébio, Milan Paul, Swati Biswas, Zhengrong Cui, Diana Costa, Ângela Sousa

P3.32 - DEVELOPMENT OF A NOVEL T-CELL ENGAGER TRISPECIFIC ANTIBODY FOR ENHANCED IMMUNE TARGETING OF NON-HODGKIN LYMPHOMA
Margarida Ferreira-Silva, Inês Rosa, Afonso P. Basto, Pedro Bule, Sara Nogueira, Rainer Storb, Luís Tavares, Frederico Aires da Silva, Joana N. R. Dias

P3.33 - TARGETED TAXIFOLIN DELIVERY TO CERVICAL CANCER CELLS THROUGH ALBUMIN-BASED DELIVERY SYSTEMS
Miguel Ferreira, Diana Gomes, Milan Paul, Swati Biswas, Diana Costa, Ângela Sousa

P3.34 - AN IMMUNOTOXIN DERIVED FROM *PSEUDOMONAS AERUGINOSA* AS A PROMISING APPROACH FOR THE TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER
Rafaela Marimon, Isa Moutinho, Ana André, Ana Leonardo, Luís Tavares, Frederico Aires-da-Silva

P3.35 - SEEKING FOR FUNGAL SIALIDASE INHIBITORS
Gonçalo Conde de Oliveira, Chantal Fernandes, Luís Rodrigues, Rita Domingues, Daniela Calheiros, Michael Brad Strader, Aishwarya L. Saha, Kavita Sharma, Christian Hadiwinarta, Jay E. Slater, Dina Murtinho, Elisa da Silva Serra, Teresa Gonçalves

P3.36 - DEVELOPMENT OF A FAST METHOD FOR THE EARLY DETECTION OF *PAENIBACILLUS LARVAE* SPORES IN BEEHIVES - A STRATEGY TO CONTROL AMERICAN FOULBROOD
Renato Mota, Paulo Carvalho, Diana Rodrigues, Artur Ribeiro, Sílvio B. Santos, Ana Oliveira

P3.37 - FAST AND SENSITIVE DETECTION OF *PSEUDOMONAS AERUGINOSA* IN CLINICAL SETTINGS USING ENGINEERED REPORTER PHAGES
Maria João Costa, Luciana Meneses, Joana Azeredo, Sílvio Santos, Diana Priscila Pires

P3.38 - RAPID DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH DRUG RESISTANCE IN MALARIA PARASITES USING ISOTHERMAL AMPLIFICATION APPROACH
Mariana Pinto, Ana Tavares, Mayreli Ortiz, Vasoula Skouridou, Miriam Jauset- Rubio, Miguel Viveiros, Ciara O'Sullivan, Ana Paula Arez, Márcia M. Medeiros

P3.39 - IMPROVED PURIFICATION STRATEGIES FOR RNA AFFORDED BY THE USE OF BIOBASED IONIC LIQUIDS AS CONSTITUENTS OF AQUEOUS BIPHASIC SYSTEMS
Ana F. Pereira, Augusto Q. Pedro, Leonor S. Castro, Maria J. Quental, Ana P. M. Tavares, Luís C. Branco, João A. P. Coutinho, Fani Sousa, Mara G. Freire

P3.40 - IMPLEMENTATION OF A *GALLERIA MELLONELLA* INFECTION MODEL AT GHTM/IHMT-NOVA
Telma Rodrigues, Mariana Andrade, Joana Marques, Liliana Rodrigues, Miguel Viveiros, Henrique Silveira, Isabel Couto, Sofia Santos Costa

P3.41 - NEAR INFRARED LIGHT RESPONSIVE THIOL-MALEIMIDE HYDROGELS AIMED FOR BREAST CANCER CHEMO-PHOTOTHERMAL THERAPY
Francisco J.P. Costa, Micaela Nave, Rita Lima-Sousa, Cátia G. Alves, Bruna L. Melo, Ilídio J. Correia, Duarte de Melo-Diogo

P3.42 - MESOPOROUS SILICA-COATED GOLD NANOCLUSTERS FOR ENHANCED CANCER CHEMO-PHOTOTHERMAL THERAPY
Natanael F. Fernandes, Soraia Borges, Carolina F. Rodrigues, Duarte de Melo-Diogo, Ilídio J. Correia, André F. Moreira

TOPIC 4

P4.1 - PHAGE THERAPY AGAINST PSEUDOMONAS AERUGINOSA BIOFILMS IN URINE
Inês Martinho, Márcia Braz, Carla Pereira, Adelaide Almeida

P4.15 - CAN WILD BOARS BE RESERVOIRS OF RELEVANT RESISTANT BACTERIA?
Manuela Oliveira, Raquel Abreu, Zita Martins Ruano, Madalena Vieira-Pinto, Ricardo Dias, Luís Tavares, Eva Cunha

P4.16 - GUT BACTERIAL EVOLUTION DURING PREGNANCY
Joana Bom, Elsa Seixas, Nelson Frazão

P4.19 - THE INFLUENCE OF COVID-19 PANDEMIC IN ANTIMICROBIAL RESISTANCE DISSEMINATION IN VETERINARY MEDICINE
Diana Gomes, Leonor Macedo, Luis Tavares, Manuela Oliveira

P4.25 - VIRULENCE PROFILE OF ESCHERICHIA COLI FROM CAPTIVE BIRDS OF PREY USED IN AVIFAUNA CONTROL AND AS EDUCATIONAL AMBASSADORS
Rita Magalhães, Raquel Abreu, Catarina Geraldes, Eva Cunha, Luís Tavares, Manuela Oliveira

P4.40 - THE HYDROGENOME OF NATURAL MINERAL WATERS: CONTRASTING CHARACTERISTICS AT HARVESTING SITES AND WATER STORAGE TANKS
Cátia Fidalgo, Catarina Milho, Maria José Alves

P4.41 - A NOVEL *LIMOSILACTOBACILLUS MUCOSAE* SUBSPECIES ISOLATED FROM THE FEMALE URINARY MICROBIOTA
Andreia Garcia, Teresa Gonçalves Ribeiro, Filipa Grosso, Luísa Peixe

P4.42 - SIALIDASE-1 AS A KEY VIRULENCE FACTOR IN *SPOROTHRIX BRASILIENSIS* INFECTION
Tiago Cardoso, Ricardo Silvestre, Maria Horta, Gustavo H. Goldman, Agostinho Carvalho, Egídio Torrado, Cristina Cunha, Fernando Rodrigues

P4.43 - POPULATION STRUCTURE AND TRANSMISSION DYNAMICS OF *MYCOBACTERIUM BOVIS* AT THE LIVESTOCK-WILDLIFE INTERFACE
André C. Pereira, Ana C. Reis, José Lourenço, Gonçalo Themudo, Ana Botelho, Mónica V. Cunha

P4.44 - IMPACT OF GUT MICROBIOTA ON THE PHENOLIC COMPOSITION AND BIOACTIVITY OF VIRGIN OLIVE OIL
Elsa Mecha, Claudia Santos, Ana Catarina Guerreiro, Rosário Bronze, Ana Teresa Serra

P4.45 - UNEQUAL DISTRIBUTION OF ANTIFUNGAL SUSCEPTIBILITY AMONG *CANDIDA* SPP. ISOLATES OF THE FEMALE GENITAL TRACT
Filipa Oliveira Castro, Francisca Bastos, Mariana Zagalo Fernandes, José Martinez- de-Oliveira, Ana Palmeira-de-Oliveira, Ana Rita Ferrão, Paula Gouveia Pestana, Joana Rolo

P4.47 - ANTIMICROBIAL BIOPROSPECTING OF ESSENTIAL OILS OF ALENTEJO' LAMIACEAE FLAVOURING PLANTS
M. Rosário Martins, Sílvia Macedo Arantes, A. Teresa Caldeira

P4.48 - CHARACTERIZATION OF THE DIFFERENTIAL ANTIMICROBIAL ACTIVITY OF HUMAN ENDOGENOUS FATTY ACIDS IN PATHOGENIC/COMMENSAL *STAPHYLOCOCCUS EPIDERMIDIS*
F Magalhães, E Morais, LG Gonçalves, M Miragaia, AV Coelho

P4.49 - PLASMID PROFILING OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ASSOCIATED WITH SKIN AND SOFT TISSUE INFECTIONS IN COMPANION ANIMALS
Catarina Morais, Sofia Santos Costa, Dennis Hanke, Henrike Krüger-Haker, Ana Santos, Constança Pomba, Andrea T. Feßler, Stefan Schwarz, Isabel Couto

P4.50 - COFFEE PULP AS A PREBIOTIC SUBSTRATE FOR *LACTOBACILLUS PARACASEI*: ASSESSING NUTRITIONAL COMPOSITION AND CHLOROGENIC ACID PROFILE
Marlene Machado, Sara Maia, Susana Machado, Liliana Espírito-Santo, M. Beatriz P. P. Oliveira, Helena Ferreira, Rita C. Alves

P4.51 - UNVEILING COVID-19 EFFECTS: SARS-COV-2 SIGNIFICANTLY ALTERS THE OROPHARYNGEAL MICROBIOTA OF ASYMPTOMATIC ADULTS
Ana Cristina Paulo, Sónia T. Almeida, Sara Handem, Alexandra S. Simões, Raquel Sá-Leão

P4.52 - GUT BACTERIAL METATAXONOMIC ANALYSIS OF ELDERLY FROM ALGARVE
José Matos, Nádia Silva, Marlene Trindade, Ana Teresa Rajado, Pedro Castelo-Branco, Leonor Faleiro

P4.53 - QUANTIFICATION OF VAGINAL LACTOBACILLI USING AN OPTIMIZED FLUORESCENCE MICROSCOPY METHOD
Ângela Lima, Christina A. Muzny, Nuno Cerca

P4.54 - *STAPHYLOCOCCUS AUREUS* FROM ATOPIC DERMATITIS HAVE SPECIFIC GENOMIC SIGNATURES
Mariana Araújo, Nuno A. Faria, Margarida Gonçalo, Joanna Nakonieczna, Carmen Torres, Floriana Campanile, Kátia Netto dos Santos, Rita Sobral, Maria Miragaia

P4.56 - POLYMER TYPE AND ENVIRONMENTAL CONDITIONS DETERMINE THE PLASTISPHERE AND ASSOCIATED RESISTOME
Inês Alçada, Isabel Silva, Marta Tacão, Isabel Henriques

P4.57 - IDENTIFICATION OF MYCOPLASMA GENITALIUM AND CHARACTERIZATION OF MACROLIDE AND FLUOROQUINOLONE RESISTANCE-ASSOCIATED MUTATIONS IN A POPULATION FROM THE LISBON AREA, PORTUGAL
Alexandra Rusu, Filomena Martins Pereira, Liliana Rodrigues

P4.58 - EVALUATION OF TOXICITY AND *IN VITRO* ACTIVITY OF CANDIDATE DRUGS FOR REPURPOSING AGAINST *NEISSERIA GONORRHOEAE*
Beatriz Santos, Ana Armada, Liliana Rodrigues

P4.60 - DETERMINATION OF FOSFOMYCIN SUSCEPTIBILITY OF ESBL-PRODUCING *E. COLI* AND CARBAPENEM-RESISTANT *K. PNEUMONIAE* ISOLATES FROM HOSPITALIZED PATIENTS
Ricardo Goulart, Sónia Ferreira, Sónia Mendo, António Maio, Tânia Caetano

P4.61 - EXPLORING THE VIRULENCE POTENTIAL OF CC152 AND CC121 *S. AUREUS* STRAINS RELATED TO PEDIATRIC COMMUNITY-ACQUIRED BACTERAEMIA IN THE MANHIÇA DISTRICT HOSPITAL, MOZAMBIQUE
Marcelino Garrine, Mariana Andrade, Inácio Mandomando, Isabel Couto, Sofia Santos Costa

P4.62 - EVOLUTION OF *ALIARCOBACTER BUTZLERI* UNDER LOW CIPROFLOXACIN CONCENTRATIONS ASSOCIATED WITH THE SELECTION OF MULTIDRUG- RESISTANT MUTANTS
Inês Martins, Alexandra Nunes, Mónica Oleastro, Susana Ferreira

P4.63 - BACTERIAL VAGINOSIS: ASSOCIATION BETWEEN ANTIMICROBIAL TOLERANCE AND *IN VITRO* BIOFILM FORMATION MODEL
Sofia A. Pereira, Lúcia G. V. Sousa, Nuno Cerca

P4.64 - COLISTIN SUBINHIBITORY CONCENTRATIONS EFFECT ON GROWTH AND NATURAL TRANSFORMATION IN *ACINETOBACTER BAUMANNII*
Tiago Lima, Victoria Stubberud, Monica Doan, Gabriela Jorge da Silva, Sara Domingues

P4.65 - INDUCED RESISTANCE TO VANCOMYCIN IMPAIRS BIOFILM FORMATION BY *S. EPIDERMIDIS*
Inês Santos, Ângela França

P4.67 - DIARRHEA IN UNDER FIVE CHILDREN IN BENGO AND LUANDA: ANTIBIOTIC SUSCEPTIBILITY AND *ROTAVIRUS*
Elsa Fortes-Gabriel, Maria de Fátima da Silva, Jocelyne Vasconcelos, Miguel Brito

P4.68 - PREDICTION OF NOVEL SMALL NON-CODING RNAs FROM *PSEUDOMONAS AERUGINOSA* INVOLVED IN ANTIBIOTIC RESISTANCE AND BIOFILM FORMATION
Joana Silva, Cecília Maria Arraiano, Vânia Pobre

P4.69 - DISCLOSING AZOLE RESISTANCE MECHANISMS IN *CANDIDA GLABRATA* CLINICAL STRAINS THROUGH OMICS ANALYSES: BEYOND CGPDR1
Maria Joana Pinheiro, Sara B. Salazar, Carolina Soares, Vitória Rodrigues, Fábio Fernandes, Nuno P. Mira

P4.70 - PRELIMINARY STUDY OF PERMEABILITY IN ENTEROCOCCI OBTAINED FROM A VETERINARY BIOLOGICAL ISOLATION AND CONTAINMENT UNIT
Catarina Geraldes, Sophie Burchael, Luís Tavares, Solange Gil, Marta Martins, Manuela Oliveira

P4.71 - DRUG REPURPOSING FOR IDENTIFICATION OF NEW EFFLUX INHIBITORS AND/OR ANTIBIOFILM AGENTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*
Joana Neves, Carolina Antunes, Mariana Andrade, Maria Bento, Isabel Couto, Liliana Rodrigues, Sofia Santos Costa

P4.72 - ATOPIC DERMATITIS MICROENVIRONMENT FAVOURS *STAPHYLOCOCCUS AUREUS* GROWTH AND PROMOTES ANTIBIOTIC RESISTANCE
Diana Caieiro, Nuno Faria, Mariana Araújo, Joanna Nakonieczna, Rita Sobral, Maria Miragaia

P4.73 - CROSS-SPECIES GENE EXPRESSION PROFILING OF THE RESISTANCE TO INFECTION BY *MYCOBACTERIUM ULCERANS*
João Fevereiro, Nuno Osório, Maria Peixoto, Ana Mendes, Laurent Marsollier, Estelle Marion, Alexandra Fraga, Jorge Pedrosa

P4.74 - TLR2-DEPENDENT ACTIVATION OF MACROPHAGES DURING *MYCOBACTERIUM ULCERANS* INFECTION
Juan Dominguez, Teresa Martins, Bruna Ribeiro, Gabriela Trigo, José Gama, António Castro, Jorge Pedrosa, Alexandra Fraga

P4.75 - CHLORHEXIDINE AND BENZALKONIUM CHLORIDE ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM SKIN AND SOFT TISSUE INFECTIONS IN COMPANION ANIMALS
Carolina Ferreira, Catarina Morais, Paula Zacharias, Patrícia Abrantes, Constança Pomba, Sofia Santos Costa, Isabel Couto

P4.76 - BIOFILM AND MULTIDRUG-RESISTANT BACTERIA: A STUDY IN BLACK-AND-WHITE RUFFED LEMUR
Carolina Silva, João F. Requicha, José J. Martins, Isabel R. Dias, Carlos A. Viegas, Aida Duarte, Maria J. Saavedra

P4.77 - *STREPTOCOCCUS PNEUMONIAE* CARRIAGE AND SEROTYPE DISTRIBUTION IN SALIVA SAMPLES FROM PRE-SCHOOL CHILDREN
Sónia T. Almeida, Alexandra S. Simões, Sara Handem, António Brito-Avô, Hermínia de Lencastre, Raquel Sá-Leão

P4.80 - LOW-COST SALIVA TESTS FOR SARS-COV-2 INFECTION CONTROL
Catarina Amaral, Sofia M. da Silva, Diana Grilo, Wilson Antunes, Elin Moe, Américo Duarte, Luís M. P. Lima, Cláudia Luís, Inês Morais, Gonçalo Afonso, Nuno Faria, Inês Gomes, Ana Margarida Henriques, Miguel Fevereiro, Raquel Sá-Leão, Maria Miragaia, Mónica Serrano, Catarina Pimentel

P4.81 - USE OF ANTISENSE OLIGOMERS FOR THE CONTROL OF TRANSCRIPTION FACTORS INVOLVED IN BIOFILM FORMATION OF *CANDIDA GLABRATA*
Daniela Araújo, Tânia Ribeiro, Joana Castro, Mariana Henriques, Sónia Silva

P4.82 - DETECTING ENTEROTOXIGENIC *ESCHERICHIA COLI* IN ANIMAL PRODUCTION: METHOD DEVELOPMENT AND VALIDATION
Maria Margarida Barros, Ricardo Oliveira, Joana Castro, Ana Campos, Gonçalo Nieto Almeida, Daniela Araújo, Sónia Silva, Divanildo Outor-Monteiro, Carina Almeida

P4.83 - ASSESSING THE ABILITY OF *LACTOBACILLUS* STRAINS TO COUNTERACT ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) INFECTION BY USING A *GALLERIA MELLONELLA IN VIVO* MODEL

Daniela Araújo, Ana Rita Silva, Ana Oliveira, Nuno Pereira Mira, Sónia Silva, Carina Almeida, Joana Castro

P4.84 - MARINE MICROALGAE MICROBIOME: A SOURCE OF POTENT ANTIFUNGAL COMPOUNDS

Teresa Pissarro, Francisco Xavier Nascimento, Catarina Pimentel

P4.85 - EXPLORING THE ANTIMICROBIAL EFFECTS OF VARIOUS HONEY TYPES FOR THE MANAGEMENT OF DIABETIC FOOT INFECTIONS

Andréa Bezerra, Maria José Alves, Maria José Saavedra, Hélder Fonseca, Francisca Rodrigues, Cristina Delerue-Matos, Irene Gouvinhas, Juliana Garcia

***P4.86* - *STREPTOMYCES LUNALINHARESII* A54A, AN ENDOPHYTIC ACTINOBACTERIA FROM SÃO PAULO COAST-BRAZIL AS A POTENTIAL BIOCONTROL AGENT OF PHYTOPATHOGENIC BACTERIA**

Jairo I. Quintana-Bulla, Gabriel Luiz Padoan Gonçalves, Roberto G. S. Berlinck, Simone Possedente de Lira

TOPIC 5

P5.20 - TIME SERIES EXOMETABOLOMICS DEPICT METABOLIC FEATURES OF *STAPHYLOCOCCUS EPIDERMIDIS* PATHOGENICITY

Elisabete Morais, Maria Miragaia, Luís G Gonçalves, Ana V Coelho

P5.21 - EFFECT OF *HTRA* GENE IN THE PATHOGENICITY OF *ALIARCOBACTER BUTZLERI*

Cristiana Mateus, Fernanda Domingues, Roland Bücker, Mónica Oleastro, Susana Ferreira

P5.22 - PHAGE HUNTING ACROSS *MYCOBACTERIUM BOVIS* GENOMES

Daniela Pinto, Inês Mendes, Mónica V. Cunha

P5.23 - GENOME-WIDE SIGNATURES OF ADAPTATION TOWARDS THE SESSILE LIFESTYLE IN *STAPHYLOCOCCUS AUREUS*

Sara Teixeira, Beatriz Ramos, Mónica V. Cunha

P5.24 - CABLE BACTERIA'S GROUNDBREAKING PROTEIN: ON THE JOURNEY FOR IMPROVED BIOELECTRICITY GENERATION AND BIOELECTRONICS COMPONENTS

Marta A. Silva, Benjamin S. Korsgaard, Pilar C. Portela, Krutika Bavishi, Thomas Boesen, Carlos A. Salgueiro

P5.25 - INSIGHTS INTO THE GENOME AND TRANSPORTOME OF THE YEAST *CYBERLINDNERA JADINII*

Maria Sousa-Silva, Ricardo Franco-Duarte, Simão Rodrigues, Margarida Casal, Pedro Soares, Isabel Soares-Silva

P5.26 - STRUCTURE-FUNCTION STUDIES OF THE *SCHIZOSACCHAROMYCES POMBE* DICARBOXYLIC ACID TRANSPORTER MAE1

Tayane Fontes, João Alves, Filipe Kuhlmann, Pedro Soares, Sandra Paiva, Margarida Casal, Isabel Soares-Silva

P5.27 - GLYCINE BETAINE ROLE IN *STAPHYLOCOCCUS EPIDERMIDIS* PH ADAPTATION AND PATHOGENICITY
Luís G. Gonçalves, Elisabete Morais, Susana Santos, Jean Armengaud, Maria Miragaia, Ana V. Coelho

P5.28 - HETEROLOGOUS EXPRESSION OF A LANM HOMOLOGOUS, FROM STRAIN *MESORHIZOBIUM QINGSHENGII* J19, INVOLVED IN YTTRIUM IMMOBILIZATION
Carina Coimbra, Rita Branco, Paula V. Morais

P5.29 - BIOCOMPATIBLE ENGINEERED POLYUREA (PURE) DENDRIMERS ARE A NEW HOPE AGAINST MULTIDRUG-RESISTANT (MDR) BACTERIA
Dalila Mil-Homens, Laura Pereira, Rita F. Pires, José M. Andrade, Marta M. Alves, Gabriel Serafim, Nuno Martinho, Manuel Melo, Arsénio M. Fialho, Vasco D.B. Bonifácio, Sandra N. Pinto

P5.30 - RNA-SEQUENCING ANALYSIS REVEALS KEY CHANGES IN *G. VAGINALIS* TRANSCRIPTOME AFTER ANTIMICROBIAL CHALLENGE IN POLYMICROBIAL BIOFILMS
Lúcia G. V. Sousa, Vânia Pinheiro, Nuno Cerca

P5.31 - EXPLORING THE STRATEGIES OF ADAPTATION OF *STAPHYLOCOCCUS EPIDERMIDIS* TO THE HOSPITAL ENVIRONMENT USING A PANGENOMIC APPROACH
Ana Botelho, Henrik Westh, Joana Rolo, Maria Miragaia

P5.32 - TOWARD COST-EFFECTIVE PRODUCTION OF RECOMBINANT HUMAN SERUM ALBUMIN FOR USE IN MYCOTOXIN CAPTURE DEVICES
Diana G. Rodrigues, Heidar Adalsteinsson, Lavínia Pinto, Tatiana Q. Aguiar, Carla Oliveira, Luís Abrunhosa, Lucília Domingues

P5.33 - EXPLORING *HANSENIASPORA GUILLIERMONDII* AS A CELL FACTORY: INSIGHTS INTO ACETATE ESTER PRODUCTION AND ETHANOL TOLERANCE MECHANISMS
Isabel Seixas, Diogo Santos, Nuno P. Mira, Ana Mendes-Ferreira

P5.34 - REDUCING POWER IN *GEOBACTER*'S PERIPLASM: NMR INTERACTION STUDIES BETWEEN TRIHEME CYTOCHROMES
Leonor Morgado, Marisa R. Ferreira, Carlos A. Salgueiro

P5.35 - EXPLORING THE BIOSYNTHETIC CAPABILITY OF A CYANOBACTERIUM FROM LEGE-CC FOR THE PRODUCTION OF NOVEL COMPOUNDS
Dora Ferreira, Leonor Ferreira, Ralph Urbatzka, Joana Almeida, Mariana Reis

P5.36 - ENHANCING LACTONE BIOSYNTHESIS IN *ASHBYA GOSSYPII*: TOWARDS SUSTAINABLE PRODUCTION FROM DIVERSE CARBON SOURCES
Carlos E. Costa, Luís Freitas, Miguel Francisco, Tatiana Q. Aguiar, Lucília Domingues

P5.37 - PHYSIOLOGICAL DIFFERENCES IN EXPONENTIAL AND STATIONARY PHASE CELLS OF *DEBARYOMYCES HANSENII* IN RESPONSE TO NACL
Tomás Gonçalves, Catarina Prista

P5.38 - INTERACTION OF *ACINETOBACTER* SPP. AND *CANDIDA* SPP. IN DUAL MIXED BIOFILMS
Ana Patrícia Abrantes, Tiago Lima, Sara Domingues, Gabriela Jorge da Silva

P5.39 - DEVELOPMENT OF GENETICALLY ENCODED BIOSENSORS FOR PLASMODIUM FALCIPARUM
Ana Pinheiro, Adriana Gonçalves, Pedro Ferreira

P5.40 - EXPLORING OXIDATIVE STRESS PATHWAYS IN G. SULFURREDUCENS: THE REDOX NETWORK BETWEEN MACA PEROXIDASE AND TRIHEME PERIPLASMIC CYTOCHROMES
Pilar C. Portela, Leonor Morgado, Marta A. Silva, Lukas Denkhaus, Oliver Einsle, Carlos, A. Salgueiro

TOPIC 6

P6.4 - RECOMBINANT HPV E7 ONCOPROTEIN BIOSYNTHESIS AND PURIFICATION: A CRUCIAL INTEGRATION STEP TOWARDS TARGETED ANTI-HPV DRUG DEVELOPMENT
Diana Gomes, Luís A. Passarinha, Ângela Sousa

P6.11 - SUSTAINABLE TECHNOLOGY TO EXTRACT AND PURIFY PROTEINS FROM INSECTS
Pedro Moleiro, Augusto Q. Pedro, Ana Paula M. Tavares, Mara G. Freire

P6.12 - ADVANCING MANUFACTURE OF HIPSC-DERIVED HEPATOCYTES WITH IMPROVED FUNCTIONALITY: A NATURE-INSPIRED BIOPROCESS
Joana I. Almeida, Pedro Vicente, Inês E. Crespo, Nikolaus Virgolini, Inês A. Isidro, Tomás Calmeiro, Henrique V. Almeida, Elvira Fortunato, Maren Calleja, Juan R. Rodriguez-Madoz, Felipe Prosper, Pedro M. Baptista, Paula M. Alves, Margarida Serra

P6.13 - PRE-SACCHARIFICATION AND HIGH SOLIDS OPERATION AS KEY STRATEGIES TO IMPROVE BIOETHANOL PRODUCTION FROM *EUCALYPTUS GLOBULUS* BARK BY SSF
Mariana S. T. Amândio, Ana M. R. B. Xavier, Jorge M. S. Rocha

P6.14 - LIPID PRODUCTION FROM NON-DETOXIFIED LIGNOCELLULOSIC BIOMASS HYDROLYSATE BY *ASHBYA GOSSYPII* PRECISION FERMENTATION
Miguel Francisco, Tatiana Quinta Aguiar, Lucília Domingues, Gabriel Abreu, Susana Marques, Francisco Gírio

P6.15 - USE OF TOMATO WASTE HYDROLYSATES FOR PIGMENTS PRODUCTION USING *RHODOTORULA GLUTINIS* CELLS
Helena Ribeiro, Boelo Schuur, Jorge F. B. Pereira

P6.16 - SYNTHESIS AND CHARACTERIZATION OF PETASIS-UGI LIGAND FOR ANTIBODY PURIFICATION
Carolina Natal, Catarina Domingos, Henrique Costa, Margarida Dias, Ana Cecília Roque

P6.17 - EVALUATION OF OCHRATOXIN A (OTA) INTERACTION WITH RECOMBINANT DOMAIN II OF BOVINE SERUM ALBUMIN TOWARD THE DEVELOPMENT OF NEW OTA EXTRACTION PLATFORMS
Tatiana Q. Aguiar, Tânia Leal, Diana G. Rodrigues, Luís Abrunhosa, Carla Oliveira, Lucília Domingues

P6.18 - ENHANCED RNA CAPTURE AND RECOVERY FROM COMPLEX SAMPLES USING CARBON NANOMATERIALS
P.L. Ferreira, Ana Videira, J.L. Faria, C.G. Silva, Fani Sousa

P6.19 - PROTEIN RECOVERY FROM BIOMASS USING INTEGRATED PROCESSES BASED ON IONIC LIQUIDS
João L. Fernandes, Leonor S. Castro, Ana F. Pereira, Ana M. Ferreira, Mara G. Freire, Pedro J. Carvalho, Augusto Q. Pedro

TOPIC 8

P8.1 - A DUAL BIOCATALYZED MICROBIAL ELECTROSYNTHESIS SYSTEM FOR CO_2 SEQUESTRATION AND SIMULTANEOUS PRODUCT VALORIZATION WITH BIOCATHODE AND BIOANODE
Khurram Tahir, Felipe Conzuelo, Inês Cardoso Pereira

P8.2 - FROM DOGS TO DOGS: FIRST NATIONWIDE SCREENING OF GIP AND AMR BACTERIA IN OFFICIAL MUNICIPAL KENNELS
Patrícia Lopes, Inês Natal, Jacinto Gomes, Mónica V. Cunha

P8.3 - EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF PLANT ORIGIN BYPRODUCTS EXTRACTS ON BACTERIA AND YEASTS
Matheus Guilherme Lemos, Ana Ferreira, Ana Rita Barata, Maria José Saavedra, Gonçalo Almeida

P8.4 - ALGARVE'S PGI CITRUS ESSENTIAL OILS: UNLOCKING THE POTENTIAL OF FOOD INDUSTRY BY-PRODUCTS
Cristina Rodrigues, Daniela Magalhães, Ana A. Vilas-Boas, Manuela Pintado

P8.5 - EXPLORING THE ANTIMICROBIAL AND DERMATOLOGICAL POTENTIAL OF *VACCINIUM MYRTILLUS L*. BIORESIDUES
Juliana Garcia, Daniela M. Correia, Sandrina Heleno, Lillian Barros, Maria José Saavedra, Maria José Alves

TOPIC 9

P9.1 - BUILDING A NETWORK OF BIOBANKS AND BIOLOGICAL RESOURCE CENTRES ACROSS PORTUGUESE-SPEAKING COUNTRIES: COLLABORATIVE EFFORTS FOR GLOBAL SUSTAINABILITY
Ana Paula Arez

MICRO BIOTEC 23

Plenary Lecture
Abstracts

PL1 - HYPES, HOPES, AND THE WAY FORWARD FOR MICROALGAL BIOTECHNOLOGY

Maria J Barbosa
Wageningen University, Netherlands

Prof. Dr. Maria Barbosa is Professor in Bioprocess Engineering and is Director of AlgaePARC at Wageningen University and Research Centre, in the Netherlands. She has been president of the Dutch Biotechnology Association (NBV). She holds a Ph.D. in Bioprocess Engineering obtained at Wageningen University. She has worked at ETH (Swiss Federal Institute of Technology), Switzerland, IBET, and at EMBO (European Molecular Biology Organisation), Germany.
She presently leads the group on microalgal biotechnology and coordinates several large research programs covering the entire microalgae production chain and applications in the food and feed sectors.
Her scientific interests are in microalgae strain improvement, process development and scale-up.

ABSTRACT

The urge for food security and sustainability has advanced the field of microalgae biotechnology. Microalgae are microorganisms able to grow using (sun)light, fertilizers, sugars, CO2, and seawater. They have high potential as a feedstock for food, feed, energy, and chemicals. Microalgae grow faster and have higher areal productivity than plant crops, without competing for agricultural land, and with 100% efficiency uptake of fertilizers. In comparison with bacterial, fungal, and yeast single-cell protein production, based on hydrogen or sugar, microalgae show higher land use efficiency. New insights are given into the potential of microalgae replacing soy protein, fish oil, and palm oil, and being used as cell factories in modern industrial biotechnology to produce designer feed, recombinant proteins, biopharmaceuticals, and vaccines.

PL2 - AFFINITY PROTEINS FOR BIOTECHNOLOGICAL AND MEDICAL PURPOSES

Sophia Hober
KTH – Stockholm, Sweden

Sophia Hober is professor in molecular biotechnology at KTH. During her career, she has worked on developing affinity molecules for in vitro and in vivo diagnostics, where recent findings have led to a very effective method for finding HER2-expressing cancers via a newly developed radioactive affinity molecule. This has been confirmed in clinical studies, where small metastases, not detectable with regular methods, could be visualized. The targeting module has also been optimized for therapeutic applications. Further, prof. Hober has focused on developing molecules for highly specific purification of monoclonal antibodies. Among other applications, this effort has led to a purification method that is used globally by the majority of the pharmaceutical companies that produce and purify therapeutic antibodies (MabSelect SuRe, sold by Cytiva). Currently, one of Hober's research focuses is the development of a novel protein domain that displays calcium-dependent binding. The ion-dependent mechanism is utilized to both develop mild affinity purification strategies and increase internalization into cells and thereby improving the therapeutic effect for cancer treatment.

ABSTRACT

Affinity proteins are crucial for life, for building structures, performing reactions and for signaling purposes. In life sciences and medicine, affinity proteins are used to generate knowledge, but also for protein purification, diagnostic and therapeutic purposes.
By using combinatorial protein engineering and protein library technologies, small protein scaffolds can be engineered and thereby equipped with various functions. The focus of our research has been on the development of protein-based systems for protein purification and detection as well as for therapeutic purposes. For this we are utilizing small, well characterized domains designed for use in various applications. By tailoring the domains differently, the specificity, biodistribution, half-life as well as the cell internalization can be manipulated, to suit the intended use more efficiently. Here, the development, evaluation and use of these affinity domains will be discussed.

PL3 - MAPPING FOOD MICROBIOMES TO IDENTIFY SOURCES AND PATTERNS OF SUCCESSION LINKED WITH QUALITY AND SAFETY TRAITS

Avelino Alvarez-Ordóñez
Department of Food Hygiene and Technology, Universidad de León, León, Spain
(*) e-mail: aalvo@unileon.es

Avelino Álvarez-Ordóñez is Associate Professor at the Department of Food Hygiene and Technology of University of León (Spain). He previously worked at University College Cork, Ireland (2010 – 2013) and Teagasc Food Research Center, Ireland (2013 – 2016). He is Principal Investigator of projects funded by the Spanish Ministry of Science and Innovation, the BBVA Foundation, Science Foundation Ireland and the European Commission. He has published more than 100 research articles in peer reviewed international journals and has participated as a speaker in numerous congresses, conferences and seminars, both nationally and internationally. He is member of the panel on biological hazards (BIOHAZ) of the European Food Safety Authority (EFSA) since July 2018. His research is focused on exploiting knowledge on food microbiomes for improving food quality and safety.

Keywords: microbiome; resistome; food safety; processing environments.

ABSTRACT

The composition and function of food microbiomes are of critical importance for food quality and safety, and this extends to the environmental microbiomes present in the facilities where food is produced, processed or stored. Despite the substantial efforts devoted in the past to study microbial succession patterns during processing and shelf life of a wide variety of foods, certain aspects relating to microbial interactions and their functional implications remain largely unknown. High throughput sequencing has transformed the way we study microbial ecology in complex ecosystems, such as those of some foods and food processing environments, as it provides access to difficult-to-culture microorganisms and facilitates the mechanistic understanding of community assembly in such a way that allows studying temporal dynamics of microbial communities at an unprecedented level of detail. It also allows going further, identifying the presence of genes responsible for potentially dangerous activities (e.g., antimicrobial resistance genes – the resistome) and reconstructing metagenome assembled genomes, which offers interesting opportunities for source tracking and hazard characterization activities. The plenary talk will summarise the research in this expanding field, including the activities undertaken by our research team in the last years aimed at deciphering the microbiome and resistome dynamics in various food production chains.

Acknowledgements:
Research in the lab of A. Alvarez-Ordóñez in the field of the plenary talk has been funded by the European Commission under the European Union´s Horizon 2020 research and innovation program (grant agreement No 818368), and the Ministry of Science and Innovation of the Spanish Government, under grant numbers AGL2016-78085 and PID2020-118813GB-I00.

PL4 - ENTERIC INFECTIONS DISRUPT GUT MICROBIOTA BIOFILMS: FROM MECHANISMS TO THERAPY

Andre G. Buret
University of Calgary, Biological Sciences, Host Parasite Interactions, Inflammation Research Network, 2500 University Dr. N.W., Calgary (AB) Canada
(*) e-mail: aburet@ucalgary.ca

The overall aim of Dr. Buret's research is to characterize microbial-host interactions in gastrointestinal tract and in the lungs, and their effects on health, inflammation, and chronic disease, in an attempt to develop novel therapeutic strategies. Dr. Buret has published over 200 articles and book chapters, and has been invited to deliver more than 330 presentations worldwide. Dr. Buret holds over 20 issued patents, and contributed to the creation of three biotech spin-off companies. He has mentored more than 100 undergraduate research projects, over 150 Ph.D and M.Sc. students, as well as 14 post-doctoral fellows. He serves on many National, and International grant and government committees, and acts as advisor, scientific committee member, and/or board member for several institutes and private companies.

Dr. Buret was inducted in the Order of the University of Calgary (2013) the institution's top honour, a Fellow of the Royal Society of Tropical Medicine and Hygiene (London UK, 2001), a Fellow of the Canadian Association of Gastroenterology (2020), and a Fellow of the American Gastroenterological Association (2009). His long list of awards include many Graduate and Undergraduate Teaching Excellence awards from 2 universities, the 2007 NSERC Synergy Award for collaborative research and innovation with industry from the Natural Sciences and Engineering Research Council of Canada, the 2007 Research Excellence Award from the Canadian Association of Gastroenterologists (for which he served as VP Research), the 2008 Robert A. Wardle Medal from the Canadian Society of Zoologists for excellence in Parasitology research, the 2019 Stoll-Stunkard award from the American Society of Parasitologists, and a Killam Professorship (2012). His research has been funded by more than 80 grants from numerous organizations, including the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. Dr. Buret has a current h index of 70, with over 17,000 citations to date (Google scholar).

ABSTRACT

Intestinal infections are common causes of diarrhea, and may lead to post-infectious complications, via mechanisms that remain obscure. A host may be co-infected with multiple diarrheal-disease causing pathogens, and the final disease outcome results from the complex interactions between the host and this polymicrobial cross-talk. Using models of human tissues and live rodent models reproducing co-infections with Escherichia-coli, Campylobacter jejuni, and the Protozoan parasite Giardia sp., our studies shed new light on why the production of symptoms may be so variable and hence point towards new therapeutic targets. Indeed, direct immunomodulatory effects of Giardia attenuate the pathophysiological responses induced by gastrointestinal pathogens that cause disease via severe inflammation. These effects can be further demonstrated in human tissues obtained from patients with Crohn's disease where administration of Giardia trophozoites significantly attenuate the contents of pro-inflammatory mediators. Co-infection with Giardia protects against intestinal disease induced by attaching-effacing enteropathogens. In addition, our findings demonstrate that high fat versus low fat diets may facilitate pathogenic factors in giardiasis, offering further support to the hypothesis that diet also plays a role in symptom variability. Finally, we have demonstrated how enteric infections can disrupt intestinal mucus barriers, fragment gut microbiota biofilms microbiota and transform commensals into invasive pathobionts that penetrate the intestinal barrier to cause intestinal inflammation. These alterations required the presence of environmental iron, and oral administration of novel therapeutics with anti-inflammatory and iron chelating properties were able to block these effects. In Giardia infections, the formation of microbiota pathobionts is triggered by a thermo-resistant and RNAse-sensitive cargo from extra-cellular vesicles. Together, these observations shed new light on the biology of polymicrobial infections in the gastrointestinal tract and underscore the multifactorial basis of the clinical variability in intestinal disorders. The findings point to new research directions in our attempts at the developing strategies to control enteric disease.

References:

1. Buret AG, Allain T. J. Exp. Med. 2023; 220(3): e20221743
2. Siddiq A et al. J. Extracell. Biol. 2023;2 (9), e109
3. Fekete E and Buret AG. Am. J. Physiology (Gastro. And Liver) 2023;324: G452-G465
4. Motta JP, et al. Nat. Rev. Gastro. Hepato. 2021;1-21
5. Allain T et al, Scientif. Rep. 2021;11(1):1-17
6. Motta JP, et al. Inflam. Bowel Dis. 2018;24 (7): 1493-1502

PL5 - BIOECONOMY AND OTHER RENEWABLES OPPORTUNITIES FOR PORTUGAL

Luuk van der Wielen
Department of Biotechnology, Delft University of Technology, The Netherlands and Bernal Institute, University of Limerick, Ireland

Prof.dr.ir. Luuk A.M. van der Wielen is the Director of the Bernal Institute at the University of Limerick, Ireland (https://bernalinstitute.com/our_people/luuk-van-der-wielen/), and Bernal Professor for Biosystems Engineering and Design since 2017. From 1998 until 2017 as Distinguished Professor in Biobased Economy (nowadays part time) at TU Delft's Dept. of Biotechnology (https://www.tudelft.nl/tnw/over-faculteit/afdelingen/biotechnology/research-sections/bioprocess-engineering/luuk-van-der-wielen), he headed the Bioprocess Engineering Section. His research interests include thermodynamics for bioprocesses, bioseparation/-conversion technologies, multifunctional bioreactors, miniaturized ('on-chip'), high-throughput technology for rapid process development, analysis and development of (bio)renewables production systems, and their societal impacts. The last Google Scholar counts over 350 publications/patents as of July 2023 (7669 citations; H-index 44), but more importantly, almost 50 PhD, 75 PDEng and over 100 MSc students have graduated under his supervision.

Since 2017 Luuk van der Wielen is also the Chair of BE-Basic Foundation Board, and was 2004-'17 director of BE-BASIC (www.be-basic.org), the globally operating private-public research organization for Biobased Sustainable Industrial Chemistry & Energy, which is based in The Netherlands with hubs in South East Asia and Brazil. He is/was member of editorial and advisory boards of several leading international scientific journals, and chaired several scientific conferences (a.o. ESBES4, BPP2005, RRB4, ECOBIO2016/-18, Braz Bioenergy S&T Conf 2017).

ABSTRACT

During the last decade, European countries have embraced a massive decarbonisation ambition, by starting to replace fossil feedstocks (coal, natural gas, crude oil) for energy use by rapidly expanding wind and solar sectors. For many domestic, industrial and transport uses, this is a strategy based on progressive electrification, replacing fossil power by renewable. For a number of societal and industrial sectors, carbon atoms are an essential part of product and product performance such as for food & feed, plastics, and liquid fuels for long haul and air transport. This poses a new question of sourcing carbon at industrial scale.

A number of studies focus on conventional, non-combustion carbon (point) sources such as cement and steel industries. These however are non-biogenic nor circular sources, and most likely not eligible in the future. EU in its various energy related policies (REFUEL EU, REPOWER EU, RED II) clearly positions itself as mandating only biogenic and circular carbon which is only a fraction of current carbon flows. Fast-forwarding scenarios to significantly decarbonised futures (2050), it is easily understood that biogenic and circular carbon will become rare commodities. Negative carbon pricing of this moment (- € 50..100 per tonne) will logically transform into positive carbon prices when recovered from biomass and other biogenic sources and even higher from Direct Air Capture at + €500..1000 per tonne with current technologies. This will seriously affect feasibility of business cases.

It should also be noted that the sources of the European biomass are significantly heterogeneous which complicates further industrial use – therefore a priority is to develop platforms that are tradable and give access to larger markets and uses. These platforms could be industrial gas feedstocks as biomethane and biogenic CO2, and / or liquid platforms (lower alcohols as methanol or ethanol). Technology for this (digestion, gas purification, fermentation technology or chemical reduction) is available at high TRL levels enabling industrialisation with modest technology risk for investors provided the infrastructural and planning hurdles are taken.

'Biogenic' will become an important qualifier, and therefore critically important is to develop and implement a certification system to guarantees industrial and other users verifiable sustainable origin of the biomass and biogenic carbon for processed products. This system doesnot exist in any of the EU countries (or beyond).

In this contribution, we will present a number of technological and economic scenarios for purposing and optimising biogenic carbon sources across the key sectors of food (especially alternative proteins and cultured meat), circular materials as plastics, long haul fuels such as Sustainable Aviation Fuel (SAF) and even advanced biopharmaceutical therapeutical products such as using a (bio)process systems approach for relevant European cases.

Recognising the longterm impact of prof Joaquim Cabral on Portuguese and European bioprocess engineering landscape, we will especially highlight how his ground breaking work in cell and gene bioengineering underlying regenerative medicine may inspire circular biobased economic development in Portugal, a country blessed with abundant solar and wind energy as well as biogenic carbon potential.

MICROBIOTEC 23

Keynote Lecture
Abstracts

KL1 - CYANOBACTERIA AND THEIR METABOLITES: IMPLICATIONS FOR HUMAN HEALTH AND THE ENVIRONMENT

Vitor Vasconcelos [1,2]
[1] *CIIMAR – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal*
[2] *FCUP - Faculty of Sciences, University of Porto, Porto, Portugal*

[(*)] *email:* ***vmvascon@fc.up.pt***

Keywords: Cyanobacteria; metabolites; toxins; biotechnology; human health, environment.

ABSTRACT

Cyanobacteria are very diverse organisms in terms of morphology, habitat and ecology and are well known for the diversity of secondary metabolites that they produce either when living isolated or in symbiosis. The benefits and risks associated with the use of cyanobacteria and microalgae as food and food supplements will be highlighted. Among those metabolites produced by cyanobacteria, toxins are extensively studied due to the harmful effects they cause on the ecosystems and on human health. Cyanotoxins can have neurotoxic, hepatotoxic, cytotoxic and dermatotoxic properties, being exposure to humans via drinking water, dermal contact during recreation or via food contaminated with the toxins. Apart from producing toxins, and due to their ancestral origin, ecological and biochemical diversity, cyanobacteria are a prolific source of compounds with potential biotechnological applications, namely in the pharmacological field. A wide range of secondary metabolites exhibiting pharmaceutical properties such as antibacterial, antiviral, antifungal, anti-inflammatory, antipsoriasis and anticancer have been described. The potential of cyanobacteria as source of new bioactive compounds is enormous, with the advantage of being applicable in many different areas of biotechnology, with many industrial applications with benefits for human health. The sustainable use of culture collections as the LEGE_CC hosted by CIIMAR will be highlighted with examples regarding bioassay guided approaches as well as a untargeted metabolomics.

KL2 - AI + BIOLOGY FOR SUSTAINABLE INGREDIENTES

Simão Soares
SilicoLife Lda., Braga, Portugal

(*) email: ***simao.soares@p-bio.org***

ABSTRACT

SilicoLife (www.silicolife.com), founded in 2010, merges computational biology with synthetic biology to optimize microorganisms and develop novel metabolic pathways, facilitating the sustainable production of valuable molecules like food ingredients, chemical intermediates, and natural products. Over the years, the company has transitioned into a full-stack synthetic biology entity capable of managing the entire R&D continuum, from molecule selection to proof-of-concept strains and validated processes. Utilizing a combination of artificial intelligence and metabolic engineering, SilicoLife reduces the time and cost associated with developing new, efficient production processes for these molecules.

The company's technology suite includes tools for microorganism modeling, the engineering of optimized cellular factories, novel metabolic pathway identification and design, and the establishment of new strain designs alongside the discovery of biosynthetic routes. This suite is underpinned by robust systems in synthetic biology, artificial intelligence, and software engineering.

SilicoLife employs computational models and proprietary algorithms to identify the most efficient pathways from raw material to end product, thereby enhancing the strain design process. These methods facilitate the exploration of non-obvious pathway modifications, which results in cost reductions across R&D programs and a decrease in the need for laboratory experimentation.

In an era where programmable cell biology is becoming increasingly important to meet consumer demands for more sustainable and scalable production methods, SilicoLife boasts an extensive track record in the development and application of computational techniques to decipher and engineer different biological systems.

Chemical synthesis of complex molecules like natural products is often difficult and may be subject to unclear mechanisms, frequently producing undesired by-products or racemic mixtures, while the complexity of some compounds may render them virtually unattainable. In contrast, nature offers a plethora of products, albeit in low concentrations, making traditional extraction costly and environmentally demanding. Microbial production, as an alternative, leverages nature's resourcefulness to produce these molecules sustainably and at scale. To align with this market trend, it is crucial to devise technologies that enable efficient biological processes that can be scaled up in an environmentally and socially responsible manner. SilicoLife has made significant strides in this direction by identifying production pathways and building cell factories for the efficient production of target compounds.

In December 2022, SilicoLife announced an investment to develop its own portfolio of production technologies for the dietary supplements industry through precision fermentation. This move underscores the company's commitment to creating a platform for the sustainable production of pure ingredients, thus revolutionizing ingredient sourcing, reducing carbon footprints, eliminating seasonal dependence, and guaranteeing consistent, high-quality production.

KL3 - PROBING BACTERIAL INTERACTIONS IN POLYMICROBIAL BIOFILMS TO BETTER UNDERSTAND BACTERIAL VAGINOSIS

Nuno Cerca [1,2]

[1] *Centre of Biological Engineering (CEB), Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
[2] *LABBELS –Associate Laboratory, Braga, Guimarães, Portugal;*

[(*)] *email: nunocerca@ceb.uminho.ptl*

Keywords: RNA-sequencing; biofilms; antibiotic tolerance; bacterial vaginosis recurrence.

ABSTRACT

Despite Bacterial vaginosis (BV) significant impact on women worldwide, we still do not know BV etiology. For long, two main theories attempted to describe BV: the single-agent and the polymicrobial theory. Recent developments have shed some light in BV etiology. The association of the biofilm phenotype to the clinically relevant clue cells, and the clarification of genomic diversity of Gardnerella that led to the description of 3 novel Gardnerella species on a total of 13 possible genomic species (most yet uncharacterized).

Despite more than 300 species have been found associated with BV, it is becoming evident that many are not directly involved in BV development. The existence of more Gardnerella species also casts a new light on the vaginal colonization on healthy women by non-virulent Gardnerella spp.

In this talk, I will address how specific bacterial interactions can contribute to BV development, and how these interactions can contribute to BV treatment failure.

Acknowledgements:
This work was partially funded by the Portuguese Foundation for Science and Technology (FCT), with the strategic funding of the unit (UIDB/04469/2020) and the project EXPL/SAU-INF/1000/2021. It was also partially funded by the National Institute of Allergy and Infectious Diseases (R01AI146065-01A1).

KL4 - MICROBIAL TECHNOLOGY FOR SUSTAINABLE DEVELOPMENT: EXPLORING BACTERIAL STRATEGIES FOR METAL INTERACTION

Paula V. Morais
University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Coimbra, Portugal

(*) email: ***pvmorais@uc.pt***

Keywords: critical metals; bio microtechnologies; biosensors; bio accumulators.

Abstract
Microorganisms are abundant in nature and their ability to transform a variety of substrates into nutrients or other valuable products enables them to play a key role in the mission for a sustainable environment. In the last decades, with the establishment of sustainable development goals by the United Nations, the challenges for sustainability were seen from a new perspective. This new perspective points to a society and an economy based on renewable processes and based on, or in the image of, biological processes. Within this transition, microorganisms are playing a significant and global role as technology drivers since they harbour the blueprints to sustainable biotechnology in their genomes.

Raw materials are essential for a more sustainable society based on green technologies to exist. In Europe, access to primary raw materials is limited, making it essential to develop solutions for the circular use of materials. To apply microorganisms to production processes, it is necessary to understand their almost infinite metabolic diversity. Microorganisms support a diversity of genes and operons, many related to metal homeostasis in the cell and toxicity management.

Bacteria can do the biological recovery of metals from the environment. Metal(oid) solubilisation using microorganisms was proven to be an applicable technology for processing primary, in particular low-grade ores. Biosorption, biomineralisation and bioaccumulation are biological strategies to recover metal(oid)s selectively. Designing new bio-microtechnologies for metal circularization by making use of these microbial abilities, involves knowledge of the physiological and molecular mechanisms present in microorganisms. In the last years, our work was focused on the study of the bacterial mechanisms to interact with Tungsten, Tellurium, Indium and Yttrium. Examples of genetically modified and non-modified bacteria and their metabolic products for the bioextraction from mine residues and biomonitoring of different European critical metals will be presented.

Acknowledgements:
The studies were supported by project 821096 – Biorecovery – H2020-SC5-2018- 2019-2020 and by the project ERA-MIN-2019_67- Reviving. The research was also sponsored by national funds through FCT under the projects UIDB/00285/2020 and LA/P/0112/2020.

KL5 - *VIBRIO NATRIEGENS* AS A NEW HOST FOR PLASMID DNA PRODUCTION

Duarte Miguel Prazeres [1,2(*)], ***Guilherme F. Mourato*** [1,3], ***Ana Rita Silva-Santos*** [1,2], ***Jorge João*** [1,2]

[1] *iBB – Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.*

[2] *Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal*

[3] *Faculdade de Ciências, Universidade de Lisboa*

(*) *email:* ***miguelprazeres@tecnico.ulisboa.pt***

Keywords: biomanufacturing; gene and cell therapy; nucleic acids; plasmid DNA; *Vibrio natriegens.*

ABSTRACT

The biopharmaceutical relevance of producing plasmids at large scale has increased steadily due to the development of direct and indirect applications. Plasmids are used as vectors to deliver genes in gene/cell therapies and DNA vaccination. Further, they play a key role as materials in the manufacturing of viral vectors, mRNA vaccines, and in the *ex vivo* modification of cells for therapeutic purposes. Unlike recombinant proteins, large scale manufacturing of plasmid DNA (pDNA) depends exclusively on one platform host – *Escherichia coli*. Modified strains of this host are available that can be grown to densities of hundreds of grams per liter and coaxed into producing up to 1-2 g pDNA per liter of culture. While this performance looks unbeatable, the high demand for pDNA vectors justifies a search for bacterial chassis that might be more favorable for manufacturing. One curious bacterium that could fit the role of a new pDNA producer host is the Gram-negative *Vibrio natriegens*. This marine bacterium has the fastest growth rate of any known organism and an admirable metabolic proficiency. Further, it can be used safely since it meets biosafety level 1 standards. Whilst most studies so far focused on the use of *V. natriegens* in cloning and protein expression, little attention has been devoted to its use for pDNA replication. The aim of the work reported here was to evaluate if the remarkable characteristics of *V. natriegens* can be tapped to develop high productivity pDNA processes capable of exceeding the performance of *E. coli*.

Acknowledgements:
This research was funded by FCT-Fundação para a Ciência e a Tecnologia, grant numbers UIDB/BIO/04565/2020, UIDP/BIO/04565/2020 and LA/P/0140/2020.

KL6 - ADHESINS IN OPPORTUNISTIC RESPIRATORY PATHOGENS: FROM SEEING TO DETERMINING THEIR BIOLOGICAL FUNCTION

Arsénio M. Fialho [1,2]

[1] *Biological Sciences Research Group, Institute of Bioengineering and Biosciences (iBB), Instituto Superior Técnico, Lisbon, Portugal*
[2] *Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal*

[(*)] *email:* ***afialho@tecnico.ulisboa.pt***

Keywords: *Burkholderia cenocepacia*, respiratory infections, adhesins, Trimeric autotransporter adhesins

ABSTRACT

The initial contact between pathogenic bacteria and the human airway epithelial cells is crucial for the success of an infection. Such bindings allow bacteria to come into tight contacts with target cells, prevent their mechanical clearing and trigger distinct signalling pathways in the host. The machinery used by bacteria is complex and several surface molecules like adhesins (fimbrial and afimbrial), flagella, outer membrane proteins or lipoproteins have been reported to mediate specific and nonspecific adhesion to host cells and extracellular matrix (ECM). Typically, they operate synergistically through binding to host protein receptors or their carbohydrate ligands or ECM components. In our laboratory at iBB (BSRG/Bioadhesion Lab), the Gram-negative contact-dependent bacterium *Burkholderia cenocepacia* has long been used as a model organism for studying the phenomena of bacterial adhesion to host cells and its significance in pathogenesis. To do that, we conducted a multidisciplinary approach including bacterial genetics, molecular and cell biology, biochemistry, and biophysics. This presentation will focus on the current knowledge about the adhesion molecules (adhesiome) described in this opportunistic respiratory pathogen. A particular emphasis will be given to a sub-class of adhesins named Trimeric Autotransporter Adhesins (TAA) defining their functions in the context of bacteria/host cell interactions and envisaging the development of new anti-adhesion therapies.

Acknowledgements: Funding received by the iBB from FCT (UID/BIO/04565/2020) and by Programa Operacional Regional de Lisboa 2020 (Project N. 007317) is acknowledged.

KL7 - DISCLOSING GUT BACTERIAL SIGNATURES IN ELDERLY FROM ALGARVE

M. Leonor Faleiro[1,2,3(*)]

[1] *Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, Faro, Portugal;*
[2] *Algarve Biomedical Center—Research Institute, Faro, Portugal;*
[3] *Champalimaud Research Program, Champalimaud Centre for the Unknown, Lisbon, Portugal*

[(*)] *email:* ***mfaleiro@ualg.pt***

Keywords: gut, elderly, *bacteriome, diet, functionality, medication*

ABSTRACT

Across the lifespan the gut microbiota undergoes changes that has the potential to disturb the health of older individuals compromising their health span and independent living. Aging population is increasing worldwide with a significant burden of age-related diseases. Is expected that by 2050 the elderly proportion of the global population will increase about 7%. The healthcare society is committed to elaborate strategies that can promote the health span by reducing frailty and comorbidities. With this purpose in mind, we are studying the gut bacteriome signatures in elderly from Algarve taking in consideration their location of living, lifestyle, diet, diseases, medication, and functionality. The bacterial patterns at level of phylum or species mainly diverge between the location of living of the older individuals. The bacterial signatures of older individuals that live in urban areas diverge from those living in rural areas. The bacterial signatures of older individuals from rural locations seem to be more related with neurological disorders, in contrast with urban individuals for which cardiovascular, digestive diseases and motion disorders are more evident. The type of medicine also clusters the older individuals differently that resembles the treatment for the main diseases. Diet and functionality map the older individuals similarly. The current approach allows us to establish the most influential factors that are shaping the gut bacteriome of the elderly from Algarve and explore interventions to promote longevity.

Acknowledgements:
The author is grateful to the financial support through the Project Algarve Regional Operational Programme (CRESC – Algarve), Reference: ALG-01-0145-FEDER-072586.

KL8 - IONIC LIQUIDS AS ADDITIVES FOR STABILIZATION AND FORMULATION OF PROTEIN- BASED PRODUCTS

Nathalia V. Veríssimo [1], Qi Han [2], Valéria C. Santos-Ebinuma [1], Tamar L. Greaves [2], Jorge F.B. Pereira [3(*)]

[1] Department of Bioprocesses Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil
[2] STEM College, RMIT University, Melbourne, Australia
[3] University of Coimbra, CIEPQPF, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, Portugal

(*) email: jfbpereira@eq.uc.pt

Keywords: Ionic liquids; Proteins; stabilization; formulation; additives

ABSTRACT

The low stability and high production costs of proteins is the main bottleneck limiting access to life- saving protein-based bioproducts in countries and communities of low income. To improve the instability of proteins, ionic liquids (ILs) have been employed as stabilizers of proteins. However, several variables can impact the effect of ILs on proteins, including the nature, biocompatibility, and concentration of ILs, environmental conditions such as temperature and pH, and the intrinsic properties of proteins. Hence, this work compiled and analyzed the effect of ILs on proteins considering the protein properties, ILs classes, type of protein stability, and IL solutions concentrations to find trends that indicate the impact of each variable in protein stability. Considering the top four major IL families in this field, imidazolium and ammonium-based ILs are the predominant classes for protein stabilization studies. However, the most compatible classes with proteins are ammonium and cholinium ILs, followed by imidazolium and pyridinium/pyrrolidinium ILs. Moreover, ILs have a great aptitude to prevent protein aggregation (more than half of samples decreased aggregation) and activity (more than 40%), including some IL families that are also adequate for the preservation of structural and thermal stability of proteins (one-third of samples). Finally, we also experimentally evaluated the effect of different concentrations and IL classes on the short and long-term stability of the Green Fluorescent Protein (GFP). For the GFP, imidazolium- and cholinium-based ILs increased GFP short and long-term stabilization at room temperature by decreasing its aggregation. This work provides a clear overview about which ILs families can be used as protein stabilizers, with the potential to help expand the applications of unstable proteins and increase access to biological products.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. J.F.B. Pereira acknowledge FCT for funding the project DRI/India/0044/2020. N. V. Veríssimo thanks the support from PROPG/PROPe call 05/2022. V. C. Santos-Ebinuma thanks the financial support of FAPESP (2021/06686-8).

KL9 - ENZYMATIC HYDROLYSIS: A TOOL TO PRODUCE FUNCTIONAL AND SUSTAINABLE FOOD INGREDIENTS

Manuela Pintado

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Portugal

(*) email: ***mpintado@ucp.pt***

Keywords: agrifood byproducts, protein hydrolysates, bioactivity, sustainable food

ABSTRACT

Enzymatic hydrolysis and fermentation are the most widely used methods for obtaining protein hydrolysates, based on the advantages that these processing techniques show. Many of the physiological and functional properties of proteins are attributed to biologically active peptides normally encoded in the parental protein sequence. Recent research based on either in vitro or in vivo studies has shown that peptides from different sources, such as dairy products, plants, animals, and seafood have a wide range of bioactivities. The food processing sector generates a large amount of waste annually and this problem could be turned into an advantage, if potentially they are considered by-products to be valorized in several added-value ingredients with Zero Waste. Enzymatic hydrolysis alone or combined with other technologies is a cost-efficient technology that allows natural production of added-value compounds, namely those with potential biological and functional properties. Most of the bioactive peptides exhibit specific bioactivity, but some peptides have been found to exhibit multifunctional properties. Several examples of enzymatic conversion of proteins from by-products into new added value ingredients/products with application in food and feed have been studied. Nevertheless, some bioactive molecules present inherent drawbacks regarding oral administration, due to the conditions prevailing throughout gastrointestinal tract.

This presentation comprises a review of recent studies to demonstrate the potential of enzymatic hydrolysis for agrifood byproducts and low value resources valorization encompassing research cases studies developed by our research group (Bioactives and Bioproducts Research Laboratory) on production of biopeptides of plant, animal and fermentation byproducts, but also low value resources (insect and algae). Hydrolysis using different types of enzymes and conditions for obtaining biopeptides with technological, nutritional value and health properties, assuring functional and sustainable ingredients, will be presented. Impact of gastrointestinal tract and oral delivery systems of biopeptides and examples of the potential application of these new ingredients will also be presented.

KL10 - NATURE INSPIRED SOLUTIONS IN HEALTH BIOTECHNOLOGY

Cecília Roque [1,2]

[1] *UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, School of Science and Technology, NOVA University of Lisbon, Portugal.*
[2] *i4HB – Institute for Health and Bioeconomy, Chemistry Department, School of Science and Technology, NOVA University of Lisbon, Portugal.*

(*) *email:* cecilia.roque@fct.unl.pt

Keywords: biomimetics; bioseparation; biosensing

ABSTRACT

Nature is a source of inspiration for science and technology, having generated several solutions that foster health biotechnology, namely through new and personalized tools in diagnostics and therapeutics.

Molecular recognition is ubiquitous in Nature. In my group, we take inspiration from dynamic molecular recognition events in Nature to engineer biological and chemical tools that give rise to advanced functional materials. We explore such materials in bioseparation and biosensing.

The first topic will address nature-inspired ligand design in bioseparation. Rationally designed chemical combinatorial libraries support the development of robust peptidomimetics that can be easily adapted to several targets and to chromatographic and non-chromatographic matrices.

The second topic will focus on the field of artificial olfaction, namely on self-assembled materials that act as molecular recognition systems for analytes in the gas phase. Together with in-house developed electronic noses and machine learning algorithms, such materials are tuned to mimic the sense of smell and used for biosensing purposes.

Acknowledgements:
This work has received funding from Fundação para a Ciência e Tecnologia (Portugal) for projects PTDC/BII-BIO/28878/2017, PTDC/CTM-CTM/3389/2021, Research Unit on Applied Molecular Biosciences – UCIBIO (UIDP/04378/2020 and UIDB/04378/2020) and Associate Laboratory Institute for Health and Bioeconomy – i4HB (LA/P/0140/2020), from INCD funded by FCT and FEDER under the project 01/SAICT/2016 nº 02215, and from the European Research Council (ERC) under the EU Horizon 2020 research and innovation programme (SCENT-ERC-2014-STG-639123, 2015−2022 and grant agreement No. 101069405 — ENSURE — ERC-2022-POC1).

KL11 - NATURAL PRESERVATIVES FOR THE FOOD AND BEVERAGE INDUSTRIES

Sandrina A. Heleno[1,2], Márcio Carocho[1,2]

[1] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[2] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

(*) email: ***sheleno@ipb.pt***

Keywords: Natural compounds; antimicrobials; food industry; preservatives

ABSTRACT

Bio-based additives can be obtained in Nature, given its richness and diversity in highly bioactive molecules.

Distinct classes of compounds such as phenolics, organic acids, tocopherols, sterols and others, reported as strong bioactive agents, exhibiting interesting antioxidant and antimicrobial properties, can be found in natural matrices, two essential attributes to be considered possible natural preservative agents, besides having low or no toxic effects. Considering the global agendas, and in line with the strategy to promote the circular economy, these molecules can also be extracted from raw material considered as biowaste from the food industry, being possible to obtain highly valuable compounds and re-include them in the value chain.

Several natural matrices namely plants, fruits, mushrooms and bioresidues have already been studied as sources of preservative compounds, exhibiting strong antioxidant and antimicrobial properties using green solvents, sustainable processes, and application of stabilization techniques, given the instability of these compounds.

The strong antioxidant and antimicrobial properties of these extracts is attributed to the presence of phenolic compounds (catechin, quercetin and luteolin derivatives), phenolic acids (rosmarinic, chicoric, lithospermic, caffeic, caffeoylquinic acids), and hydrolysable tannins (trigalloyl-HHDP-glucoside); and organic acids, namely citric acid, that were subjected to extraction optimization, bioactive evaluation for further incorporation in foods and beverages to act as natural preservatives. The main achievements were the development of innovative products with extended shelf life, decreasing/substituting the use of artificial additives as it is the case of yogurts, cheese, muffins and nutraceuticals, highlighting the ChestWine and SpraySafe formulations. ChestWine is a preservative natural extract with outstanding antioxidant ad antimicrobial activities for application in wines; SpraySafe in an edible biofilm applied in foods, to substitute the use of plastic wrap. These projects have been important to contribute to the development of natural alternatives to artificial preservatives and can also be applied in other industrial sectors.

Acknowledgements:
The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). M. Carocho thanks FCT for his individual employment program–contract (CEEC-IND/00831/2018), S.A. Heleno also thanks to the national funding by FCT through the institutional scientific employment program–contract for their contracts.

KL12 - MICROBIAL CULTURE COLLECTIONS: STRAINS, SERVICES, PROJECTS AND CONSORTIA TO UNDERPIN THE MICROBIOTECH INNOVATION

Nelson Lima [1,2(*)]

[1] *CEB - Centre of Biological Engineering, Micoteca da Universidade do Minho (MUM), University of Minho, Braga, Portugal*
[2] *LABBELS - Associate Laboratory, University of Minho (Braga/Guimarães), Portugal*

[(*)] *email:* ***nelson@ie.uminho.pt***

Keywords: Culture Collections; mBRC, MIRRI-ERIC; MIRRI-PT/Pt-mBRCN.

ABSTRACT

Over the years, microbial culture collections (CCs) have been providing services to the scientific community, acting as reservoirs and providers of microorganisms, including their living cells, genomes, and information, being key players in the development of new and more sustainable products, compounds, and practices. When all these activities are performed under a legal framework and within a quality management system put in place the more advanced concept of microBiolological Resource Centre (mBRC), as defined by the Organisation for Economic Cooperation and Development (OECD), is applied. The OECD recognized the mBRC as “a key component of the scientific and technological infrastructure of the life sciences and biotechnology”. Taking this into consideration, at the European level the mBRC started to work together to establish the European Resource Research Infrastructure (MIRRI, www.mirri.org). After a preparatory phase and the recognition as a “Landmark” by the European Commission on the last ESFRI Roadmap, MIRRI became a non-for-profit international organization with its headquarters at the University of Minho. This Research Infrastructure has evolved as an ERIC legal entity and is now in the operational phase and involved, so far, in 13 EU projects, with a 100% success rate. The organizational structure of MIRRI-ERIC will be presented, and the ongoing projects as well as the services, including the Transnational Access, will be presented. Finally, the Portuguese node MIRRI-PT/Pt-mBRCN, coordinated by Micoteca da Universidade do Minho (MUM), and how it is implementing several cutting-edge technologies for the benefit of microbiology and biotechnology and to respond to user communities' demands will be also presented.

Acknowledgements:
This study is partially supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems (LA/P/0029/2020); and by “MIRRI-PT (Polo Norte)” project (PINFRA04/84445/2020) funded by the European Regional Development Fund under Norte2020-Programa Operacional Regional do Norte.

KL13 - UNLEASHING THE POWER AGAINST SUPERBUGS: UNRAVELING GLOBAL CHALLENGES AND INNOVATIONS IN ANTIMICROBIAL RESISTANCE

Luísa Vieira Peixe
UCIBIO – Applied Molecular Biosciences Unit, Faculty of Pharmacy, University of Porto, Porto, Portugal

ABSTRACT

Antimicrobial resistance (AMR) poses a staggering global health threat, with estimated substantial increase of deaths for the next years if left unchecked. AMR now affects every region, with overuse and misuse of antimicrobials in human and animal health, and food production systems accelerating resistance. Despite infection prevention efforts, resistant superbugs easily spread across borders and environments while antibiotic development stagnates. Increased AMR research has expanded knowledge, although gaps persist in surveillance and transmission patterns. Combating AMR requires a coordinated global response under a One Health approach, as recognized by different organizations or regulatory bodies pressing to the action on this respect. Exciting new directions bring hope, from antimicrobial alternatives to rapid diagnostics improving antibiotic stewardship, vaccine pipelines targeting resistant pathogens, behavioral interventions promoting optimal antimicrobial prescribing, and big data innovations enhancing surveillance. Stewardship and infection control interventions also show promise. Realizing the full potential of these innovations necessitates sustained multisectoral investments, open access data sharing, and global partnerships. While the road ahead is long, strategic collaborations and a relentless focus on research priorities such as improved diagnostics and transmission interruption will help unlock innovations to overcome the threat of superbugs. Our collective efforts can curb the tide of AMR worldwide and safeguard antimicrobials for future generations. But the window for action is closing fast, requiring immediate and coordinated steps globally.

KL14 - NEW GENERATION OF DNA VACCINES AGAINST CANCERS INDUCED BY HPV INFECTION

Ângela Sousa

CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

(*) email: angela@fcsaude.ubi.pt

Keywords: Biotechnological platform; cervical cancer; DNA vaccines; minicircle DNA.

ABSTRACT

Cervical cancer (CC) is the fourth most frequently diagnosed cancer in women worldwide and the first one in low-income countries, with 99% of cases related to Human Papillomavirus (HPV) infection and becoming a big public health problem. Nucleic acid vaccines proved to be a revolutionary technology to give an efficient, safe, and rapid response against pandemics, like the coronavirus disease 19. DNA vaccines are more stable and can encode several antigens than messenger RNA, present low-cost manufacturing, thermostability and easy distribution than conventional vaccines. In addition, they induce strong preventive and therapeutic immune responses after presentation of antigen genetic information to antigen-presenting cells (APCs), being considered a potential solution for CC treatment. The innovative minicircle DNA (mcDNA) vector overcome some limitations of plasmid DNA (pDNA) due to the cutting-edge *in vivo* recombination process, which excises prokaryotic sequences when the pDNA amplification is completed.

This work proposes the implementation of a biotechnological platform to obtain the first immunotherapeutic mcDNA vaccine, encoding mutant HPV16 E6/E7 antigens (E6/E7mut). After the parental plasmid (PP-E6/E7mut) construction and *Escherichia coli* ZYCY10P3S2T transformation, its recombination into mcDNA-E6/E7mut was optimized by design of experiments, and the mcDNA biosynthesis was scaled-up in bioreactor. After alkaline lysis, crude lysate was directly treated with a new SARTORIUS product, to eliminate precipitated impurities, centrifugation steps and the use of organic solvents/high salt concentrations. The mcDNA was purified in the cadaverine-monolith, according to requirements of regulatory agencies. Finally, ternary delivery systems based on mcDNA/PEI/R8-mannose were developed and characterized to efficiently complex, protect and transfer mcDNA into APCs and adequately express E6/E7mut antigens. This work offers a fast, simple, universal, economic, and attractive technology to the biopharmaceutical industry.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2021.

KL15 - NUCLEIC ACID BIOSENSING PLATFORM FOR SARS-COV-2 DETECTION

Paula Martins-Lopes [1,2(*)]***, Alexandra Lino*** [1,2]***, Helena Gonçalves***[3,4]
[1] *DNA & RNA Sensing Lab, University of Trás-os-Montes and Alto Douro, School of Life Science and Environment, Blocos Laboratoriais bdg, 5000-801 Vila Real, Portugal.*
[2] *BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisbon, Portugal.*
[3] *Biosensor NTech – Nanotechnology Services, Lda, Avenida da Liberdade, 249, 1º Andar 1250-143 Lisboa, Portugal.*
[4] *REQUIMTE, Instituto Superior de Engenharia do Porto, Porto, Portugal.*

(*) *email:* ***plopes@utad.pt***

Keywords: Nanoparticles; SARS-CoV-2; Nucleic Acids; Certification.

ABSTRACT

The recent COVID-19 pandemic has led to the need for the development of quick and reliable diagnostic tools that can be widely available for general use. The resource to different biosensing solutions were largely explored, with rapid antigen testing being extensively used to limit the virus dissemination [1]. Nevertheless, this type of testing was not suitable for an early detection that can only be achieved through the use of the SARS-CoV-2 direct detection, achieved through point-of-care technologies such as RT-qPCR. On the other hand, the high mutation rate of the virus constitutes an added problem of the detection success required in the pandemic control [2].

In order to overcome some of the problems, we aimed to develop a biosensor nucleic acid base platform that could be applied directly to saliva samples. Based on the *ORF1ab*, *N* and *E* genes, widely used in RT-qPCR, nanoparticles were functionalized with specific probes and were used to test the presence of SARS-CoV-2 in direct oropharyngeal swab samples. The analyses were all validated using the RT-qPCR. The developed biosensor proved to be more sensitive than the recommended RT-qPCR and was submitted to IVD European Union certification. This technology was patented and licensed to the industry.

The versatility of the biosensing platform allows the possibility to apply to other human diagnostic applications, as well as to other areas.

References:

[1] Lino A.; Cardoso M.A.; Gonçalves H.M.R.; Martins-Lopes P., Chemosensors, 2022, 10(6), 221, https://doi.org/10.3390/chemosensors10060221
[2] Lino A.; Cardoso M.A.; Martins-Lopes P.; Gonçalves H.M.R., Rev Med Virol., 2022 Jul;32(4):e2358, https://doi: 10.1002/rmv.2358

Acknowledgements:
We would like to acknowledge everyone that where directly and indirectly involved in this project. A special acknowledgment to the institutions that participated in the development of this biosensor, namely, Universidade de Trás-os-Montes e Alto Douro, Rede de Química e Tecnologia (REQUIMTE) and Instituto Superior de Engenharia do Porto. Further we would like to acknowledge the COVID19 Testing Center of UTAD for all the support given with the validation of this biosensor platform. **Funding:** Cdots Biosensing COVID19, nº 041_596518523, financiado pelo RESEARCH COVID-19 da Fundação para a Ciência e Tecnologia.

MICROBIOTEC 23

Oral Presentation
Abstracts

OP1.1 - MUCUS OF THE PHOTOSYNTHETIC SEA SLUG *ELYSIA CRISPATA*: PROTEOME AND ANTI-BACTERIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGINOSA*

Diana Lopes [1], Susana S. Aveiro [2], Eva Cunha [3,4], Sónia Cruz [5], Manuela Oliveira [3,4,6], Pedro Domingues [7], Paulo Cartaxana [5]

[1] ECOMARE – Laboratory for Innovation and Sustainability of Marine Biological Resources, CESAM – Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Aveiro, Portugal

[2] GreenCoLab – Associação Oceano Verde, University of Algarve, Campus de Gambelas, Faro, Portugal

[3] CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

[4] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

[5] ECOMARE, CESAM, Department of Biology, University of Aveiro, Aveiro, Portugal

[6] cE3c—Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Science Faculty, University of Lisbon, Lisbon, Portugal

[7] Mass Spectrometry Centre, LAQV-REQUIMTE- Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology, Department of Chemistry, University of Aveiro, Aveiro, Portugal

(*) e-mail: ***lopes.diana@ua.pt***

ABSTRACT

Elysia crispata (Sacoglossa, Gastropoda) is a tropical sea slug known for its ability to incorporate functional chloroplasts from various macroalgae, a phenomenon termed kleptoplasty[1]. This sea slug, amenable to laboratory cultivation, produces mucus, a viscous secretion that serves diverse purposes including lubrication, protection, and facilitating locomotion[2]. In this study, we present a comprehensive analysis of the mucus proteome of *E. crispata* employing gel electrophoresis and HPLC-MS/MS techniques and explore its potential anti-bacterial properties. A total of 306 proteins were identified in the mucus secretions of *E. crispata*, despite the limited information available for this species in the Uniprot database. Through Gene Ontology for functional annotation, the mucus proteome of *E. crispata* was shown to encompass proteins involved in various functions, including hydrolase activity (molecular function), carbohydrate-derived metabolic processes (biological processes), and cytoskeletal organization (cell component). Notably, a significant proportion of the identified proteins in *E. crispata* mucus exhibited putative enzymatic activity, suggesting potential biotechnological applications. The substantial presence of hydrolases additionally suggests potential antimicrobial properties prompting us to conduct preliminary tests to assess this sea slug mucus' antimicrobial activity. Initial results revealed strong inhibitory activity against *Pseudomonas aeruginosa*, a bacterial species classified by WHO as a critical priority pathogen and associated with high-risk infections due to its frequent multidrug-resistant profile[3]. Further investigations will help elucidate the full range of applications associated with the mucus derived from this unique marine organism.

References:

1. Cruz, S. & Cartaxana, P. (2022) Plos Biol. **20**, e3001857.
2. Smith, A. M. (2002). Integr. Comp. Biol. **42**, 1164–1171.
3. Raman, G., Avendano, E.E., Chan, J., Merchant, S., & Puzniak, L. (2018) Antimicrob. Resist. Infect. Control. **7**, 79.

OP1.2 - THE MICROBIOME OF OCTOCORALS: FROM COMMUNITY STRUCTURE, FUNCTION AND METABOLIC INTERACTION TO BLUE BIOTECH OPPORTUNITIES

Tina Keller-Costa [1,2*], Sandra G. Silva [1,2], João Almeida [1,2], Daniela Silva [1,2], Ângela Taipa [1,2], Jorge Gonçalves [3], Asunción Lago-Lestón [4], Nikos C. Kyrpides [5], Ulisses Nunes da Rocha [6], Rodrigo Costa [1,2]

[1]Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal;
[2]Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal;
[3]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal;
[4]Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico;
[5]Department of Energy, Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California, USA;
[6]Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany;

(*) e-mail: tinakellercosta@tecnico.ulisboa.pt

Keywords: Marine Microbiology, Symbiosis, Corals, Metagenomics, Metagenome-assembled genomes (MAGs), Chitin, Chitinases, Cross-feeding, Heterologous expression

ABSTRACT

Octocorals (Octocorallia, Cnidaria) are an integral part of benthic marine ecosystems. They increase habitat complexity and biodiversity and play key roles in coastal food chains. They are found in association with diverse microorganisms, including micro-eukaryotes, prokaryotes, and viruses. Our research on temperate octocorals showed that their microbiome is distinct from the environmental surroundings, host genus-specific, and undergoes complex structural changes in the transition to the dysbiosis state [1]. However, the role of microbial symbionts that populate octocorals is still poorly understood. To shed light on their metabolic capacities, we examined 66 high-quality metagenome-assembled genomes (MAGs) spanning 30 prokaryotic species, retrieved from microbial metagenomes of three octocoral species and seawater [2]. Symbionts of healthy octocorals were affiliated with *Endozoicomonadaceae*, *Candidatus* Thioglobaceae, and *Metamycoplasmataceae,* among others. Phylogenomics showed that the *Endozoicomonadaceae* MAGs represent a novel genus unique to temperate octocorals, denoted *Candidatus* Gorgonimonas. Their genomes revealed metabolic capacities to thrive under suboxic conditions and high numbers of genes related with host colonization and aggregation. All *Candidatus* Gorgonimonas symbionts harboured chitinase and chitin-binging protein-encoding genes, indicating that they can hydrolyse the most abundant polysaccharide in the ocean. This indicates a thus-far unanticipated role for dominant *Endozoicomonadaceae* symbionts in the processing of chitin, a major component of the natural plankton feed of octocorals. Other symbionts possessed genes to assimilate smaller chitin-oligosaccharides resulting from chitin breakdown, suggesting possibilities for cross-feeding and a role for the coral microbiome in overall chitin turnover. Since Candidatus *Gorgonimonas* symbionts remain unculturable, we currently employ gene synthesis and heterologous expression to harness and characterize their enzymes. Indeed, chitinases hold excellent opportunities for upcycling of sea-food waste as chitin-derived added-value products find applications in the pharmaceutical sector, biomedicine, food industry, aquaculture, and agriculture.

References:

[1] Keller-Costa et al., 2021, Microbiome, 9, 1-21 https://doi.org/10.1186/s40168-021-01031-y

[2] Keller-Costa et al., 2022, Microbiome 10, 151 https://doi.org/10.1186/s40168-022-01343-7

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) through the research project grants 'ChicoBionts' - EXPL/BIA-MIC/0286/2021 and EXPL/MAR-EST/1664/2013 and by the Fundo Azul program of Direção-Geral de Política do Mar (DPGM; Ministry of the Sea, Portugal) through grant FA_05_2017_032. The authors also acknowledge FCT funds in the scope of projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and project LA/P/0140/2020 of i4HB. T.K.C. is the recipient of an investigator contract (CEECIND/00788/2017) conceded by the FCT.

OP1.3 - ANTIBIOTIC-RESISTANT BACTERIA HITCHHIKING MICROPLASTICS IN A RIVER: DETAILED ANALYSIS OF CARBAPENEMASE-PRODUCING ENTEROBACTERALES IN THE PLASTISPHERE

Isabel Silva [1,2 *]***, Marta Tacão*** [2]***, Isabel Henriques*** [1]

[1] *Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal*
[2] *Department of Biology University of Aveiro, CESAM (Centre for Environmental and Marine Studies) University of Aveiro, Aveiro, Portugal*

[(*)] *e-mail:* ***silva.isabel.ua@gmail.com***

Keywords: Antibiotic resistance; Plastic pollution; Freshwater; Whole-genome sequencing;

ABSTRACT

Microplastics (MPs, < 5 mm) might accumulate antibiotic-resistant bacteria (ARB) in aquatic systems. We assessed whether MPs accumulate priority antibiotic-resistant pathogens and used a whole genome sequencing-based approach to characterize selected strains. For this, particles of polypropylene (PP), polyethylene (PE) and a mixture of different MP types [MPs Mix, composed of PP, PE and polyethylene terephthalate (PET)] were submerged in a Portuguese river. Sand particles were used as a natural control substrate. After 30 days, bacteria colonizing these particles were cultivated in m-FC supplemented with ciprofloxacin, cefotaxime or meropenem. The number of resistant CFUs/1000 particles was significantly higher in MPs, compared to sand. Meropenem-resistant CFUs were found colonizing MPs (7±1 in MPs Mix and 8±3 in PET) but not sand. Carbapenem-resistant Enterobacterales (CRE) were detected colonizing MPs and were identified as Klebsiella pneumoniae (n=3), Klebsiella quasipneumoniae (n=3), Raoultella ornithinolytica (n=2), Enterobacter kobei (n=1) and Citrobacter freundii (n=1). All isolates harbored carbapenemase-encoding genes [blaKPC-3 (n=8) and blaGES-5 (n=6)]. Two KPC producers were able to transfer this gene to a receptor strain through conjugation, resulting in a multi-drug resistance phenotype of the transconjugants. Consistent with the genome analysis, all CRE demonstrated a multi-drug resistant phenotype. One K. pneumoniae isolate co-harbored the blaKPC-3 and the extended-spectrum β-lactamase-encoding gene blaCTX-M-15. This strain belonged to ST15 - a clonal lineage recognized as a high-risk clone. Two K. quasipneumoniae isolates carried the plasmid- mediated colistin resistance gene mcr-9. Virulence genes were predicted for all isolates and all strains presented at least one of the virulence traits tested (biofilm formation, haemolytic activity and siderophore production). Our results indicate a preference of ARB, including carbapenemase producers, in colonizing MPs over a natural substrate and that these particles may act as carriers, potentially facilitating the proliferation and dissemination of critical priority antibiotic-resistant pathogens harboring ARGs of great concern

Acknowledgements:
This work was financed by Fundação para a Ciência e a Tecnologia (FCT) through individual grants to Isabel Silva (SFRH/BD/144902/ 2019) and Marta Tacão (CEECIND/00977/2020), and through the strategic projects granted by FCT to Centre for Functional Ecology and Associate Laboratory TERRA (UIDB/04004/2020, LA/P/0092/2020) and to CESAM (UIDP/50017/ 2020 + UIDB/50017/2020 + LA/P/0094/2020).

OP1.4 - PEPTIDES FROM ENTOMOPATHOGENS AS A SOURCE OF NEW BIOINSECTICIDES

Duarte Toubarro [1]***, Jorge Frias*** [1]***, Tiago Paiva*** [1]***, Nelson Simões*** [1(*)]
[1] *Centro de Biotecnologia dos Açores (CBA) e Faculdade de Ciências e Tecnologia, Universidade dos Açores.*

[(*)] *e-mail:* ***nelson.jo.simoes@uac.pt***

ABSTRACT

One of the green deal challenges is the protection of plants using plant protection products (PPPs) with reduced risks. This aim imposes great challenges particularly in the control of insect pests which natural enemies tends to reduce view actual agricultural practices and climatic changes. Insect pathogens

release virulence factors that all together induces insect death. Based on genetic and transcriptomic analysis of virulence factors we identified in an insect pathogen many virulence factors acting at different targets in insects, however none of them has an effective insecticidal activity, which is mandatory for an efficient control of the pest. However, a more detailed analysis of the excreted secreted proteins

combined with its biological activity in insects, allowed the identification of ShK domains as effectors targeting the Shaker channels of the insect. A library of analogs of ShK domains was then produced and the interaction of these analogs with the channel studied using IA approaches. One of these domains

was produced recombinantly in Escherichia coli. data on toxicity assays using Drosophila melanogaster as a model, show that injection of this peptide can kill insects in a dose-dependent manner with an LD50 of

16.9 µM per adult within 24 hours. Oral administration of the fusion protein significantly reduced the locomotor activity of insects after 48 h ($p < 0.05$, Tukey's test). These data show the potential of these new peptides as biopesticides.

Acknowledgements:
This research was done under the project NanoNema financed by Programa Operacional AÇORES 2020 (ACORES-01-0145-FEDER000113).

OP1.5 - SCREENING FOR PLASTIC-DEGRADING POTENTIAL IN MARINE BACTERIA ASSOCIATED WITH NET BIOFILMS AND HYDROCARBON DEGRADATION

Rafaela Perdigão [1,2(*)]***, Maria F. Carvalho***[1,2]***, Catarina Magalhães***[1,3]***, Sandra Ramos***[1]***, C. Marisa R. Almeida***[1,3] ***and Ana P. Mucha***[1,3]

[1] *CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.*

[2] *ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Portugal.*

[3] *FCUP - Faculty of Sciences, University of Porto, Porto, Portugal.*

[(*)] *e-mail:* ***rafaela.mendes1@ciimar.up.pt***

Keywords: Marine bacteria; plastic; screenings; biodegradation; plastic-degrading enzymes.

ABSTRACT

Marine litter and microplastics impact severely our ocean and its wildlife. Biodegradation of plastics by microorganisms, especially bacteria, is gaining increasing attention, where a link between hydrocarbon and plastic-degradation has been hypothesized. In this study, a screening for plastic-degrading potential was performed in 19 bacterial strains isolated from one–month-old biofilms of 3 plastic fishing nets (Braided Polyethylene, Braided Nylon, Thin Nylon). Plus, 3 highly capable hydrocarbon-degraders were also tested. The strains were grown in solid minimal media supplemented with each net (as sole carbon source) for one month, both in discs and wells cut within the media. Since many plastic-degrading enzymes fall in the group of esterase/lipase, another culture-dependent approach -a tributyrin agar assay - was employed. Afterwards, genes encoding hydrocarbon or plastic-degrading enzymes (*alkB homologs, almA, cut190 and PETase*-like encoding genes) were detected by PCR, for the previously mentioned bacteria, but also for other strains (~100) grown in the net biofilms. Considering the screening assays, the genomes of 6 promising strains were sequenced in the Illumina platform, for general functional inference and mining of plastic-degrading enzymes, which is currently ongoing. Overall, 12 bacteria grew better in the presence of net polymers, when compared to the control minimal media, namely from the genera *Erythrobacter*, *Sulfitobacter*, *Rhodococcus*, *Bacillus* and *Pseudomonas*, and 9 of which demonstrated as well esterase/lipase activity. Amplification of the selected genes was observed mainly in Actinobacterial strains. The workflow employed here allowed us to select some marine bacteria with plastic-degrading potential, collected from plastic fishing nets or hydrocarbon enrichments. However, future microcosm experiments will be assembled to validate these results.

Acknowledgements:
PhD grant 2020.04689.BD(FCT); This work was partially supported by project BIOSYSMO (EU funded under the GA no 101060211). FCT is acknowledged by CIIMAR Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through national funds provided by FCT and the ERDF.

OP.1.6 - BACTERIA-BASED BIOREFINERY: GENERATING BIOACTIVE OLIGOSACCHARIDES FROM A SINGLE FERMENTATION OF SEAWEED

Duarte Toubarro [1(*)], ***Duarte Seixas*** [1,2], ***Nelson Simões*** [1]

[1] *CBA - Açores Biotechnology Center (CBA), Faculty of Science and Technology, University of Açores, Ponta Delgada, Portugal.*

[2] *Azores Life Science, S.A, Ponta Delgada, Portugal*

(*) e-mail: duarte.nt.tiago@uac.pt

Keywords: deconstruction of seaweed; bioactive compounds; Bacteria-Based Biorefinery.

ABSTRACT

The enzymatic deconstruction of seaweed can result in enhanced extraction of bioactive compounds with applications within food, cosmetic and health. So far, commercially available enzymes have been used with success, with increased efficiency when combination of enzymes are employed. However, the cost remains a subject of debate in industrial applications. To enhance the cost-effectiveness of the process, our current focus is on identifying bacterial capable of efficiently deconstructing seaweed cell walls. The gut microbiomes of sea urchins and microbiome of marine shallow sediments were enriched using a growth-selective medium containing algae, resulting in the construction of 24 consortia. Two consortia, AU14 and P5, were selected based on their capacity to break down Gelidium microdon and Ulva rigida, as well as the amount of reduced sugars and proteins produced. From these consortia, 100 bacterial isolates were obtained and analysed for enzyme production. The isolate AU-i14, identified as Bacillus subtilis, displayed the highest activity, exhibiting simultaneous cellulase, xylanase, β-glucosidase, and β-xylosidase production. Utilizing AU-i14, we achieved solubilization rates of 80% and 65% for Ulva and Gelidium, leading to the production of 8.35 g/kg and 12 g/kg of oligosaccharides, respectively. These oligosaccharides displayed valuable functional properties both in vitro and in a Drosophila model, making them promising candidates for further investigation in cellular assays.

Acknowledgements:
This research was supported by Vertical Algae project, Subproject 3, funded by the Blue Bioeconomy Pact through funds from the Recovery and Resilience Plan (PRR).

OP1.7 - VALORIZATION OF *GELIDIUM CORNEUM* BY-PRODUCT THROUGH SOLID-STATE FERMENTATION

Marta Ferreira [1(*)], ***José Manuel Salgado*** [2], ***Helena Peres*** [3,4], ***Isabel Belo*** [1,5]

[1] *Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal.*

[2] *Biotecnia Group, Department of Chemical Engineering, Campus Agua, University of Vigo (Campus Ourense), As Lagoas s/n, Ourense, 32004, Spain*

[3] *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal*

[4] *CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal*

[5] *LABBELS –Associate Laboratory, Braga, Guimarães, Portugal*

[(*)] *e-mail:* ***martaf824@gmail.com***

Keywords: red seaweed by-product, chemical composition, solid-state fermentation, enzyme activities analysis, bioreactor, scale-up

ABSTRACT

Industrial agar extraction of red seaweed *Gelidium* produces a significant amount of by- products (red seaweed by-product - RSB), which are often discarded. Nevertheless, their rich composition in carbohydrates and proteins makes them appropriate for value-added compound production through solid-state fermentation (SSF). In this study, RSB was utilized as substrate for SSF with *Aspergillus ibericus* MUM 03.49 and *Aspergillus niger* CECT 2915. RSB was initially characterized, and after was used as substrate for SSF as unsupplemented RSB, RSB supplemented with Mandel salt solution, and in a 50% (w/w) mixture of RSB with agro-industrial by-products (rice bran, sunflower cake, rapeseed cake, and corn gluten feed) and green seaweed *Ulva rigida*. The changes in crude protein content and carbohydrases production in the fermented biomass were assessed. The maximum xylanase activity (498 ± 49 U g^{-1}) was achieved with SSF of RSB mixed with sunflower cake using *A. niger*, while the mixture between RSB and rapeseed cake led to the production of the highest cellulase activity (382 ± 37 U g^{-1}). Additionally, protein content increased after SSF with *A. niger* in RSB mixed with rice bran (30%), rapeseed (18%), an sunflower cakes (15%). As a proof of concept, an SSF scale-up of up to 20-fold of dry substrate was done with *A. niger* using a mixture of RSB and sunflower cake. The effect of aeration and agitation on xylanase and cellulase production was studied using two types of bioreactors. In tray- type bioreactors enzyme activities were similar with values obtained at small scale, while in the stirred-drum bioreactor, forced aeration and low agitation enhanced both enzymes production. SSF-based bioprocessing of RSB mixed with agro-industrial by-products was demonstrated to be a cost-effective and sustainable approach for producing high-value enzymes and valorizing this seaweed by-product.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit; by the project "Macroalgae biorefinery: a novel approach to produce sustainable feedstuffs and functional additives towards low carbon footprint aquafeeds", reference 2022.06587.PTDC; and by LABBELS Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems (LA/P/0029/2020). Marta Ferreira was supported with a PhD grant, SFRH/BD/143614/2019, funded by the Portuguese Foundation for Science and Technology (FCT).

OP1.8 - EXPLORING THE FATE OF CHIRAL PHARMACEUTICALS IN AN AGS SYSTEM UNDER SALTWATER INTRUSION PHENOMENA

Catarina Miranda [1(*)], ***Catarina Amorim*** [1], ***Clara Piccirillo*** [2], ***Maria Elizabeth Tiritan*** [3,4], ***Paula Castro*** [1]

[1] *Universidade Católica Portuguesa, Centro de Biotecnologia e Química Fina – Escola Superior de Biotecnologia, Porto, Portugal*

[2] *CNR NANOTEC, Institute of Nanotechnology, Campus Ecoteckne, Lecce, Italy*

[3] *Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia do Porto, Portugal*

[4] *Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Portugal*

(*) *e-mail:* ***cmiranda@ucp.pt***

Keywords: Chiral pharmaceuticals; Aerobic granular sludge; Wastewater; Salt intrusion; Adsorption.

ABSTRACT

Aerobic granular sludge (AGS) is a robust technology, largely adopted in wastewater treatment plants (WWTPs) worldwide. However, there is a lack of knowledge regarding how this technology deals with saltwater intrusion and variable daily wastewater salinity loads. With the sea level rise, in coastline WWTPs, seawater infiltration into sewers is a growing problem. In addition, the increase in pharmaceutical production and consumption led to their accumulation in wastewater. Many of these are chiral pharmaceuticals (CPs) whose enantiomers can differ in their degradation ratio and toxicity in the environment. The fate of CPs in AGS systems is scarcely reported, especially if combined with variable salt concentration in wastewater.

In this study, an AGS reactor was operated for 132 days for the treatment of urban saline wastewater sporadically containing a mixture of CPs namely: tramadol and venlafaxine and its metabolites o-desmethyltramadol and o-desmetylvenlafaxine, respectively, at concentrations near those found in the environment (8 µg/L). Both daily salinity fluctuations and the presence of CPs in wastewater did not affect the biological removal of COD, N, and P. However, the AGS system was not able to remove the CPs that ended up in the effluent. To address this challenge, a parallel experiment was performed using a bone char material derived from fish-food waste (tuna bones) to adsorb the pharmaceuticals tramadol and venlafaxine. The bone char exhibited removal efficiencies of around 40%, as such in combination with AGS systems it can help to decrease the release of CPs into the environment.

Acknowledgments:
This work was financed by Norte 2020 through Project 3BOOST (POCI-01-0246-FEDER-181302). The authors thank the CBQF and CIIMAR scientific collaboration under the FCT projects UIDB/50016/2020 and UIDB/04423/2020 + UIDP/04423/2020, respectively. C. Miranda thanks the research grant from FCT, Portugal (2020.06577.BD) and POCH, supported by the European Social Fund and MCTES national funds.

OP1.9 - THE QUEST FOR NATURAL MICROBIOMES: TRANSFORMING CO_2 INTO VALUABLE BIOPRODUCTS THROUGH PHOTOSYNTHESIS

André Freches [1,2(*)], ***Inês Cardoso*** [1,2], ***Joana Fradinho*** [1,2], ***Maria Reis*** [1,2]
[1] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal*
[2] *UCIBIO - Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal*

(*) *e-mail:* ***a.freches@campus.fct.unl.pt***

Keywords: Photosynthesis, Microbiomes, Biopolymers, Bacteriochlorophyll.

ABSTRACT

In the pursuit of circular bioeconomy approaches, the effective integration of carbon capture technologies with the synthesis of value-added substances stands as a critical objective. Anoxygenic photosynthetic bacteria present a compelling avenue for achieving this goal, given their unique capacity to utilize inorganic carbon to produce various valuable compounds, including pigments and biopolymers. These bacteria thrive in diverse environments, with notable abundance in natural hot springs where carbonate and sulfide resources are readily accessible.

In this study, four distinct Portuguese hot spring locations (Unhais da Serra, Manteigas, Caldas da Rainha, and São Miguel) were chosen to collect microbiomes adapted to unique site-specific conditions. Following collection, these microbiomes were cultivated in a nutrient-rich medium. Subsequently, different conditions regarding pH and temperature were systematically tested to assess the performance of anoxygenic photosynthesis. This evaluation involved tracking carbonate uptake and sulfide removal, and concurrently determining the production of bacteriochlorophyll, carotenoids, glycogen and polyhydroxyalkanoates.

The results of our investigation demonstrated the capacity of these four microbiomes to efficiently transform inorganic carbon into value-added bioproducts. Moreover, the study identified a particularly high-performing microbiome collected from one hot spring which displayed higher capability for CO_2 conversion and polyhydroxyalkanoates synthesis. This microbiome was subsequently selected for further exploration at reactor scale targeting the production of biopolymers using CO_2 and sulfide as carbon and electron donors, respectively.

This research underscores the potential of natural microbiomes, especially anoxygenic photosynthetic bacteria, in facilitating the transformation of carbon dioxide into valuable bioproducts. The outcomes of this investigation offer valuable insights into harnessing these microbiomes for sustainable biotechnological applications and contribute to the ongoing quest for environmentally responsible and economically viable solutions in the circular bioeconomy paradigm.

Acknowledgements:
This work was supported by the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT (UIDB/04378/2020) and Fundação para a Ciência e Tecnologia for doctoral grant SFRH/BD/145555/2019.

OP1.10 - AN ALLOSTERIC REDOX SWITCH ENABLES OXYGEN PROTECTION OF A FORMATE DEHYDROGENASE FROM *DESULFOVIBRIO VULGARIS* HILDENBOROUGH

Ana Rita Oliveira [1¥], Cristiano Mota [2,3¥], Rita Rebelo Manuel [1*], Guilherme Vilela-Alves [2,3], Neide Pedrosa [1], Vincent Fourmond [4], Kateryna Klymanska [2,3], Christophe Léger [4], Bruno Guigliarelli [4], Maria João Romão [2,3], Inês Cardoso Pereira [1]

[1] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal
[2] Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal
[3] UCIBIO, Applied Molecular Biosciences Unit, Departament of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal
[4] Aix Marseille Univ, CNRS, BIP, Laboratoire de Bioénergétique et Ingénierie des Protéines, Marseille 13402, France
¥ contributed equally

(*) e-mail: rs.manuel@itqb.unl.pt

Keywords: Redox Switch, Formate Dehydrogenases, *Desulfovibrio vulgaris* Hildenborough, CO2 Reduction, Oxygen Tolerance.

ABSTRACT

The use of CO_2 as feedstock for a circular economy is an intense area of research. Formate is an added-value product considered as an alternative liquid fuel [1]. Metal dependent formate dehydrogenases (Fdhs) reduce CO_2 to formate with high efficiency and selectivity. The Fdh from *Desulfovibrio vulgaris* Hildenborough (FdhAB) is a tungsten containing Fdh that is the main responsible for CO_2 reduction to formate in vivo [2]. This enzyme is an excellent model system for the study of Fdhs since it has a simple structure, it can be handled under aerobic conditions and its CO_2 reduction activity is one of the highest reported [3]. However, the catalytic mechanism for CO_2 reduction and the molecular basis for its unusual oxygen tolerance was not elucidated yet.

To better understand these two FdhAB features, we generated some enzyme variants. The effect of single-point mutations was assessed by kinetic assays and spectroscopic and electrochemical studies. In parallel, the structural impact of these mutations was studied by x-ray crystallography.

We found that FdhAB activity is controlled by a redox switch based on an allosteric disulfide bond. If the bond is closed, the enzyme is in a resting state, with very low formate affinity, almost no catalytic activity but high oxygen tolerance. Opening of the disulfide bond triggers large conformational changes that propagate to the active site, resulting in enzyme with high activity and high affinity for formate, but also higher formate-induced oxygen sensitivity. This mechanism is reversible *in vivo* and prevents reduction of the enzyme by physiological formate levels, likely conferring a fitness advantage during transient O_2 exposure [4].

References:

[1] Pereira, (2013). *Science*, **342**(6164):1329-1330.
[2] Silva et al., (2013). *Microbiology*, **159**(8):1760-1769.

[3] Oliveira et al., (2020). *ACS Catal*, **10**(6):3844-3856.
[4] Oliveira et al. (2023) *Nature Chem. Biol., in press*

Acknowledgements:
This work was financially supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) through fellowships SFRH/BD/116515/2016 and DFA/BD/7897/2020, COVID/BD/151766/2021, grant PTDC/BIIBBF/2050/2020, and R&D units MOSTMICRO-ITQB (UIDB/04612/2020 and UIDP/04612/2020) and UCIBIO (UIDP/04378/2020 and UIDB/04378/2020), and Associated Laboratories LS4FUTURE (LA/P/0087/2020) and i4HB (LA/P/0140/2020).

OP1.11 - HARNESSING *SACCHAROMYCES CEREVISIAE* FOR SUSTAINABLE FDCA PRODUCTION: OPTIMIZING 5-HMF DETOXIFICATION AND DERIVATIVE SYNTHESIS

Cristiana Martins [1], ***Carlos E. Costa*** [1,2], ***Joana Cunha*** [1], ***Lucília Domingues*** [1,2*]
[1] *CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2] *LABBELS - Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail:* ***luciliad@deb.uminho.pt***

Keywords: 5-HMF, FDCA, cell surface display, *Saccharomyces cerevisiae*, biotransformation.

ABSTRACT

The escalating demand for energy and chemicals necessary in various daily routines allied to climate change and the increase in greenhouse gas emissions has been pushing the need to substitute their source from fossil fuels to biological processes. The establishment of a biorefinery for the manufacture of biofuels and other chemicals from renewable feedstocks, such as lignocellulosic biomass is essential to this goal. The yeast *Saccharomyces cerevisiae* has been widely studied for its ability to detoxify 5-HMF, a toxic by-product of hexoses degradation. However, its capability as a whole-cell biocatalyst to produce 5-HMF derivatives has not been sufficiently investigated [1]. 5-HMF is a versatile compound and an attractive chemical because of its unique properties and multiple applications, being reduced or subsequently oxidized to a variety of products. These HMF-derivatives have a wide range of applications such as synthesising polymers (BHMF), synthesising surfactants and resins (FFCA) and producing plasticisers, polyurethanes, polyesters or polyamides (FDCA), among others [2]. Here, a robust industrial *S. cerevisiae* strain was used as host for the development of genetically engineered strains to produce heterologous enzymes, HMF/Furfural oxidoreductase or HMF oxidase, envisioning FDCA production. We further investigated and optimised the ability of the modified strains to convert 5-HMF into its derivatives, especially FDCA. All recombinant strains were screened for their ability to detoxify 5-HMF, with the best-performing strain exhibiting efficient detoxification of 40 mM of 5-HMF, which led to the production of 12 mM of FDCA. Afterwards, bioconversion conditions were optimised by assessing the impact of oxygenation conditions, as its fine-tuning is deeply connected to 5-HMF detoxification and FDCA production. Lastly, a synthetic hemicellulosic hydrolysate, with glucose and fructose as carbon sources, was successfully used to generate FDCA. This study helps to the establishment of a sustainable bio-based process to produce 5-HMF derivatives.

References:

[1] J. T. Cunha, A. Romaní, and L. Domingues, "Whole Cell Biocatalysis of 5-Hydroxymethylfurfural for Sustainable Biorefineries," Catalysts, vol. 12, no. 2, pp. 1–15, 2022, doi: 10.3390/catal12020202.
[2] K. Saikia et al., "Recent advances in biotransformation of 5-Hydroxymethylfurfural: challenges and future aspects," J. Chem. Technol. Biotechnol., vol. 97, no. 2, pp. 409–419, 2022, doi: 10.1002/jctb.6670.

OP2.1 - TRADITIONAL CHEESES AS BIO-FACTORIES FOR PROBIOTIC BACTERIA

Susana Serrano [1,2*] †, Maria V. Ferreira [3] †, Cinthia Alves-Barroco [1,2], Maria Teresa Barreto-Crespo [4,5], Teresa Semedo-Lemsaddek [1,2]

[1]CIISA – Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS).

[3]Faculty of Sciences (FCUL), University of Lisbon, Portugal.

[4]IBET, Institute of Experimental Biology and Technology, Oeiras, Portugal.

[5]ITQB, Institute of Chemical and Biological Technology António Xavier, Nova University of Lisbon, Republic Avenue, Oeiras, Portugal.

† Equal contributors to this study

[(*)] e-mail: ***sserrano@fmv.ulisboa.pt***

Keywords: Traditional cheeses, autochthonous microbiota, *Enterococcus*, probiotic potential, safety.

ABSTRACT

Portuguese traditional cheeses produced with raw milk and harbouring Protected Designation of Origin (PDO), are delicacies appreciated by many. Azeitão and Nisa regions produce PDO cheeses, which unique organoleptic characteristics are attributed to the autochthonous microbiota, mainly lactic acid bacteria, among them *Enterococcus* spp. However, these bacteria are known to possess a dual role, acting as opportunistic pathogens in healthcare settings but also harbouring technological and probiotic features. Hence, the present study performed a comprehensive characterization of PDO-cheese enterococci, including the evaluation of desirable traits and underlying safety.

Briefly, *Enterococcus* were isolated from Azeitão and Nisa cheese-samples (2016-2022) and compared by RAPD-PCR. Seventy-two genetic representatives of the microbial collection were further characterized regarding hemolytic ability and antibiotic resistance (here considered exclusion features, not desirable in “food-grade” enterococci), growth under various conditions (e.g., different pHs and temperatures, in the presence of NaCl or bile salts) and biofilm-forming ability (inclusion traits for putative probiotics).

Resistance among the enterococci were as follows: quinupristin/dalfopristin (92%), streptomycin (74%) and tetracycline (71 %), while lower levels of resistance (< 20%) were observed for ampicillin, erythromycin, chloramphenicol, ciprofloxacin and vancomycin. All the isolates were sensitive to gentamicin, apart from one resistant, and linezolid, but approximately 16% showed a multidrug resistant (MDR) phenotype. As for multiplication in different conditions, all isolates were able to survive in all salt concentrations tested, as well as on the presence of bile salts. Regarding biofilm formation, the majority were considered biofilm-producers, but several differences were observed, both considering the accumulation of biofilm (after crystal violet coloration) and viable cell estimation (using resazurin as indicator). Overall, although confirmatory assays are currently ongoing, data already attained indicates the putative probiotic potential of the enterococcal community recovered from Azeitão and Nisa PDO cheeses.

Acknowledgements:
This project was supported by National Funds through FCT – Foundation for Science and Technology under the projects UIDP/00006/2020, LA/P/0059/2020 - AL4AnimalS and PTDC/OCE-ETA/1785/2020 [EMOTION].
Teresa Semedo-Lemsaddek is financially supported by national funds through FCT under the Transitional Standard — DL57/2016/CP1438/CT0004.
Susana Serrano holds a fellowship supported by national funds through FCT - UI/BD/153073/2022.

OP2.2 - GENOTYPIC DIVERSITY AND OFF-FLAVORS PRODUCTION IN *DEKKERA BRUXELLENSIS* AS DRIVERS FOR SELECTION OF BIOCONTROL AGENTS

João Sousa [1,2], Emanuel Peixoto [1], Rogério Tenreiro [2], Nuno Mira [3] and Ana Mendes-Ferreira [1,2(*)]

[1] WM&B - Wine Microbiology & Biotechnology Laboratory, Universidade de Trás-os-Montes e Alto Douro

[2] BioISI - Biosystems and Integrative Sciences Institute

[3] Department of Bioengineering - Instituto Superior Técnico; iBB - Institute of Bioengineering and Biosciences

[(*)] e-mail: anamf@utad.pt

Keywords: *Dekkera bruxellensis*; *Metschnikowia pulcherrima*; POFs; Bioagents.

ABSTRACT

Dekkera (Brettanomyces) bruxellensis continues to pose a major concern for wine producers due to its ability to produce volatile phenolic off-flavors (POFs). These compounds, such as 4-ethylphenol and 4-ethylguaicol, result from the decarboxylation of hydroxycinnamic acids naturally present in musts/wines being highly variable among strains. This yeast can survive wine´s hostile environment characterized by its low pH and high alcohol content, making it difficult to control its growth. Chemical products are normally used to restrain *D. bruxellensis*, but new biobased approaches are emerging with the introduction of other yeasts with antagonistic proprieties being promising tools.

In this work, the genomic diversity of a set of 30 isolates of *D. bruxellensis* was assessed by PCR-*fingerprinting*, using three primers: csM13, $(GTG)_5$ and $(GACA)_4$. Based on the dendrogram obtained, representative strains were selected to assess their spoilage potential. In this line, the yeasts were grown in YPD supplemented with the different hydroxycinnamic acids - ferulic and coumaric, and with or without 6% of ethanol. The production of volatile phenols was strain-dependent, and it was not possible to establish a relationship between genomic diversity and production of POFs.

Next, the antagonistic activity of a set of 96 strains of *Metschnikowia pulcherrima* against a *D. bruxellensis* strain, that stood out for its rapid growth and efficiency in producing POFs, was assessed. The killer-assays performed revealed that the inhibitory effect of *M. pulcherrima* is highly dependent on the medium pH, making a strong case for the use of this criterium in the selection of biocontrol agents.

Acknowledgements:
This work was financially supported by SMARTWINE 2.0 project with the reference 2022.06777.PTDC, financed by FCT - Portuguese Foundation for Science and Technology. João Sousa is a recipient of a PhD grant with the reference 2022.11749.BD, from the FCT Doctoral Program in Chemical and Biological Sciences (UTAD).

OP2.3 - CHARACTERIZATION OF FOODBORNE PATHOGENIC *VIBRIO SPP*. IN ENVIRONMENTAL AND SEAFOOD SAMPLES COLLECTED FROM ANGOLA

Inês C Leal [1+(*)] **, *Babak Najafpour*** [1+]**, *Eunice Cassoma*** [2]**, *Isaac Bumba*** [2]**, *Carmen dos Santos*** [2]**, *Adelino VM Canário*** [1]**, *Deborah M Power*** [1,] ***João CR Cardoso*** [1]

[1] *Comparative Endocrinology and Integrative Biology, Algarve Centre of Marine Sciences (CCMAR-Algarve), Universidade do Algarve, Faro, Portugal*
[2] *Universidade do Namibe, Campus Farol de Noronha, Moçâmedes, Angola*
+ shared first authorship

[(*)] *e-mail: ifleal@ualg.pt*

Keywords: Bacteria, marine microbiota, pathogens, human health, *Vibrio*

ABSTRACT

Marine microbiota play a vital role in ocean ecology however, some are primary or opportunistic pathogens that cause infection in marine organisms and humans. The majority of the abundant bacteria of the Vibrio genus are non-pathogenic microorganisms of the aquatic environment, but a few are pathogenic and responsible for disease outbreaks in aquaculture and seafood poisoning.

Seafood poisoning is a major public health burden and is frequent in Africa causing 137,000 deaths and making 91 million people ill every year. Nonetheless, the link between microbial risks, Vibrio spp. and foodborne illness remain unclear. The aim of this study was to characterize marine microbiota and identify the frequency of pathogenic bacteria that pose a risk to human health in Namibe, Angola. Food insecurity is high in the Namibe region and food of marine origin is important in the local economy and to sustain local inhabitants. A common bivalve, the mussels were collected along with seawater from 6 coastal sites and microbial taxonomic profiles were characterized using 16S rRNA gene sequencing technology. Putative pathogenic Vibrio bacteria were also isolated from mussel samples using classical microbiological approaches (ISO 21872-1: 2017) to identify the species. Microbiome analysis of 36 biological samples and 4 environmental water samples revealed that Protobacteria was the most abundant phylum and uncovered the presence of several pathogens, including Vibrios. Isolation of bacteria by conventional microbiology and 16S rRNA PCR revealed that the pathogenic species, V. alginolyticus and V. parahaemolyticus, were the most abundant pathogens and represented 35% of the isolates in the samples. This study revealed for the first time that pathogenic bacteria of the Vibrio genus are abundant in the seawaters and mussels in Namibe and suggests they may be responsible for seafood-borne infections.

Acknowledgements:
This study received Portuguese national funds from FCT - Foundation for Science and Technology through Institutional projects UIDB/04326/2020/2021/2022/2023, and LA/P/0101/2020 and the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121 and from FCT funded project AGAKHAN/541666287/2019 HealthyBi4Namibe.

OP2.4 - FIRE4CAST – AN INTEGRATIVE STRATEGY FOR ACCURATE PREDICTION OF FIRE BLIGHT DISEASE OUTBREAKS IN PORTUGUESE ORCHARDS

Daniel McGuire [1]***, Francisco Pinto*** [2]***, Telma Costa*** [2]***, Joana Cruz*** [1]***, Rui Sousa*** [3]***, Miguel Leão de Sousa*** [3]***, Carmo Martins*** [4]***, Ana Tenreiro*** [2]***, Rogério Tenreiro*** [2]***, Margarida Gama-Carvalho*** [2]***, Leonor Cruz*** [1,2(*)]

[1] *Instituto Nacional de Investigação Agrária e Veterinária, Unidade de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, Laboratório de Fitobacteriologia, Oeiras, Portugal;*

[2] *Universidade de Lisboa, Faculdade de Ciências, Instituto de Biossistemas e Ciências Integrativas (BioISI), Lisboa, Portugal;*

[3] *Instituto Nacional de Investigação Agrária e Veterinária, ENFVN, Portugal;*

[4] *Centro Operativo e Tecnológico Hortofrutícola Nacional, Estrada de Leiria, S/N 2460-059 Alcobaça.*

[(*)] *e-mail: leonor.cruz@iniav.pt*

Keywords: Plant pathology; Pear trees; Immuno-flow cytometry; Epidemiology; Forecast model.

ABSTRACT

Fire blight affects pear and apple production areas worldwide and is caused by the plant pathogenic bacterium *Erwinia amylovora,* listed as a regulated pest by the EU phytosanitary legislation. In Portugal, this disease has a devastating impact on the production of apple and pear fruits, with the economically important variety 'Rocha' being particularly susceptible. Lack of effective control measures has led to disease outbreaks in production regions, resulting in the potential decimation of orchards. Conventional outbreak predictive models such as Cougarblight and BIS98 have failed to accurately adapt to Portuguese disease progression. Based on this need, we developed a new epidemiological model to predict fire blight outbreaks fitted to Portuguese agroecological conditions. Using a systems biology approach, we integrated microbiological, cytological and genomic pathogen data with phenological host development and climatic variables. Two pear orchards with fire blight history, located in Alcobaça, were monitored between February and June, from 2019 to 2022. In 440 samples collected from the surveyed trees, we monitored the presence, and viability of *E. amylovora* populations in natural settings, regardless of disease symptoms. Through conjugation of EPPO standard diagnostic protocols with a newly devised immune flow cytometry (IFCM) test, we detected viable *E. amylovora* populations in asymptomatic tissue, in February, long before the known disease high risk period. The largest number of positive samples were observed in April, May, and June, with *E. amylovora* viability being highest in April, coinciding with early flowering of pear trees. Furthermore, the integration of the whole data set allowed the development of the Fire4CAST predictive model, able to set outbreak alarms with an 83% precision rate, much higher than CougarBlight (40%) and BIS98(43%). Hopefully, this tool will contribute to a more effective management of *E. amylovora* in Portuguese orchards and inspire similar systems approach methodologies for distinct pathosystems forecast models.

Acknowledgements:
This study was funded by FCT/MCTES from Project Fire4CAST (PTDC/ASP-PLA/28305/2017) and BioISI Research Unit grants (UIDB/04046/2020 and UIDP/04046/2020).

OP2.5 - MORE THAN COLORING AGENTS: NATURAL PIGMENTS WITH ANTIMICROBIAL ACTIVITY

Nuno Ribeiro da Silva [1,2(*)], ***Lina F. Ballesteros*** [1,2], ***Carla Fonseca*** [1], ***Bruna Basto*** [1], ***José A. Teixeira*** [1,2], ***Sara C. Silvério*** [1,2]

[1] *CEB – Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga, Portugal.*

[2] *LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, Braga, Portugal.*

(*)e-mail: nuno.r.dasilva@gmail.com

Keywords: *Penicillium*; Natural pigments; Antioxidants; Antimicrobial activity.

ABSTRACT

Natural pigments interest has rapidly increased due to a risen awareness concerning sustainability and green economy policies, and as an attempt to comply with changes in consumer demands. Health and safety concerns due to synthetic pigments also prompt the crescent global market trend for natural pigments, valued at 5862 million USD in 2022 and estimated to reach 9824.5 million USD by 2032. Natural pigments are non-toxic, biocompatible coloring agents that can also provide additional biological properties (antioxidant, antimicrobial, anti-inflammatory, anticancer activity, etc.). For this reason, they have been attracting several industrial sectors and have gradually replaced synthetic pigments and dyes.

Among natural sources, microbes, particularly Fungi, have gained special attention over the last years as valuable producers of natural pigments. Recently, we have described the ability of a *Penicillium* strain to produce pigments under different growth conditions (fermentation type and medium). Also, its ability to produce pigments in low-cost, alternative media composed only of agro-industrial byproducts like cheese whey and corn steep liquor was demonstrated. Interestingly, according to the growth condition, different pigment mixtures were produced, presenting distinct antioxidant potential and antimicrobial activity.

Outstanding results were achieved with the mixture of pigments ethanolic extracted from the *Penicillium* mycelium grown in agar plates. This crude extract had the highest concentration of flavonoids and phenolic compounds, despite its low antioxidant potential. Nevertheless, it was effective in inhibiting (0.31-0.62 mg/mL) and/or killing (> 0.62 mg/mL) gram-negative and gram-positive bacteria, yeast, and filamentous fungi, according to the agar diffusion method and the minimum inhibitory concentration (MIC) assays.

Our research showed alternative and sustainable approaches to produce natural pigments with proven antimicrobial activity and antioxidant potential. Those can be attractive for the food, beverages, pharmaceutical, and cosmetics industries not only as coloring agents but also as alternatives to the common antimicrobial and disinfectant agents.

References:

Basto, B., da Silva, N., Teixeira, J., Silvério, S. (2022) Production of natural pigments by Penicillium brevicompactum using agro-industrial byproducts. Fermentation 8:536. doi: 10.3390/fermentation8100536.

Fonseca, C., da Silva, N., Ballesteros, L., Basto, B., Abrunhosa, L., Teixeira, J., Silvério, S. (2022) Penicillium brevicompactum as a novel source of natural pigments with potential for food applications. Food and Bioproducts Processing 132:188-199. doi: 10.1016/j.fbp.2022.01.007.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

OP2.6 - Sustainable film solutions: Exploring cutin recovery from tomato by-products for hydrophobic applications

Andreia Simões [1]*, Isabel M. Coelhoso [1], Vítor D. Alves [2], Carla Brazinha [1]

[1] LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal;
[2] LEAF-Linking Landscape, Environment, Agriculture and Food, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

(*) e-mail: ***afm.simoes@campus.fct.unl.pt***

Keywords: tomato pomace; cutin; fatty acids; chitosan-cutin blend films.

ABSTRACT

Tomato pomace, a readily available and cost-effective resource, has garnered attention as a potential source for extracting cutin, a biopolyester primarily composed of long-chain hydroxy fatty acids. These fatty acids have the potential to be used as building blocks for the development of hydrophobic biopolymers.

In this study, the extraction and isolation of cutin monomers from tomato pomace were undertaken, and these monomers were subsequently utilized in the production of cutin-based films.
Various strategies were explored to depolymerize and isolate monomeric cutin. Differences emerged in the initial state of the raw material, the inclusion of a tomato peel dewaxing step, the type of alkaline hydrolysis, and the method for isolating cutin monomers. These strategies enabled the production of extracts rich in fatty acids, including 16-hydroxyhexadecanoic, hexadecanedioic, stearic, and linoleic acids, among others.

Subsequently, cutin and chitosan-based films were successfully cast using cutin extracts in combination with commercial chitosan. The films produced exhibited promising characteristics. Notably, the cutin and chitosan blend films demonstrated high malleability, thickness values of 0.103 ± 0.004 mm and 0.106 ± 0.005 mm, water contact angles of 93.37 ± 0.31° and 95.15 ± 0.53°, and water vapor permeabilities of (3.84 ± 0.39) $x10^{-11}$ mol·m/m²·s·Pa and (4.91 ± 1.33) $x10^{-11}$ mol·m/m²·s·Pa. This study highlights the potential of tomato pomace as a sustainable source for cutin extraction, offering a pathway to harness valuable hydroxy fatty acids for the development of hydrophobic biopolymers. The successful production of chitosan and cutin-based films presents an eco-friendly solution for addressing tomato processing waste while offering versatile materials for potential food packaging applications as well as others.

References:

Simões, A.; Coelhoso, I. M.; Alves, V. D.; Brazinha, C. Recovery and purification of cutin from tomato by-products for application in hydrophobic films. *Membranes* **2023**, *13*, 261.

Acknowledgements:
Andreia Simões acknowledges the company Sumol and Compal, S.A., for kindly providing the tomato pomace for this work. This research was supported by the Associate Laboratory for Green Chemistry—LAQV and LEAF—Linking Landscape, Environment, Agriculture, and Food research units, which are financed by national funds from FCT/MCTES (UIDB/50006/2020, UIDP/50006/2020, and UID/AGR/04129/2020). This research was also funded by Sociedade Ponto Verde—Sociedade Gestora de Resíduos de Embalagens, S.A., through the project entitled “Bioplastics and Edible, Vegan Films”.

OP3.1 - AVIAN ANTIBODY PRODUCTION FOR SARS-COV-2 SPIKE PROTEIN: EPITOPE-SPECIFIC INSIGHTS

Tiago Ochôa-Pires [1,2], ***Rafaela Seabra*** [1,3], ***Beatriz Vaz*** [1,2], ***Ana Beatriz Marques*** [1,3], ***Guilherme Gabriel*** [1], ***Rui Jorge Nobre*** [1,4,5], ***Luís Pereira de Almeida*** [1,5,6], ***Ricardo S. Vieira-Pires*** [1,7(*)]

[1] *Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal*

[2] *Doctoral Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal*

[3] *Vasco da Gama University School (EUVG), Coimbra, Portugal*

[4] *Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal*

[5] *ViraVector, Viral Vectors for Gene Transfer Core Facility, University of Coimbra, Coimbra, Portugal*

[6] *Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

[7] *HBT – Saúde e Biotecnologia Lda, Coimbra, Portugal*

(*) e-mail: ricardo.pires@biocant.pt

Keywords: IgY antibody; SARS-CoV-2; Avian host; Immunodominant epitope.

ABSTRACT

Following the pandemic outbreak of the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), researchers approached the issue by developing antibodies targeting the viral Spike glycoprotein, a critical component for host-cell entry and proliferation. One possible host for the development of these antibodies, are avian hosts, which offer unique advantages for antibody production, such as generating distinct antibody repertoires and enabling cost-effective, high-yield production of polyclonal IgY antibodies in eggs.

Grounded on these, we employed a proprietary scaffold to recombinantly produce five Spike epitopes previously identified as immunodominant in avian systems. These epitopes were used to immunize ISA-Brown chickens with three distinct vaccine formulations: a mixture containing all five recombinant epitopes (Ep1-5) and two separate protocols for individual epitopes (Ep2, Ep3).

Over a 90-day period post-immunization, daily egg collection enabled the evaluation of anti-Spike antibody titers via ELISA assays, leading to the selection of hyperimmune yolk pools. Subsequently, we purified total IgY polyclonal antibodies using a water-dilution and PEG precipitation methods. The final anti-Spike IgY polyclonal antibodies were analysed for titer, target specificity, and sensitivity using ELISA and Western blotting (WB).

Examination of the five ELISA assay response profiles revealed that the epitope Ep3 played a significant role in the response observed with the mixed formulation. Conversely, epitope Ep2 appeared responsible for prominent peaks in both individual and mixed formulations. In contrast, epitope Ep5 displayed a low overall titer, suggesting it was the least immunogenic among the peptides tested.

These findings underscore the varied responses observed across the five epitopes, with particularly promising outcomes observed for Ep2 and Ep3. However, it is crucial to emphasize the necessity for additional testing, including ongoing viral neutralization assays, to confirm the effectiveness of

these epitope-derived antibody formulations against SARS-CoV-2. These assays are pivotal in assessing practical applicability and efficacy of such novel avian antibodies.

Acknowledgements:

This work was supported by Regional Operational Program Center 2020, Competitiveness Factors Operational Program (COMPETE 2020), Project Centro de Neurociências e Biologia Celular POCI-01-0145-FEDER-007440 (Ref. FCT UID/NEU/04539/2013) and National Funds through FCT (Foundation for Science and Technology) and when applicable co-financed by FEDER under the PT2020 Partnership Agreement. BYDRUG Project (POCI-01-0145-FEDER-030550); BrainHealth2020 projects (CENTRO-01-0145-FEDER-000008), ViraVector (CENTRO-01-0145-FEDER022095), CortaCAGs (PTDC/NEU-NMC/0084/2014| POCI-01-0145-FEDER-016719), SpreadSilencing (POCI-01-0145-FEDER-029716), as well as SynSpread, ESMI and ModelPolyQ under the EU Joint Program – Neurodegenerative Disease Research (JPND), the last two co-funded by the European Union H2020 program, GA No.643417; by National Ataxia Foundation (USA), the American Portuguese Biomedical Research Fund (APBRF) and the Richard Chin and Lily Lock Machado-Joseph Disease Research Fund. The work was also supported by industry partner HBT - Saúde e Biotecnologia Lda.

OP3.2 - KERATIN-BASED MATERIALS FOR BIOMEDICAL APPLICATIONS

Cariny Polesca [1(*)], Bruno Neves [2], Jason Hallett [3], João Coutinho [1], Helena Passos [4], Mara Freire [1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

[2] Department of Medical Sciences and Institute of Biomedicine-iBiMED, University of Aveiro, Aveiro, Portugal

[3] Department of Chemical Engineering, Imperial College London, London, United Kingdom

[4] Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

(*) e-mail: ***carinypolesca@ua.pt***

Keywords: chicken feather, ionic liquids, keratin films.

ABSTRACT

The poultry-processing industry generates a significant amount of feather waste, which is generally disposed of through incineration or landfilling. However, this waste comprises around 90 wt % of keratin, a fibrous protein and one of the most abundant biopolymers in the environment. While keratin recovery from human hair and wool has been highly reported in the literature, its recovery from feather waste has not been thoroughly explored. From this perspective and considering the low solubility of this protein in the most common organic solvents, in this study, acetate-based ionic liquids (ILs) were used to dissolve chicken feathers and recover keratin, achieving a yield of 93 wt % under optimal conditions. Furthermore, the IL recovery and reuse were investigated, finding that it could be effectively reused for at least four cycles without affecting keratin yield. Aiming to understand the potential applications of keratin in the biomedical field, keratin films were developed, and their biological properties were investigated. Cytotoxicity tests were performed using different cells (macrophages, monocytes, keratinocytes, and fibroblasts), indicating that the keratin film has no toxicity for the evaluated cells. Additionally, the antioxidant and anti-inflammatory properties of the keratin films were demonstrated. Finally, in vitro wound healing assays were performed, demonstrating the effectiveness of keratin films in promoting wound healing in 16h. The developed research highlights the possibility of feather waste valorisation while contributing to the development of sustainable biomaterials with application in the biomedical field.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). C. Polesca acknowledges FCT- Fundação para a Ciência e a Tecnologia for the PhD grant with the reference UI/BD/151282/2021. H. Passos acknowledges FCT, I.P., for the researcher contract CEECIND/00831/2017, under the Scientific Employment Stimulus-Individual Call, 2017.

OP3.3 - A NOVEL BACTERIOPHAGE RECEPTOR BINDING PROTEIN FOR IMPROVED *SALMONELLA* DETECTION

Ana Brandão, Sílvio Santos *(*)*
CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal
LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

(*) e-mail: ***silviosantos@deb.uminho.pt***

Keywords: *Salmonella*, Diagnosis, Detection, Bacteriophages, RBP.

ABSTRACT

Salmonella is one of the most important foodborne pathogenic bacteria which can cause serious public health problems. The substantial health and economic impact of *Salmonella* infections, coupled with its antibiotic resistance demands the fast and reliable identification of this pathogen. Conventional approaches are time-consuming, present high detection limits, low specificity, and inability to differentiate viable from non-viable cells. These facts call for urgent improved detection methods to prevent the introduction of Salmonella into the food-chain and to provide timely guidance for clinical treatment and avoid disease progression. Due to their high specificity and binding ability, bacteriophages and their proteins can circumvent these limitations and provide the foundations for the development of novel cost-effective and improved diagnosis.

In this study, we employed bioinformatic tools to identify potential receptor-binding proteins within the genome of a sequenced bacteriophage. Selected genes were cloned into *Escherichia coli*, fused with a green fluorescent protein (EGFP) and assessed for their ability to bind and decorate *Salmonella* cells.

Results showed that phage protein gp27 exhibits strong binding affinity for *Salmonella* cells, enabling their identification under a fluorescent microscope. This protein demonstrated high specificity by effectively binding to *Salmonella* and not to other related genera. Its exceptional specificity minimizes the occurrence of false-positive results. Notably, this protein binds to *Salmonella* cells within a rapid 15-minute timeframe.

Our research unveils a novel bacteriophage receptor-binding protein with remarkable specificity for *Salmonella*. This breakthrough paves the way for the development of novel advanced diagnostic tools, promising faster and more reliable *Salmonella* detection. These advancements will significantly enhance food safety measures and mitigate the impact of *Salmonella*-related infections on public health and the economy.

Acknowledgements:
FCT under UIDB/04469/2020 unit and LA/P/0029/2020. Agendas/Alianças mobilizadoras para a Reindustrialização under SMARTgNOSTICS.

OP3.4 - CYTOPROTECTIVE GUARDIANS: QUANTIFYING THE CYTOPROTECTIVE EFFICIENCY OF EXTRACELLULAR VESICLES (EVS) AGAINST *STAPHYLOCOCCUS AUREUS* ALPHA HEMOLYSIN (HLA)

Diogo Gonçalves [1], Ricardo Silva [1], Beatriz Avó [1], Nuno Bernardes [1,2], Ana M. Azevedo [1,2], Sandra N. Pinto [1,(*)], Fábio Fernandes [1,2]

[1] iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Portugal

[2] Bioegineering Department, Instituto Superior Técnico, Portugal

*[(*)] e-mail: **sandrapinto@ist.utl.pt***

Keywords: Extracellular vesicles (EVs)*; Staphylococcus aureus infections*; *Alpha Hemolysin (Hlα)*; *Antivirulence therapeutic strategies.*

ABSTRACT

Methicillin-resistant *S. aureus* (MRSA) infections are a major public health concern, particularly in the context of hospital-acquired infections, being associated with high rates of mortality. The production of bacterial pore forming toxins (PFT) such as alpha hemolysin (Hlα) plays an important role in MRSA pathogenicity. Notably, a role of extracellular vesicles (EVs) as part of the innate immune system, and more specifically as decoys to sequester and inactivate bacterial toxins has been recently proposed.

Therefore, in this work we aimed to quantify the efficacy of EVs in inhibiting the toxicity of *S. aureus* alpha hemolysin (Hlα). Cytotoxicity assays were carried out against the L-929 mouse fibroblast cell line, employing commercially available *S. aureus* Hlα, and LC50 values were determined. *S. aureus* Hlα was found to have an LC50 of 174 nM in complete medium and 72 nM in EV-depleted medium, confirming that EV depletion promoted Hlα mediated cell death. Dramatic inhibition of Hlα permeabilizing activity by EVs was confirmed in permeabilization assays of both L-929 cells and giant unilamellar vesicles (GUV), in the presence and absence of isolated EVs. Importantly, modification of lipid composition of isolated EVs was found to potentiate the Hlα neutralization efficacy of these vesicles, further validating the potential of EVs in the framework of antivirulence therapeutic strategies for drug-resistant bacterial infections.

Acknowledgements:
This study was supported by funds received from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects SentinelEVs (EXPL/BTM-MAT/0910/2021) and DENDRICARE (2022.03627.PTDC); the project supporting the Research Unit Institute for Bioengineering and Biosciences (UIDB/04565/2020) and the project supporting Institute for Health and Bioeconomy, i4HB (LA/P/0140/2020).

OP3.5 - CAT DERIVED ANTIBODY FRAGMENTS AS A PROMISING STRATEGY FOR TREATMENT AND PREVENTION OF SARS-COV-2 INFECTION

Isa Moutinho [1,2,3]*, Mafalda Henriques [1,2], Sara Cardoso [1,2], Teresa Coutinho [1,2], Luís Tavares [1,2], Solange Gil [1,2], Telmo Nunes [1,2], Frederico Aires-da-Silva [1,2]

[1] Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)
[3] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Portugal

*(*) e-mail: **isasoraia@fmv.ulisboa.pt***

Keywords: SARS-CoV-2; COVID-19; cats; recombinant antibodies; One Health

ABSTRACT

SARS-CoV-2 and the resulting disease, COVID-19, are one of the greatest public health concerns worldwide. Antibodies against the virus have been detected in several species. Cats have received special attention due to their close contact with humans and high susceptibility to infection. According to published studies, cats exhibited rapid recovery and a low incidence of severe disease, suggesting the development of a potent immune response against the virus. In this study, we aimed to characterize the humoral immune response of cats to SARS-CoV-2 to develop potent and broadly neutralizing antibody fragments for treatment and prevention of COVID-19 disease.

A biobank of serum samples was constructed from cats admitted to the Veterinary School Hospital (HEV) of FMV-ULisboa. These samples were tested against SARS-CoV-2 Spike- S and RBD proteins from alpha, delta and omicron variants by ELISA (Enzyme-Linked Immunosorbent Assay). Positive samples were subjected to a Surrogate Virus Neutralization Test (sVNT). Finally, serum samples with high neutralizing activity were tested in an infection assay against viral particles pseudotyped with the S protein from different SARS-CoV-2 variants.

Of the 614 cat serum samples collected, 97 (15.8%) tested positive in the ELISA assay. These 97 samples were tested in the sVNT and 21 (21.6%) presented neutralizing antibodies, with four serum samples neutralizing the alpha, delta and omicron variants. The serum samples highlighted in the ELISA and sVNT as having a higher antibody titer showed a greater ability to inhibit infection in pseudotyped virus assays.

Our study attests to the susceptibility of cats to SARS-CoV-2 infection and the development of potent antibodies in these animals. Thus, from cats naturally infected with SARS-CoV-2, we are developing a new generation of antibodies for COVID-19, a novel therapeutic strategy, not only for humans, but also for the broad spectrum of susceptible species.

Acknowledgements:
This work was supported by the Portuguese Funding Agency, Fundação para a Ciência e Tecnologia, FCT IP, (PTDC/CVTCVT/0149/2021 and 2020.08209.BD,). CIISA has provided support via Project UIDB/00276/2020, funded by FCT, and LA/P/0059/2020-AL4AnimalS.

OP3.6 - NEW FLUOROQUINOLONE-PHENOTHIAZINE HYBRIDS WITH ANTIBACTERIAL PROPERTIES AGAINST *STAPHYLOCOCCUS AUREUS*

Marina Cassago Posso [1(*)], ***João Lourenço Serrano*** [1,2], ***Paulo Almeida*** [1,2], ***Fernanda Domingues*** [1,2], ***Samuel Silvestre*** [1,2,3], ***Susana Ferreira*** [1,2]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *Departamento de Química. Facultade de Ciências. Universidade da Beira Interior, Covilhã, Portugal*

[3] *CNC- Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.*

(*) *e-mail: marina.posso@ubi.pt*

Keywords: Hybrids fluoroquinolone-phenothiazine, Biofilm inhibition, Antimicrobial resistance.

ABSTRACT

The One Health concept recognizes the interconnectedness of human, animal, and environmental health, emphasizing the need for collaboration and integration across these sectors to effectively address health challenges, such as antimicrobial resistance (AR). Efflux pumps are specialized membrane transporters that are crucial in conferring resistance to antimicrobial agents, including multidrug resistance, and are therefore implicated in the challenge of combating drug-resistant infections. Therefore, targeting efflux pumps and developing inhibitors are promising approaches to combat antimicrobial resistance and restore the effectiveness of antibiotics in treating bacterial infections. Phenothiazines, known for their efflux pump inhibitory and anti-biofilm formation properties, may act as effective antibiotic adjuvants. Thus, in this work, two fluoroquinolones, ciprofloxacin and norfloxacin, were hybridized with phenothiazines to develop molecules with antibacterial and efflux pump inhibitory properties. This strategy enabled molecules to be designed to act synergistically while allowing them to simultaneously reach their intended target. The synthesis of these hybrid molecules involved nucleophilic substitution reactions and posterior conversion into their maleate salts to improve aqueous solubility. Then, the antimicrobial activity of fluoroquinolones, alone and their hybrids was evaluated, focusing on their minimum inhibitory concentrations (MIC), time-kill curves, post-antibiotic effects, mutation frequency, inhibitory activity of efflux pump, and anti-biofilm activity. The findings highlight the potential of six of the eight synthesized hybrids as effective agents against bacterial growth and as potent inhibitors of biofilm formation. Additionally, these novel molecules led to a lower mutation frequency compared to the reference fluoroquinolone, and showed to improve ethidium bromide accumulation, which indicates that the synthesized hybrids can inhibit efflux pumps. The results from this work can contribute to the ongoing efforts to develop innovative strategies to combat bacterial infections and provide potential alternatives in combating antimicrobial resistance, recognizing the multifaceted nature of this critical issue.

Acknowledgements:
Staphylococcus aureus SA1199 and SA1199A were kindly provided by Professor Dr. Lorena Tuchscherr de Hauschopp (Jena University Hospital-Germany). João L. Serrano acknowledges a doctoral fellowship grant from the FCT (SFRH/BD/148028/2019). This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

OP4.1 - CONTROLLING DIARRHEAGENIC *E. COLI* WITH BACTERIOPHAGES: FACTS AND CHALLENGES

Francisca Rodrigues [1,2]***, Isidro García-Meniño*** [3,4,5]***, Azucena Mora*** [3,4]***, Luís Daniel Melo*** [1,2]***, Ana Oliveira*** [1,2(*)]

[1] *CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS- Associate Laboratory, 4800-122 Guimarães, Portugal*

[3] *Laboratorio de Referencia de Escherichia coli (LREC), Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain*

[4] *Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago, Spain*

[5] *Department for Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany*

[(*)] *e-mail:* ***anaoliveira@deb.uminho.pt***

Keywords: Enterotoxigenic *E.coli*; bacteriophages; anti-phage defense systems.

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) colonizes the intestine, causing severe diarrhoea in humans and animals. The rise of antibiotic resistances and limitation on their use demands news strategies to tackle this pathology.

Bacteriophages (phages), viruses specifically infecting bacteria and harmless to animals and plants, are a promising antibacterial tool. Although studies support their ability to efficiently overcome ETEC infections, they have been shown to be highly strain-specific. If this can be associated with the presence of anti-phage defense systems (APDS) in ETEC genomes, it is also true that phages can counter-evolve to escape APDS.

This work aimed to define phage cocktail with broader lytic spectra, capable of overcoming APDS of ETEC, enhancing phage efficacy.

We firstly sequenced 29 ETEC strains from our collection to search for the presence of APDS in their genomes. Then, we performed phage isolation and the subsequent *in vitro* and genomic characterization: i) evaluation of lytic spectra against ETEC collection; ii) whole-genome sequencing and phage safety evaluation (absence of undesirable genes) iii)presence of proteins responsible for escaping the main APDS.

We were able to identify distinct mechanisms supporting APDS. Bacterial proteins that prevent the entry of DNA from phages (CRISPR-Cas-related proteins) or that enable the cut of phage nucleic acids (restriction-modification enzymes) were detected, however, most of them were related with the induction of “abortive infection” events (e.g. toxin-antitoxin systems).

We also isolated 3 phages, SUS35, SUS42 and SUS65, which proved to be safe for therapy and to encode proteins enabling to escape APDS, inclusively against “abortive infection”.

Phage-host interaction mechanisms must be considered when preparing phage- based products for therapy. This work clearly indicates that a strict selection of phages, or the construction of synthetic phages with desired traits will be a turning point in their versatility to fight against ETEC infections.

OP4.2 - A PORTRAIT OF THE INTERACTION ESTABLISHED BETWEEN VAGINAL COLONIZING *LACTOBACILLUS* SPECIES AND THE PATHOGENIC YEASTS *CANDIDA ALBICANS* AND *CANDIDA GLABRATA*

Nuno A Pedro [1,2], Gabriela Fontebasso [1,2], Sandra N Pinto [1,2], Dalila Mil-Homens [1,2], Arsénio Fialho [1,2], Nuno Pereira Mira [1,2]

[1] iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal

[2] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Portugal

(*) e-mail: nuno.mira@tecnico.ulisboa.pt

Keywords: OMICS analyses; vaginal micro and mycobiomes; anti-Candida treatments; ecological interference.

ABSTRACT

The success of *C. glabrata* and *C. albicans* as human commensals or pathogens depends on their ability to cope with a competing bacterial microflora that, in the vaginal tract, is dominated by *Lactobacillus*. In this work we have studied, in detail, the interaction established between *C. albicans/C. glabrata* and the poorly characterized vaginal species *L. gasseri*. While in co-culture (both in planktonic or in biofilms) *L. gasseri* reduced growth rate, viability and pathogenesis of *C. albicans*/*C. glabrata* against epithelial cells and the wax *Galleria mellonella* (with the first species being more affected by co-cultivation in planktonic growth and the second in the mixed biofilm). For the first time we could also demonstrate that this anti-*Candida* effect prompted by *L. gasseri*, both in planktonic and in biofilms, is greatly potentiated by the availability of acetate in the environment. Gathering results from dual RNA-seq and phenOMICs experiments (undertaken in planktonic and in biofilms) we could identify a set of *C. glabrata* mutants highly sensitive to the presence of *L. gasseri*, while also identifying the genes of both species more actively transcribed during the co-cultivation. The results of these global analyses will be discussed in this work, providing a portrait into the ecological interference existing between vaginal dominant lactobacilli species and pathogens in vivo. The results herein obtained also provide many new potential therapeutic targets that can be used to reduce *Candida* competitiveness in the vaginal tract and foster new anti-*Candida* treatments based on probiotics.

Acknowledgements:
This study was supported by funds received from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project LactoCan (PTDC/BIA-MIC/31515/2017); the project supporting the Research Unit Institute for Bioengineering and Biosciences (UIDB/04565/2020) and the project supporting Institute for Health and Bioeconomy, i4HB (LA/P/0140/2020). FCT is also acknowledged for funding a PhD grant (PD/BD/143026/2018) to NAP through the doctoral program DP_AEM.

OP4.3 - PROBE-BASED METAGENOMICS: AN ADDED VALUE FOR CLINICAL DECISION?

Luís Coelho [1(*)], Rita Ferreira [1], Daniel Sobral [1], João Dourado [1], Joana Isidro [1], Verónica Mixão [1], Miguel Pinto [1], Alexandra Nunes [1], Sílvia Duarte [2], Luís Vieira [2], Vítor Borges [1], João Paulo Gomes [1]

[1] Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal

[2] Technology and Innovation Unit, Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal

*[(*)] e-mail: **luis.coelho@insa.min-saude.pt***

Keywords: Metagenomics, NGS, Pathogen detection, Difficult Diagnoses, CSF.

ABSTRACT

In clinical settings, metagenomics Next-Generation Sequencing (mNGS) may detect accurately and rapidly unsuspected, unidentified and uncultivatable organisms, resulting in higher recovery outcomes due to timely and more specific treatments. However, mNGS presents several challenges, such as high costs per sample, a small microbial/human DNA ratio, and demanding laboratorial and computational infrastructures.

We tested two recently developed Illumina panels for probe-based pathogen enrichment and detection, the Respiratory and the Urinary Pathogen ID/AMR panels (RPIP and UPIP), which together target 383 pathogenic agents (virus, bacteria, fungi and parasites). We selected 81 clinical samples of different nature (e.g., CSF, plasma, serum, urine, swabs, biopsies, etc.), for which at least one pathogen, mostly virus, had been previously identified by PCR in INSA's national reference laboratories. Data analysis was performed using both Illumina and in-house bioinformatics pipelines, with panels' performance being assessed in a combined fashion to mimic a real scenario where the panels would be used simultaneously to cover all 383 pathogens.

Herein, the Illumina's pipelines detected 66.3% of the pathogens (highest value was 74.6%, for virus). Since we had previously developed an in-house bioinformatics pipeline for viral metagenomics detection (INSaFLU-TELEVIR, https://insaflu.insa.pt/), we assessed its usefulness to improve viral detection rate. This strategy increased Illumina accuracy for virus detection from 74.6% to 84.7%. As such, we are currently developing a TELEVIR-like pipeline for bacteria to assess if a similar increment in detection is achieved. Finally, we observed some heterogeneity in the pathogen identification accuracy among different sample types, ranging from 67% in CSF to 100% in plasma.

Although these results rely on preliminary data, they provide strong evidence that these Illumina panels, coupled with complementary downstream pipelines, may constitute a powerful tool to help difficult diagnosis and support clinical decision, in which timely and specific treatment may be decisive for patients' clinical recovery.

OP4.4 - HOW DO METALLOENZYMES HELP IN THE FIGHT AGAINST CLOSTRIDIOIDES DIFFICILE'S INFECTION?

Filipe Folgosa [1]*, Maria C. Martins [1], Nicolas Kint [2], Carolina A. Feliciano [1], Claire Morvan [2], Susana F. Fernandes [1], Bruno Dupuy [2], Isabelle Martin-Verstraete [2], Miguel Teixeira [1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

[2] Laboratoire Pathogenèses des Bactéries Anaérobies, Institut Pasteur, UMR CNRS 2001, Université de Paris, Paris, France

(*) e-mail: f.folgosa@itqb.unl.pt

Keywords: Oxidative stress, Hospital acquired infections, metalloproteins, human pathogens

ABSTRACT

Clostridioides difficile is the most prevalent pathogen among all healthcare-associated infections. This anaerobic bacterium can colonize the human gut, typically after exposure to agents that disrupt the normal gut microbiota, such as antibiotics. In the gut, *C. difficile* is subjected to oxygen, which it has to eliminate for survival. Its genome encodes for several proteins that should be capable to tackle the effects of oxygen as well as its derived reactive species. This group of proteins includes two types of flavodiiron proteins (FdpA and FdpF), two reverse rubrerithryns and catalases[1]. Besides, some FDPs also reduce NO to N_2O, which is a relevant resistance mechanism towards the human innate immune system[2,3]. Our goal is to understand how these proteins work together in the cellular context, leading to *C. difficile* infection.

Extensive biochemical studies demonstrated that FdpF and both revRbrs harbor O_2- and H_2O_2-reductase activities *in vitro*, whereas FdpA mainly acts as an O_2-reductase[4,5]. Similar analyses were performed in cellular extracts of wild-type and multiple deletion mutant strains, to understand the effect of each protein in the entire cellular metabolism upon oxygen exposure. We also showed, *in vivo*, that the growth of a *C. difficile* fdpA mutant is affected at 0.4% O_2, whereas inactivation of both revRbrs leads to a growth defect above 0.1% O_2[5]. Our results demonstrate a key role for revRbrs, FdpA, and FdpF proteins in the ability of *C. difficile* to grow in the presence of physiological O_2 tensions such as those encountered in the colon.

References

[1] Morvan *et al*, Environment. Microbiol., 23, 4112-4125, 2021
[2] Romão *et al*, J. Biol. Inorg. Chem., 21,39-52, 2016
[3] Martins *et al*, Free Rad. Biol. Med., 140, 36-60, 2019
[4] Folgosa et al, Sci. Rep., 8, 10164, 2018
[5] Kint *et al*, mBio, 11, e01559-20, 2020

OP4.5 - UNVEILING THE PROTECTIVE MECHANISMS OF PYRUVATE KINASE DEFICIENCY AGAINST MALARIA: TRANSFORMING VULNERABILITY INTO RESILIENCE

Ana Balau [1], Daniel Sobral [2], Patrícia Abrantes [1], Maria Carvalho [1], Inês Morais [1], Verónica Mixão [2], Márcia M. Medeiros [1], Filomena A de Carvalho [3], Maria Alexandra Antunes [4], João Paulo Gomes [2], Sandra Antunes [1], Ana Paula Arez [1(*)]

[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA Lisboa, Lisbon, Portugal

[2] Genomics and Bioinformatics Unit, Department of Infectious Diseases, Instituto Nacional de Saúde Dr Ricardo Jorge, INSA, Lisbon, Portugal.

[3] Instituto de Medicina Molecular, Faculdade Medicina, Universidade Lisboa, Lisbon, Portugal

[4] Centro de Química Estrutural, Instituto Superior Técnico, Universidade Lisboa, Lisbon, Portugal

[(*)] e-mail: aparez@ihmt.unl.pt

Keywords: *Plasmodium falciparum*; trophozoite; transcriptome; Nanopore technology; infection; erythrocyte; enzymopathy; 2,3-diphosphoglycerate.

ABSTRACT

Malaria remains a global health challenge, and innovative strategies to control it are urgently needed. Exploring the interplay between the malaria parasite and host red blood cells (RBCs) offers opportunities for novel antimalarial interventions. Pyruvate kinase deficiency (PKD), characterized by reduced intracellular ATP levels and heightened 2,3-diphosphoglycerate (2,3-DPG) concentration, has been associated with malaria resistance. Elevated 2,3-DPG levels, a specific mammalian metabolite, may hinder glycolysis, prompting us to hypothesise its potential contribution to PKD-mediated protection. We investigated the impact of the extracellular supplementation of 2,3-DPG on the *Plasmodium falciparum* intraerythrocytic developmental cycle *in vitro*. Results showed a hindrance of parasite growth, likely attributed to an impediment in parasite maturation, resulting in significantly less progeny from 2,3-DPG-treated parasites. Untargeted metabolomic analysis revealed that the metabolic profile of treated infected cells became more similar to that of non-infected cells. To assess whether heightened 2,3-DPG affected RBC membrane properties and parasite invasion, we analysed alterations in membrane structure, cell morphology, and biomechanics using Atomic Force Microscopy. 2,3-DPG treatment induced mild modifications in RBC membranes compared to the profound influence exerted by the parasite on host cells. Mild modification of non-infected RBCs' height and stiffness did not impact the egress or invasion of parasite. Since these findings strongly imply a direct influence of 2,3-DPG on the parasite, we analysed differential gene expression and the transcriptomic profile of schizogonic *P. falciparum* trophozoites, the most metabolically and transcriptionally active parasite asexual stage, from *in vitro* cultures submitted or not submitted to the action of 2,3-DPG, using Nanopore Sequencing Technology. 71 genes exhibited significant differential expression from the non-exposed parasites to 2,3-DPG and the parasite response seem to influence several cellular components and binding and channel activity molecular functions.

Acknowledgements:
Fundação para a Ciência e a Tecnologia for funds to projects PTDC_BIA-CEL_28456_2017, GHTM - UID/04413/2020 and LA-REAL – LA/P/0117/2020

OP4.6 - CHARACTERIZATION OF BIOFILM FORMATION OF BACTERIA ASSOCIATED WITH THE MIDGUT OF ANOPHELES MOSQUITO VECTOR OF MALARIA

Margarida Marques [1,2]***, Sofia Santos Costa*** [1]***, Sandra N Pinto*** [2]***, Henrique Silveira*** [1]*

[1] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal*

[2] *iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico - Lisbon (Portugal)*

(*) *e-mail:* ***hsilveira@ihmt.unl.pt***

Keywords: malaria, microbiota, *Pseudomonas spp., Serratia marcescens*, bacterial biofilms

ABSTRACT

Malaria is the most prevalent vector-borne disease, caused by *Plasmodium* parasites, transmitted by female *Anopheles* mosquitoes. One malaria control approach involves the *Anopheles* midgut microbiota manipulation. According to the literature, *Pseudomonas* spp. may reduce *Plasmodium* infection in *Anopheles* mosquitoes, which could be associated with their high capacity of biofilm production. Bacterial biofilms at the midgut epithelium may restrict the ookinetes' movement, therefore with potential application as a malaria transmission blocking tool.

Our work focuses on the capacity of bacteria isolated from *Anopheles* midguts to form biofilms and their characterization, with emphasis on *Pseudomonas* and *Serratia* species.

Anopheles midguts were dissected and macerated in phosphate buffered saline. Aliquots were taken, diluted and plated in different selective and non-selective media. Morphologically distinct colonies were selected, characterized by Gram staining and oxidase test, and identified by 16S rDNA Sanger sequencing. One *Pseudomonas mendocina* and several *Serratia marcescens* isolates were further studied regarding their antibiotic susceptibility profiles by disc diffusion/E-tests and their ability to produce biofilms in different media. Biofilms were characterized by microbiological and biophysical tools, including quantification of biofilm maximum height using confocal microscopy. We identified several bacterial genera colonizing the midgut of six *Anopheles* species, including *Serratia*, *Pseudomonas* and *Elizabethkingia*. We observed, using different tools (e.g., crystal violet assay and confocal microscopy), that *S. marcescens* isolates formed poorly structured biofilms. In contrast, *P. mendocina* produced highly thick and heterogenous biofilms (39.00±22.44 μm maximum height) and that was highly dependent on the growth conditions. Moreover, the ability of the *P. mendocina* isolate to efficiently colonize the *An. stephensi* midgut was confirmed in 72-hour colonization assays.

Overall, our results highlight that the isolated *P. mendocina* has potential as a tool for the development of malaria control strategies. Further research is needed to comprehend biofilm formation mechanisms and its impact on malaria transmission.

Acknowledgements:
Fundação para a Ciência e a Tecnologia (FCT, Portugal) for support to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020), and for support to iBB (2022.03627.PTDC).

OP4.7 - DECIPHERING HOST-RICKETTSIA INTERACTIONS: MOONLIGHTING APRC RECRUITS HUMAN COMPLEMENT REGULATOR C4BP ACTING AS AN EVASIN

Ana Luísa Matos [1,2*]***, Isaura Simões*** [1,3]

[1]*CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.*
[2]*PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal*
[3]*IIIUC-Institute of Interdisciplinary Research, University of Coimbra, Coimbra, Portugal.*

(*) *e-mail:* ***almatos@cnc.uc.pt***

Keywords: *Rickettsia*, host-pathogen interactions, APRc, complement, immune evasion, retropepsin

ABSTRACT

Rickettsia is a genus of obligate intracellular bacteria transmitted by arthropod vectors, causing from mild to life-threatening diseases. The re-emergence and increased geographical distribution of these pathogens alongside the lack of protective vaccines are causing increased concern due to their impact on global health. One potential therapeutic target is the aspartic protease from *Rickettsia conorii* (APRc), a highly conserved membrane embedded retropepsin-like homologue. APRc modulates *Rickettsia* surface virulence factors, binds immunoglobulins, mediates serum resistance, and preliminary data indicates that APRc targets other serum components. Herein, we provide evidence on the complement regulator C4 binding protein (C4BP) as an APRc interactor and reveal how this interaction may facilitate rickettsiae immune evasion.

APRc interaction with C4BP was evaluated by pull-down assays and Western blot. APRc-C4BP binding was also evaluated by native gel electrophoresis/SEC. C4BP deposition at the surface of *R. massiliae* was analyzed by flow cytometry, whole-cell ELISA, and surface-binding assays. C4BP cofactor activity with Factor I was assessed in the presence of APRc and purified C3 or C4 by Western blot. APRc impact on complement activation was assessed at C5b-9 complex formation levels using a modified ELISA.

We demonstrated that APRc binds to and forms complexes with C4BP. Moreover, we confirmed C4BP-binding at rickettsial surface in the presence of NHS and purified C4BP. Our results showed that C4BP maintains its cofactor activity in the presence of APRc, resulting in the cleavage of complement C3 and C4. Additionally, APRc inhibits activation of the classical and lectin complement pathways, being independent of APRc's catalytic activity.

We confirmed that APRc targets the complement inhibitor C4BP, maintaining its cofactor activity, and impacts complement activation. We showed C4BP binding at the surface of *Rickettsia,* contributing to acknowledging the multiple functions of APRc and revealing a novel immune evasion mechanism used by *Rickettsia*.

Acknowledgements:
Funding:UIDB/04539/2020,UIDP/04539/2020,LA/P/0058/2020,FCT-Fellowship SFRH/BD/12805/2022

OP4.8 - THE SMALL NON-CODING RNA NCRNA3 IS INVOLVED IN ANTIBIOTIC RESISTANCE IN BACTERIA OF THE BURKHOLDERIA CEPACIA COMPLEX

Gonçalo R. Matos [1]*, Joana R. Feliciano [1,2], Beatriz Jesus [1], Jorge H. Leitão [1,2]

[1] Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Oeiras, Portugal.
[2] Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Avenida Padre Cruz, Lisbon, Portugal.
[3] Laboratory of Microbiology & Infectious Diseases, The Rockefeller University, New York, New York, United States of America.

(*) e-mail: ***goncalormatos@tecnico.ulisboa.pt***

Keywords: *Burkholderia cepacia* complex; Antibiotic resistance; Small non-coding RNAs

ABSTRACT

Bacteria of the *Burkholderia cepacia* complex (Bcc) are a group of human opportunistic pathogens that are responsible for severe clinical prognosis in immunocompromised patients and can chronically persist in the infected host. These bacteria are particularly feared because of their intrinsic resistance to multiple antibiotics. Until now, the multidrug resistance exhibited by these opportunistic pathogens was largely attributed to the existence of a considerable number of efflux pumps in these organisms. Several antibiotic resistance genes have been shown to be subject to coordinated regulation through complex mechanisms mediated by small non-coding RNAs (sRNAs) that promote activation of gene expression in response to antibiotic exposure. These RNA molecules can regulate gene expression by interacting with mRNA transcripts and with proteins. Although several sRNAs have been identified in *B. cenocepacia*, their influence in antibiotic resistance is still poorly characterized. In this study, bioinformatics tools predicted that 78 *B. cenocepacia* sRNAs have at least one target involved in antibiotic resistance. The sRNA ncRNA3 was predicted to target *dfrA*, a gene involved in trimethoprim resistance. When overexpressed, the sRNA ncRNA3 increased the *B. cenocepacia* resistance to trimethoprim. The *dfrA* gene was also upregulated when this sRNA was overexpressed, and the interaction between them was confirmed *in vitro* by Electrophoretic mobility shift assay. Furthermore, at subinhibitory trimethoprim concentrations, the expression levels of ncRNA3 and its *dfrA* target were increased in *B. cenocepacia*. These results corroborate the importance of sRNAs in the regulation of antibiotic resistance, and to the best of our knowledge this is the first report of a sRNA directly involved either in trimethoprim resistance or in *B. cenocepacia* antibiotic resistance.

Acknowledgements:
This work was partially funded by FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project FCT 2022.07091.PTDC; the projects UIDB/04565/2020 and UIDP/04565/2020 of the Research Unit Institute for Bioengineering and Biosciences – iBB and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. GRM acknowledges the BL150/2023_IST-ID grant in the scope of the project FCT 2022.07091.PTDC.

OP4.9 - METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* (MSSA) NASAL SCREENING AT ICU ADMISSION AS A PREDICTIVE TOOL FOR SUBSEQUENT MSSA RESPIRATORY INFECTION

Teresa Conceição [1]*, Mafalda Felgueiras [2], Silvia Ferreira [2], Marisa Simões [2], Daniela Carvalho [2], Angelina Lameirão [3], Ana Marques [3], Dulce Pascoalinho [4], Maria Paula Gonçalves [4], Ana Fernandes [4], Hermínia de Lencastre [1,5], Valquíria Alves [2]

[1]Laboratório de Genética Molecular, Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa (UNL), Oeiras, Portugal;
[2]Unidade de Saúde Local de Matosinhos – Hospital Pedro Hispano, Matosinhos, Portugal; [3]Centro Hospitalar Vila Nova de Gaia/Espinho (CEVNG-Espinho), Vila Nova de Gaia, Portugal; [4]Unidade Local de Saúde do Litoral Alentejano, EPE (ULSLA), Santiago do Cacém, Portugal;
[5]Laboratory of Microbiology & Infectious Diseases, The Rockefeller University, New York, USA.

(*) e-mail: ***teresagc@itqb.unl.pt***

Keywords: Methicillin-sensitive *Staphylococcus aureus*, nasal carriage, respiratory infection, ICU

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal screening is a well-established practice in healthcare settings, guiding infection control and support to de-escalating therapeutic decisions. Conversely, methicillin-sensitive *Staphylococcus aureus* (MSSA) screening is not commonly performed, resulting in limited data to support MSSA colonization as a predictor of invasive infections. This study aimed to determine MSSA/MRSA nasal colonization rate among intensive care units (ICU) patients, assess the risk for MSSA respiratory infections, and explore genetic relatedness between colonizing and infecting isolates.

For one year (March 2021-2022), a multicenter study was conducted in three Portuguese hospitals, involving 1,394 ICU patients. MSSA/MRSA colonization screening was performed at admission, and infection development was further monitored to calculate MSSA/MRSA colonization and infection rates. Molecular characterization of MSSA included *spa* typing and PCR detection of *mecA* and Panton Valentine leucocidin determinants.

Among the 2,089 patients admitted to the ICUs during the study, 66.7% (n=1,394) went through MSSA/MRSA screening. Of the 366 positive cases, 24.8% (n=345) were colonized with MSSA, while only 1.5% (n=21) carried MRSA. The overall MSSA respiratory infection rate was 3% (n=65), with 23% (n=15) developing MSSA bacteremia. Half (51%, n=26) of the 51 MSSA infected patients studied so far, were already MSSA carriers upon ICU admission and in nearly all of them (96.1%, n=25) MSSA infection isolate shared the same *spa* type as the initial colonizing strain. The majority of MSSA (56%) belonged to three clonal lineages: CC398 (n=8, 30.8%), CC5 (n=4, 15.4 %) and CC8 (n=2,7.7%). None of the isolates contained PVL. Characterization of additional MSSA isolates from colonization in non-infected patients is ongoing.

Our results showed a high MSSA colonization rate in ICU patients (24.8%). In most cases, MSSA infection and carriage isolates belonged to the same clonal lineage, underscoring the potential of MSSA nasal screening as a predictive tool for subsequent invasive infections.

OP4.10 - SCC*mec* ACQUISITION IS LINKED TO *STAPHYLOCOCCUS EPIDERMIDIS* BLOODSTREAM INFECTION OUTBREAKS IN A NEONATE INTENSIVE CARE UNIT (NICU)

Nuno Alexandre Faria [1*], ***Roberta Filipini Rampelotto*** [1,2], ***Rosmari Hörner*** [2], ***Maria Miragaia*** [1]
[1] *Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB-NOVA, Oeiras, Portugal.*
[2] *Department of Clinical and Toxicological Analysis, Federal University of Santa Maria, Brazil*

(*) *e-mail:* ***nfaria@itqb.unl.pt***

Keywords: *Staphylococcus epidermidis*, MRSE, neonatal sepsis.

ABSTRACT

Bloodstream infections caused by *S. epidermidis* are the most frequent and serious neonatal infections. However, *S. epidermidis* population structure and evolution in NICU is poorly explored. Herein, we aimed to characterize *S. epidermidis* transmission dynamics and genomic events occurring in NICUs.

S. epidermidis collected from bloodstream infections in newborns in a hospital in Brazil (n=55), over one year (2016/2017) were analyzed. Species were identified by *tuf* sequencing and the whole genome sequencing obtained by Illumina. Antimicrobial resistance and virulence genes content were determined using ResFinder and Abricate. Multilocus sequence type (ST) and staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined *in silico*, and isolates relatedness inferred by SNPs and BLAST analysis.

The methicillin resistant *S. epidermidis* (MRSE) rate in the NICU was 83.64% and mortality reached 14.9%. ST2 was the prevalent clonal type (52%). The remaining isolates belonged to 9 STs, belonging to the A/C cluster. ST2 isolates carried genes involved in biofilm production (*ica embp*, *sdrF* and *sdrH*) and antibiotic resistance (*mecA*, *bla*Z, *fosB*, *dfrC*, and aac(6')-aph(2'')). At least 4 different outbreaks (<44 SNPs) occurred overtime caused by MRSE strains of ST2 or ST2 SLVs (ST6). Each ST2 outbreak was associated to a distinct SCC*mec* type structures.

During outbreaks, *S. epidermidis* acquired/lost *ermC*, pUB110/pHD104-2, SE-RI-Fus and Sh- FabI, conferring resistance to macrolides, aminoglycosides, fusidic acid and triclosan, respectively, and the ACME, involved in virulence. Moreover, we observed a partial deletion of repeat regions of Aap and SdrH - proteins involved in biofilm formation.

NICU bloodstream infections resulted from multiple ST2 *S. epidermidis* outbreaks. During outbreaks *S. epidermidis* suffered multiple deletion/acquisition events with implication in antimicrobial resistance and pathogenicity. In particular, SCC*mec* acquisition was in the origin of every *S. epidermidis* NICU outbreak, suggesting this is a key event for *S. epidermidis* pathogenicity.

OP4.11 - EMERGING THREAT: RESISTANCE TO CEFTAZIDIME-AVIBACTAM AND OTHER LAST-LINE ANTIBIOTICS AMONGST CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* IN PORTUGAL

Ana Beatriz Gonçalves [1,2]***, Valquíria Alves*** [3,4]***, Isabel Neves*** [4]***, Maria Antónia Read*** [3]***, Luísa Peixe*** [1,2]***, Ângela Novais*** [1,2(*)]

[1] *Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal.*

[2] *UCIBIO – Applied Molecular Biosciences Unit, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.*

[3] *Clinical Microbiology Laboratory, Local Healthcare Unit, Matosinhos, Portugal.*

[4] *Infection Control Unit, Local Healthcare Unit, Matosinhos, Portugal.*

(*) e-mail: aamorim@ff.up.pt

Keywords: Multidrug resistance; Carbapenemase; β-lactam/β-lactamase inhibitor; High-risk clones; last-resource antibiotics.

ABSTRACT

Portugal registered a ca. 40 times increase (from 0.3% to 11.6%) in infection rates by carbapenem-resistant *Klebsiella pneumoniae* (CR-*Kp*) between 2011 and 2021. As a consequence, ceftazidime-avibactam (CAZ-AVI) is increasingly used to treat infections by CR-*Kp*. We investigated phenotypic/genotypic CAZ-AVI resistance among CR-*Kp* infection isolates identified in a Portuguese hospital in a recent four-year period. We screened CAZ-AVI susceptibility by E-test (gradient diffusion) in 772 CR-*Kp* isolates resistant to at least one carbapenem (May2019-Aug2023). Species identification and AST were performed by MALDI-TOF MS and VITEK 2 (bioMérieux). Isolates resistant to CAZ-AVI identified in hospitalized patients were further characterized by extended antibiotic susceptibility testing (disk diffusion), Fourier-transformed Infrared spectroscopy, *wzi* gene sequencing, and MLST. Resistance mechanisms were investigated by WGS in representative isolates. Epidemiological/clinical data were collected and analyzed.We detected 1.4% (11/772) of CR-*Kp* isolates resistant to CAZ-AVI (MIC=12-256mg/L, EUCAST) in hospitalized patients. CAZ-AVI resistance was confirmed by disk diffusion and molecular methods in 7/11 isolates (64%; MIC=24-256mg/L). All isolates were resistant to ertapenem and variably resistant to imipenem (43%)/meropenem (58%). Of note, isolates were additionally resistant to other last-resource options as cefiderocol (86%), imipenem-relebactam (43%) or meropenem-vaborbactam (29%), concomitantly with fosfomycin (100%), ciprofloxacin and piperacillin-tazobactam (57% each), and trimethoprim-sulfamethoxazole (43%). Resistance to CAZ-AVI was associated with production of IMP-22, KPC-3 variants (KPC-31, KPC-66) or KPC-3/DHA-1 combined with porin deficiencies (OmpK35/OmpK36). Two isolates were identified after treatment with CAZ-AVI. Isolates belonged to six *Kp* lineages (ST147-KL64, ST37-KL38, ST469-KL139, ST29-KL30, ST45-KL62, ST323-KL21). CAZ-AVI resistance is associated with diverse resistance mechanisms and clones, including in MDR high-risk lineages (e.g. ST147-KL64). The detection of strains resistant to most available therapeutic options, including last-resource antibiotics not yet introduced in clinical practice (e.g. cefiderocol, imipenem-relebactam) represents.

OP4.12 - CEFOTAXIME-RESISTANT *ESCHERICHIA COLI* FROM URINE INFECTIONS AND ENVIRONMENTAL WATERS: A COMPARATIVE ANALYSIS

Joana Abreu Silva [1(*)]***, Catarina Ferreira*** [1]***, Célia M. Manaia*** [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal.*

(*) e-mail: jpsilva@ucp.pt

Keywords: *Escherichia coli*; Isolation habitat; Phylogeny; Genetic diversity.

ABSTRACT

Aquatic environments are key niches for the evolution and dissemination of antibiotic resistant bacteria. However, the drivers of and bottlenecks for the survival of clinically important lineages of *Escherichia coli* (*E. coli*) in wastewater and freshwater are not fully understood, limiting our capacity to assess transmission risks.

We used phenotypic characterization and comparative genomics to infer phylogenetic and genomic relationships among clinical (n=52, routine urine samples) and (waste)water (n=50, municipal wastewater, hospital effluent or surface water samples) cefotaxime-resistant *E. coli* isolates. We screened stress tolerance, antibiotic resistance, virulence, and plasmid profiles. The survival rate of bacterial cultures exposed to temperatures >50 ºC (30 min), ultraviolet (UV-C) irradiation (30 to 90 sec), or up to 400 mM of hydrogen peroxide (H_2O_2, 15 min) was not significantly different between isolation sources, nor was it associated with tested phenotypic or genotypic traits.

Whole genome sequence analysis (n=54) confirmed the high phylogenetic proximity of clinical and (waste)water isolates. Were identified 20 multilocus sequence types (ST), 11 of which were represented by a single strain. Inter-phylogroup comparisons of Gene Repertoire Relatedness values revealed distinct gene repertoires. Antibiotic resistance genes were identified using CARD and ResFinder databases. Carbapenem and colistin resistance encoding genes were found only in two *E. coli* isolated from urine infections (ST12, phylogroup B2) and surface water (ST345, phylogroup B1), respectively. High diversity of virulence genes (VFDB) was observed for the isolates of phylogroup B2, mostly clinical (63%), but also comprising wastewater isolates after secondary or tertiary treatments. Slightly higher clustering (lower diversity based on Shannon indexes) was observed across phylogroups than across isolation sources for both plasmid replicon types (PlasmidFinder) and stress-response genes (EcoCyc database). This study highlights the potential of virulent *E. coli* to thrive in (waste)water habitats, while traits associated with multidrug resistance can be stabilized in the genomes.

OP4.13 - BREAKING THE WALL: MYCOBACTERIUM BOVIS TRANSMISSION AT THE ANIMAL- ENVIRONMENT INTERFACE

André C. Pereira [1,2]***, Daniela Pinto*** [1,2]***, Mónica V. Cunha*** [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

(*) e-mail: mscunha@ciencias.ulisboa.pt

Keywords: *Mycobacterium bovis*; Animal tuberculosis; Indirect transmission; Environmental contamination; Environmental genomics

ABSTRACT

The spread of *Mycobacterium bovis*, the etiological agent of animal tuberculosis (TB), among multi-host mammal communities is mainly attributed to animal interactions. Exposure of susceptible hosts to natural substrates contaminated by animal shedders has also been widely admitted, but the burden of this environmental component in TB transmission dynamics has remained unknown due to historic inability to isolate environmental *M. bovis*. We have recently developed an innovative single-cell workflow, including flow cytometry, fluorescence in situ hybridization, and fluorescent-activated cell sorting, to detect, quantify and sort *M. bovis* cells in environmental matrices. We applied this modular procedure to 65 natural substrates (sediments, sludge, water, and food) collected in the official animal TB hotspot area in Portugal. Most samples collected from animal aggregation points contained metabolically active or dormant *M. bovis* cells. Sludge samples had a higher cell burden, showing a concentration of viable *M. bovis* cells compatible with the infectious dose. We generated the first-ever whole genome sequences of *M. bovis* from the environment after a whole-genome enrichment strategy by RNA baiting. Besides establishing methodological innovations, we established epidemiological links at the environment-animal interface by phylogenomic comparison of these *M. bovis* genomes detected in the environment with numerous genomes obtained from livestock and wild ungulates in the same geographic area. We show that environmental and animal *M. bovis* genomes are highly intertwined, with genomic data supporting several events of environmental substrate contamination by infected animals.

This study provides definitive evidence of *M. bovis* excretion into the environment, the maintenance of its viability, and supports the environment as a potential source of new animal infection. These ground-breaking insights have clear implications for policy formulation, demanding environmental surveillance in *M. bovis* eradication programmes.

Acknowledgements:
Fundação para a Ciência e a Tecnologia, IP [PTDC/CVT- CVT/29783/2017, UIDB/00329/2020 UIDB/04046/2020, LA/P/0121/2020], PORLisboa [LISBOA-01-0145-FEDER- 029783], COMPETE [POCI-01-0145- FEDER-029783].

OP4.14 - UNRAVELLING MECHANISMS OF PNEUMOCOCCAL INTRA-SPECIES INTERACTIONS

Carina Valente [1,2(*)], Ana R. Cruz [1#], Adriano O. Henriques [3], Raquel Sá-Leão [1]
[1] *Laboratory of Molecular Microbiology of Human Pathogens, ITQB NOVA, Oeiras, Portugal*
[2] *Escola Superior de Saúde Dr. Lopes Dias, IPCB, Castelo Branco, Portugal*
[3] *Laboratory of Microbial Development, ITQB NOVA, Oeiras, Portugal*
[#] *Current affiliation: Laboratory of Host-Microbiota Interaction, Institut Necker Enfants Malades, Paris, France.*

[(*)] *e-mail: cvalente@itqb.unl.pt*

Keywords: *Streptococcus pneumoniae*; intra-species interactions; bacteriocins.

ABSTRACT

Streptococcus pneumoniae is responsible for high morbidity and mortality worldwide. Disease is incidental and is preceded by asymptomatic nasopharyngeal colonization in the form of biofilms. Intra-species interactions during colonization, however, remain poorly characterized. We have identified a strong negative interaction between two pneumococcal natural isolates in which a serotype 19F strain inhibits a non-encapsulated (NT) strain within biofilms, causing a 10^4 decrease in viability. Here, we aimed at understanding the molecular mechanisms underlying this interaction.

Biofilms were used to explore the role of diffusible molecules in the interaction by growing the NT strain in 19F-conditioned medium (cell-free supernatant, CFS). Bacteriocin loci were identified *in-silico* and the corresponding deletion mutants were tested for inhibition. *In-silico* analyses and gene expression assays were used to study how the production of lantibiotic Streptolancidin-D (*sld* operon) is regulated. A transwell system was used to evaluate whether contact-dependent mechanism(s) were needed for the inhibition.

19F conditioned medium was sufficient to inhibit NT biofilms. Protease treatment of CFS resulted in attenuation of the inhibition observed. Five out of seven bacteriocin loci identified in the 19F genome were predicted to be functional. Deletion of the *sld* locus (19FΔ*sld)* resulted in lower inhibitory activity. The same was observed in transwell experiments. In dual-strain biofilms, however, 19FΔ*sld* displayed strong inhibitory activity against NT, comparable to that of wt 19F. The expression levels of the *sld* locus do not differ during biofilm and planktonic growth and the locus seems to be constitutively expressed in both conditions.

We provide evidence that Streptolancidin-D mediates competition between pneumococci, although a contact-dependent mechanism might also be involved in the inhibitory effect of the19F strain. The results highlight the importance of using biofilms when studying the mechanisms underpinning bacterial interactions.

Acknowledgements:
This work was supported by projects PTDC/BIA-MIC/30703/2017 and 2022.10980.PTDC from Fundação para a Ciência e a Tecnologia (FCT), MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020). CV was supported by post-doctoral fellowship from FCT SFRH/BPD/115280/2016 and by ESCMID grant 2022 funded by European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

OP4.15 - CHARACTERIZATION OF A NOVEL QUORUM SENSING SYSTEM IN *BACTEROIDES THETAIOTAOMICRON*

*Carina R. Galhofa [1], **Kai Papenfort [2], Karina B. Xavier [1]***
[1] 1Instituto Gulbenkian de Ciência, Portugal
[2] Institute of Microbiology, Friedrich Schiller University, Germany

[(*)] e-mail: kxavier@igc.gulbenkian.pt

Keywords: bacterial signalling.

ABSTRACT

Bacteroides thetaiotaomicron (B. theta) is a member of the human gut microbiota with important roles in host nutrition and the immune system. One reason for its success in this competitive environment, which is home to hundreds of different bacterial species, is due to its remarkable capacity to degrade and utilize dietary and host polysaccharides. Production of saccharolytic enzymes is among the several collective behaviours regulated by quorum sensing (QS). QS is a cell-cell signalling mechanism used by bacteria to coordinate group behaviours in communities that depends on the production and detection of molecules called autoinducers that accumulate in the environment in function of cell density.
The only autoinducer known to be produced by *Bacteroides spp* is the autoinducer-2 (AI-2), which is produced by the enzyme LuxS. However, *B. theta* does not possess the *luxS* gene and the production of other known autoinducers hasn't been reported in this bacterium.
Recently, the autoinducer 3-5-dimethylpyrazin-2-ol (DPO) has been identified as a virulence regulator in *Vibrio cholerae*. DPO synthesis is dependent on the enzyme threonine dehydrogenase (Tdh), present in many bacteria but has never been studied in *Bacteroides*.
Here, we showed that culture supernatants of *B. theta* activate a *V. cholerae* reporter strain for the DPO QS signal, providing evidence that *B. theta* produces autoinducer molecules with DPO activity. This activation was abolished in mutants carrying a deletion in *bt1370*, a gene codifying for an homologue of *tdh,* which showed increased biofilm formation in the presence of bile acids, compared with the WT strain. These results indicate that in *B. theta,* biofilm formation is regulated by pyrazinones as it happens in *Vibrio cholerae*.
Characterization of QS systems in *B. theta* will enable us to understand the molecular mechanisms underlying group behaviors that allow mammalian gut symbionts to colonize the gut microbiota.

Acknowledgements:
We would like to thank the members of the Bacterial signalling lab and FCT (fundação para a ciência e tecnologia) for funding (under the projects PTDC/BIA-MIC/30487/2017, PTDC/BIA-MIC/6990/2020 and individual fellowship SFRH/BD/145374/2019)

OP5.1 - THE UNIQUE ABILITY OF *STAPHYLOCOCCUS EPIDERMIDIS* PHAGE SEP1 ACTIVATING DORMANT CELLS

Maria Daniela Silva [1,2], Graça Pinto [1,2], Ângela França [1,2], Joana Azeredo [1,2], Luís Melo [1,2]*

[1] CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal
[2] LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

(*) e-mail: lmelo@deb.uminho.pt

Keywords: bacteriophage; stationary cells; dormant cells

ABSTRACT

Most bacteriophages fail to replicate in dormant hosts. The lower metabolic activity of these cells, together with their thicker cell wall and the fewer adsorption sites available impair the action of phages against them. The SEP1 phage, isolated from *Staphylococcus epidermidis*, has the rare ability to reduce the number of cells in a stationary (dormant) state.
To uncover how cells respond to SEP1 infection, both exponential and stationary cultures were challenged with phage. RNA was extracted from samples collected before and after infection (5, 15 and 30 min) and the transcriptomes analyzed by total RNA sequencing.
SEP1 transcripts gradually increased over time, corresponding to 88-95% and 59-76% of the total transcriptome in exponential and stationary cultures, respectively, at 30 min. In exponential cells, there was a logical temporal progression of expressed phage genes, from host adaptation to DNA replication genes and, finally, structural and lysis genes. In stationary cells, SEP1 transcription was delayed, with a significant expression of genes putatively involved in host takeover (gp142 – gp152) observed, mainly at 5 min. Exponential cells responded to SEP1 infection only by upregulating 3 genes involved a DNA restriction and modification system at 5 min post-infection. Two of these genes were substantially overexpressed 15 min after SEP1 infection in stationary cells, with the 3 being upregulated at 30 min post-infection. While on exponential cells, 70 and 78 genes were differentially more expressed at 15- and 30-min post-infection, in stationary cells, 894 and 1309 genes were shown to be upregulated at the same time points.

Functional enrichment analysis grouped these genes in structural constituents of the ribosome, involvement in ribosome and purine nucleoside biosynthetic processes, translation, RNA metabolic processes, etc, demonstrating for the first time that a phage can activate the metabolic machinery of stationary cells.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and Project PTDC/BIA-MIC/2312/2020. Luís D. R. Melo acknowledges funding from the FCT through the Scientific Employment Stimulus Program (2021.00221. CEECIND).

OP5.2 - HIGH AND HOMOGENEOUS LEVELS OF OXACILLIN RESISTANCE IN MRSA THROUGH THE ACTIVATION OF THE STRINGENT STRESS RESPONSE LEADS TO UP- REGULATION OF THE CAPSULAR POLYSACCHARIDE

Catarina Milheiriço [1]*, Vítor Borges [2], João Paulo Gomes [2], Beatriz Silva [1], Alexander Tomasz [3], Hermínia de Lencastre [1,3]

[1] Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Oeiras, Portugal.
[2] Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Avenida Padre Cruz, Lisbon, Portugal.
[3] Laboratory of Microbiology & Infectious Diseases, The Rockefeller University, New York, New York, United States of America.

(*) e-mail: ***cicm@itqb.unl.pt***

Keywords: *Staphylococcus aureus*, Beta-lactams heteroresistance, Stringent stress response.

ABSTRACT

Most methicillin-resistant *Staphylococcus aureus* (MRSA) exhibit a beta-lactam resistant heterogeneous phenotype, where the majority of cells exhibit variable but usually low-level resistance; however, cells with homogeneous and high-level resistance (H*R) are also present. The stringent stress response (SR) has been implicated in the oxacillin H*R phenotype in MRSA; but the exact molecular mechanism remains unclear. In this study we wanted to explore the means by which the activation of SR may lead to increased beta-lactam resistance in MRSA through the assessment of global gene expression dynamics. The global gene expression was assessed using RNA-sequencing. RNA samples of D3 (H*R MRSA) and its parental strain D0 were collected under three different conditions, growth in TSB: a) without supplementation; b) in the presence of sub-inhibitory concentration (sub-MIC) of oxacillin; or c) in the presence of sub-MICs of oxacillin and mupirocin. D3 differs from D0 by a unique frameshift mutation after the *relA* synthase domain, which most likely affect its regulatory domains' function, rendering the cell with increased levels of (p)ppGpp, the SR effector molecule.

As expected, in the presence of oxacillin sub-MIC, genes associated with beta-lactam resistance, namely *mecA*, *blaR1* and *blaZ* are up-regulated (> 2 fold-change) in both strains (FDR <0.05). Addition of mupirocin sub-MIC has no effect on the expression profiles of the two strains grown in the presence of oxacillin sub-MIC. Comparing the expression profile of D3 vs D0 we saw that: *mecA* is poorly up-regulated even in the presence of oxacillin sub-MIC; *mnt* transporter and energy metabolism genes are down-regulated; siderophore and capsular operons are up-regulated. These data seem to indicate that oxacillin H*R phenotype in D3 is not due to increased expression of *mecA*, but probably with the over-expression of the cap operon.

Acknowledgements: This work was supported by project LISBOA-01-0145-FEDER-007660 ("Microbiologia Molecular, Estrutural e Celular") funded by FEDER funds through COMPETE2020 – "Programa Operacional Competitividade e Internacionalização" (POCI); ONEIDA project LISBOA-01-0145-FEDER-016417 co-funded by FEEI – "Fundos Estruturais e de Investimento" from "Programa Operacional Regional Lisboa 2020" and by national funds from FCT—"Fundação para a Ciência e a Tecnologia", Portugal and by LS4FUTURE Associated Laboratory (LA/P/0087/2020) funded by the FCT.

OP5.3 - GENOME MINING AND BIOCHEMICAL CHARACTERIZATION OF POLYHYDROXYALKANOATES PRODUCED BY BACTERIAL ISOLATES FROM UCCCB

Paula V. Morais [1], Diogo Vicente [1], Diogo Neves Proença [1(*)]

[1] *University of Coimbra, Department of Life Sciences and CEMMPRE, ARISE, Coimbra, Portugal*

(*) *e-mail: diogo.proenca@uc.pt*

Keywords: Polyhydroxyalkanoates (PHA); *phaC* gene; genome mining; biochemical characterization; culture collection.

ABSTRACT

Polyhydroxyalkanoates (PHA) are a class of biodegradable biopolymers with great potential as sustainable alternative to traditional petroleum-based plastics. PHAs can be naturally synthesized, and accumulated as granules inside bacteria cells, through the action of the *phaC* gene, which encodes the key enzyme for PHA production, the PHA synthase. Harnessing the potential of PHAs for eco-friendly plastics and other applications relies on identifying new PHA-producing strains, which can be achieved through genome mining. Their physicochemical properties depend on each producer organism and cultivation conditions. This study aimed to find new bacterial-polymers producers and new types of polymers. In total, 106 bacterial strains from University of Coimbra Bacteria Culture Collection (UCCCB), belonging to 5 different phyla, 11 classes, 24 orders, and 50 families, were screened for PHA production. The optimization of producing parameters, such as pH, temperature, carbon sources and incubation time, was performed and products were characterized. The 33 selected polymers-producing strains belonged to 5 different classes. A deep characterization of the producers revealed four strains belonging to genera that have never been reported as PHA producers. The genomic analysis of these strains revealed the presence of *phaC* gene in all strains, although belonging to different phylogenetic classes, with a diversity in the organization and the number of additional genes. The chemical characterization of the polymers revealed structural differences when comparing polymers produced by strains belonging to different genera. Combining the genomic information and biochemical screening, the best four bacterial producers showed different producing parameters as optimal production conditions. The PHA produced for each strain at optimal conditions varies from 0.019 ± 0.001 to 0.088 ± 0.007 $g.L^{-1}$ of PHB equivalents/OD600. This work highlighted the relevance of UCCCB as a valuable source of novel and unique strains that are an important resource for the discovery of new compounds with biotechnological applications.

OP5.4 - EXPLORING THE FUNCTIONAL AND STRUCTURAL ASPECTS OF THE CITRATE TRANSPORTER CEXA FROM *ASPERGILLUS NIGER*

João Alves [1,2(*)], ***Maria Sousa-Silva*** [1,2], ***Pedro Soares*** [1,2], ***Michael Sauer*** [3,4], ***Margarida Casal*** [1,2], ***Isabel Soares-Silva*** [1,2]

[1] *Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal*

[2] *Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal*

[3] *University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, Muthgasse 18, 1190 Vienna, Austria*

[4] *Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria*

(*) e-mail: id9021@alunos.uminho.pt

Keywords: Carboxylic acids; Transporter Proteins; Site-directed mutagenesis.

ABSTRACT

The CexA transporter in *Aspergillus niger*, belonging to the DHA1 (Drug-H+ antiporter) family, plays a pivotal role in citrate transport [1]. In this study, we expressed CexA in *Saccharomyces cerevisiae*, revealing its capability to interact with isocitric acid and transport citrate at pH 5.5 with low affinity, independently of the proton motive force, utilizing a facilitated diffusion mechanism. To delve into the structural aspects of CexA, we conducted site-directed mutagenesis on 21 specific residues, selected based on their conservation within the DHA1 family, 3D structural predictions, and substrate docking analysis. We assessed the functional consequences of these mutations by examining the growth of *S. cerevisiae* expressing mutant CexA alleles on carboxylic acid-containing media and measuring radiolabeled citrate uptake. Additionally, GFP tagging was employed to determine the subcellular localization of CexA mutants, revealing seven substitutions that influenced CexA's presence at the plasma membrane [2]. Notably, the P200A, Y307A, S315A, and R461A mutant alleles exhibited complete loss-of-function phenotypes. Furthermore, a majority of the substitutions affected citrate binding and translocation. Interestingly, the S75 residue displayed no effect on citrate export but enhanced its import, as the alanine substitution increased the transporter's affinity for citrate. Conversely, the expression of CexA mutant alleles in the *Yarrowia lipolytica cex1*Δ strain highlighted the involvement of residues R192 and Q196 in citrate export. In summary, our study offers valuable insights into the functional and structural dimensions of CexA, shedding light on crucial amino acid residues influencing its subcellular localization, export capacity, and import affinity.

References:

1. Steiger et al. (2019) *Metabolic Engineering, 52*, 224-231
2. Alves et al. (2023) *Computational and Structural Biotechnology Journal, 21*, 2884–2898

Acknowledgements:

This work was supported by the strategic program UID/BIA/04050/2020 and the project LA/P/0069/2020 granted to the Associate Laboratory ARNET, funded by Portuguese funds through the FCT I.P., and by River2Ocean NORTE-01-0145-FEDER-000068, co-financed by the European Regional Development Fund (ERDF) through Programa Operacional Regional do Norte (NORTE 2020).

OP5.5 - EXPLORING GENOMIC PLASTICITY IN *CANDIDA GLABRATA* AND ITS IMPACT ON BIOFILM EVOLUTION IN CLINICAL ENVIRONMENTS

Inês Vieira Costa [1,2(*)]***, Maria Zolotareva*** [1,2]***, Mafalda Cavalheiro*** [1,2]***, Mónica Galocha*** [1,2]***, Miguel Cacho Teixeira*** [1,2]

[1] *iBB - Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa – Lisboa*
[2] *I4HB – Institute for Health and Bioeconomy - Laboratório Associado, Portugal*

[(*)] *e-mail: inesvcosta@tecnico.ulisboa.pt*

Keywords: *Candida glabrata*, Biofilms, Genomics, Evolution.

ABSTRACT

Candida species are one of the primary causes of fungal infections on a global scale. Notably, *Candida glabrata* prevalence is rising, posing a significant concern due to its inherent resistance to azoles and the emergence of resistance to other established antifungal treatments. Its ability to adhere to host mammalian cells, as well as to other microorganisms and abiotic surfaces like catheters, leading to biofilm formation, further exacerbates its virulence. However, despite the advantages biofilms offer to pathogenic yeasts, there has been limited research on the evolutionary aspects of biofilm formation among clinical isolates.

To shed light on this issue, we conducted an analysis of the phenotypic variability in biofilm formation among a collection of clinical isolates of *C. glabrata*. We then combined a comparative genomics approach with experimental microevolution of these clinical isolates toward a more robust biofilm phenotype, aiming to pinpoint key regulators and effectors of biofilm formation in *C. glabrata*.

Our findings reveal that the specialization toward enhanced biofilm formation occurs rapidly, accompanied by genome alterations, adhesin modifications, and the accumulation of variations in effectors operating at various regulatory levels, spanning from epigenetic to post-translational mechanisms. We also conducted comprehensive assessments of clinical and evolved isolates through virulence and adhesion assays. While certain evolved isolates displayed a substantial increase in adherence to epithelial cells, no clear-cut correlation was observed between biofilm formation and virulence/adhesion across all clinical isolates. Nonetheless, we successfully identified genes with predictive roles in biofilm formation, including uncharacterized adhesins from the EPA and PWP gene families, as well as transcription factors and telomeric silencing proteins. These genes hold promise for future exploration as targets for disrupting biofilms and as markers of biofilm evolution.

Acknowledgements:
This work was supported by “Fundação para a Ciência e a Tecnologia” (FCT) [Ph.D. grant 2021.05051.BD]. Funding received by iBB-Institute for Bioengineering and Biosciences from FCT-Portuguese Foundation for Science and Technology (UID/BIO/04565/2020) as well as by Programa Operacional Regional de Lisboa 2020 [LISBOA-01-0145-FEDER-022231—The BioData.pt Research Infrastructure and Project No. 007317 is acknowledged.

OP6.1 - CONTINUOUS PRODUCTION OF INFLUENZA VLPS AS VACCINE CANDIDATES: A MULTI-STAGE BIOREACTOR APPROACH

Ricardo Correia [1], Taja Zotler [2], Miguel Graça [1], Bárbara Fernandes [1], Gorben Pijlman [2], Netherlands Paula M. Alves [1], António Roldão [1(*)]
[1] iBET and ITQB-NOVA, Oeiras, Portugal
[2] Wageningen University Research, Wageningen, The Netherlands

[(*)] e-mail: aroldao@ibet.pt

Keywords:

ABSTRACT

Insect cells (IC) are excellent hosts to produce recombinant proteins for many different biomedical applications, in particular when seconded by the baculovirus expression vector system (BEVS). However, the lytic nature of IC-BEVS processes limits its transition from traditional batch-based bioprocessing to more intensified continuous bioprocessing. Cascaded bioreactor setups allow to overcome the limitations of viral systems for continuous operation [1], but so far have not been efficiently conjugated with IC-BEVS.

In this study, a continuous multi-stage bioreactor process was established to produce influenza hemagglutinin- displaying virus like particles (HA-VLPs) using IC-BEVS. A set-up consisting of one bioreactor (for cell growth) simultaneously feeding non-infected insect cells to a second bioreactor (for HA-VLPs production) was implemented. Two different designs were tested:

• Design 1: neutral pH-adapted High Five cells and a baculovirus generated via Bac-to-Bac technology (rBACbacmid), a combination previously shown to allow 3-fold higher productivity [2];

• Design 2: Sf9 cells and a baculovirus generated with flashBACTM technology (rBACflashBAC).

Both designs were efficiently operated in continuous mode for up to 20 days. Cell concentration and viability profiles were similar in both designs, contrarily to baculovirus titers; design 2 retrieved consistent, high baculovirus titers (108-109 pfu/mL), unlike design 1. Importantly, in design 1, production of HA-VLPs remained steady over time (34 ±14 HA titer/mL), whereas in design 2, a decline in HA-VLPs was noticed. The presence of HA-VLPs was confirmed by electron microscopy in both designs. Altogether, these data demonstrate that changing from High Five to Sf9 cell line, and from rBACbacmid to rBACflashBAC, was essential to maximize process performance.

This work shows the implementation of a process for continuous HA-VLPs production using IC-BEVS, and paves the way for establishing continuous, integrated setups using this expression system.

References:

[1] Tapia et al., 2016. DOI:10.1007/s00253-015-7267-9
[2] Correia et al., 2020. DOI:10.3390/vaccines8040589

OP6.2 - ASSESSMENT OF MICROALGAE SIDE STREAMS POTENTIAL FOR VFA PRODUCTION IN BATCH FERMENTATION

Claudia Duarte [1,2], Marisa Cardoso [1,2(*)], Mariana Matos [1,2], Joana Fradinho [1,2], Bruno Ferreira [3,4], Jorge Pereira [5], and Maria Reis [1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal

[2] UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal

[3] Biotrend SA – Biocant Park, Cantanhede, Portugal

[4] A4F – Algae for Future, Lisboa, Portugal

[5] CIEPQPF, Department of Chemical Engineering, Univ Coimbra, Coimbra, Portugal

[(*)] e-mail: mfd.cardoso@campus.fct.unl.pt

Keywords: batch fermentation; microalgae side streams; volatile fatty acid production.

ABSTRACT

The microalgae industry generates wastewaters and side streams requiring treatment prior to disposal. Applying biorefinery and circular economy concepts, these side streams can be valorized transforming into valuable products, notably polyhydroxyalkanoates (PHAs). PHAs can be produced by a process carried out in three different phases: 1) acidogenic fermentation of the feedstock to obtain volatile fatty acids (VFAs); 2) enrichment of a PHA accumulating mixed microbial culture (MMC) using the produced VFA; 3) PHA accumulation to a maximum value using VFA as PHA precursors. The final goal of this work is to produce PHAs using microalgae side streams as feedstock. Thus, the success of PHA production strongly depends on the fermentation step efficiency.

This study evaluated the fermentation potential of three different microalgae side streams- *Nannochloropsis, Chlorella,* and a mixed phototrophic consortium. The batch reactors were inoculated with anaerobic sludge in a ratio of substrate to inoculum (S/I) of 2gVS/gVS at pH 5.5. The tests were performed for 32 days at 30 ºC and 180 rpm. Each test was conducted in triplicate. Additionally, for each microalgae feedstock a corresponding control test, without inoculum addition, was carried out.

Throughout the experiment, the absence of methane indicated effective inhibition of methanogenic activity. Results showed that all microalgae streams underwent fermentation, yielding a spectrum of fermentation products including ethanol, acetic acid, propionic acid and butyric acid. Fermentative products were observed in control tests for each stream, indicating the presence of fermentative microorganisms within these streams and their inherent fermentative potential. Nonetheless, the introduction of anaerobic sludge inoculum into the experiments facilitated the synthesis of a more diverse range and higher concentrations of VFAs from the various microalgae feedstocks. These findings highlight the efficacy of microalgae side streams as a source of VFA production, which can serve as valuable precursors for the subsequent PHA production.

Acknowledgements:
This work was financially supported by Project Pacto da Bioeconomia Azul – PBA, Ref. C644915664-00000026 –financed under Invitation Notice n.º 02/C05-i01/2022 regarding the development of projects within the scope of the Mobilizing Agendas for Business Innovation, framed and financed under component n.º 5 - Capitalization and Business Innovation of the Recovery and Resilience in Portugal. Claudia Duarte acknowledges FCT for PhD Grant PRT/BD/152855/2021

OP6.3 - MONITORING PRODUCTIVITY AND PREDICTING CRASH OF PHAEDACTYLUM TRICORNUTUM CULTURES USING SPECTROSCOPY AND MACHINE LEARNING

Pedro Brandão [1,2]***, Constança Bertrand*** [1,3]***, Rodrigo Martins*** [1,3]***, Francisco Nunes*** [1,3]***, Claudia Galinha*** [2]***; Francisco Nascimento*** [1]

[1] Instituto de Biologia Experimental e Tecnológica (iBET), Oeiras, Portugal
[2] Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (FCT-UNL), Caparica, Portugal
[3] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB-NOVA), Oeiras, Portugal.

[(*)] *pedro.brandao@ibet.pt*

Keywords: Machine Learning, Monitoring, Fucoxanthin, 2D Fluorescence, *Phaeodactylum tricornutum*, Absorbance

ABSTRACT

The industrial cultivation of *Phaeodactylum tricornutum* for fucoxanthin production requires optimal monitoring and control for achieving stable performance and prevent productivity reductions. Although accurate, the standard analytical methods are time consuming, expensive and pollutant. On the other hand, optical sensors are rapid and may be applied in situ and online, providing instant process data without interfering with it. Combined with Machine Learning (ML) tools, relevant spectroscopic information provided by these sensors can be selected while overcoming noise and non-linear dynamics, having been successfully applied in a variety of bioprocesses. In this work, fluorescence and absorbance spectroscopy are shown to provide information on the content and status of *Phaeodactylum tricornutum* cultures through ML. Cell Count (CC), Fucoxanthin (Fx) and Chlorophyll a (Chl a) were predicted, along with culture crash prediction. ML algorithms Projection to Latent Structures (PLS), Convolutional Neural Networks (CNN), Random Forests, and Support Vector Machines (SVM) were used in their regression and classification forms, together with spectral variable selection algorithms Moving-Window and Variable Importance to Projection. The results of this work consist of ML models that take either absorbance or fluorescence spectroscopy data as input for predicting CC, Fx and Chl a with $R^2 > 0.8$ of validation accuracy, and for predicting culture crash with 90% accuracy and 80% precision and recall. Moreover, these models only require up to 25% of the original spectroscopy data.

Acknowledgements: This work was supported by the PHYCO-µ-BIOME project (PTDC/BAABIO/1262/2020) and the PhD study grant 2021.07927.BD514, both funded by Fundação para a Ciência e a Tecnologia, Portugal (FCT) and by the European project "MULTI-STR3AM" funded by the Bio- Based Industries Joint Undertaking under grant agreement no. 511887227. The Associate Laboratory for Green Chemistry (LAQV), financed by national funds from FCT/MCTES516 (UIDB/50006/2020 and UIDP/50006/2020) also provided support to the work.

OP6.4 - NEW SHUTTLE VECTOR-BASED EXPRESSION SYSTEM TO IMPROVE THE BIOSYNTHESIS AND PURIFICATION OF MEMBRANE BOUND CATECHOL-O- METHYLTRANSFERASE

Ana Margarida Gonçalves [1,2,3] *, ***Márcia Correia*** [2,3], ***Claúdio Jorge Maia*** [1], ***João Queiroz*** [1], ***Maria João Romão*** [2,3], ***Luís António Passarinha*** [1,2,3,4]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*
[2] *Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal*
[3] *UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal*
[4] *Laboratório de Fármaco-Toxicologia, UBI Medical, Universidade da Beira Interior, Covilhã, Portugal*

(*) *e-mail:* ***ggmargarida@gmail.com***

Keywords: bacteriophage; stationary cells; dormant cells

ABSTRACT

Membrane bound catechol-*O*-methyltransferase (MBCOMT), an integral membrane protein present in the brain, is involved in the main pathway of catechol neurotransmitters deactivation. Due to its biological role, the protein has been associated with different mental dementias and considered an important therapeutic target. Thereby, establish an effective production and purification platform, that ensure proper amounts of active protein is the main goal for several biomedical applications. As a membrane protein, properties like solubility, protein denaturation and activity lost are always points of concern when a biosynthesis strategy is re-designed. Using AlphaFold2, a model of the MBCOMT structure (AF-P21964-F1) can be predicted, with a very high per-residue confidence score (pLDDT) above 90, with only the anchor membrane region displaying values between 90 and 70 pLDDT. The analysis of the predicted protein structure revealed the amphipathic features of the first anchor region helices. On the basis of the analysis of the AF2 model, a new MBCOMT construct was designed, in an attempt to increase the protein stability, without compromising its biological activity. A depletion of the first 30 amino acids (aa) (N-terminus) and the last 5 aa (C-terminus) was performed and to increase the construct solubility and expression, a dual tag of MBP-His6 was incorporated. The sequence was cloned into the pETM41 vector. Cultures of IPTG induced *Escherichia coli* BL21 was then evaluated, with a time course (2, 4, 6 and 8h) and three protein recovery flowcharts (glass beads, freeze/thaw and sonication lysis). All the implement strategies were able to produce active MBCOMT. Therefore, a simple MBCOMT sequence manipulation, coupled with the use of MBP tag, ensured the overexpression of a biologically active MBCOMT, essential to perform further structural studies.

Acknowledgements:
This work was developed within the scope of the CICS-UBI (projects UIDB/00709/2020, UIDP/00709/2020) and Applied Molecular Biosciences Unit UCIBIO (UIDB/04378/2020 and UIDP/04378/2020) and the Associate Laboratory Institute for Health and Bioeconomy—i4HB (project LA/P/0140/2020) which are financed by National Funds from FCT/MCTES. Researchers also acknowledge funding by FEDER through COMPETE 2020—Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES from the project "IL2BioPro"-PTDC/BII-BBF/30840/2017. Ana Margarida Gonçalves's individual PhD Fellowship (SFRH/BD/147519/2019).

OP6.5 - A MOLECULAR MODELING PERSPECTIVE ON THE DEVELOPMENT OF SYNTHETIC AFFINITY LIGANDS

Carlos Costa, Carolina Natal, Jéssica Rodrigues, Ana Margarida Dias, Ana Cecília Roque, Arménio J. M. Barbosa *(*)*
Associate Laboratory i4HB - Institute for Health and Bioeconomy, Chemistry Department, NOVA School of Science and Technology, Campus Caparica, Caparica, Portugal
UCIBIO – Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Caparica, Portugal

(*) e-mail: aj.barbosa@fct.unl.pt

Keywords: Affinity ligands; Bioseparation; Ligand design.

ABSTRACT

Synthetic affinity ligands have garnered increased attention within the area of biomolecular purification. Understanding molecular interactions between affinity ligands and their targets forms the cornerstone of effective molecular capture and isolation. While the utilization of molecular modeling techniques in this field is still rising, their application in crucial phases of the ligand development pipeline yields essential data for ligand refinement and elucidating binding modes and interactions. This presentation outlines the strategies employed to design synthetic ligands for the purification of various biomolecules, including antigen-binding fragments, Human Serum Albumin (HSA), and VLPs-capsid proteins, namely SARS-CoV-2 spike protein.

Computational techniques enabled the identification of potential binding sites between synthetic ligands and target biomolecules (such as antibodies and HSA) through blind molecular docking calculations. For instance, the ligand B1Al2A2, designed for antibody molecules, demonstrated an affinity for non-CDR regions within Fab and Nanobody structures. Similarly, the HSA adsorbent A6A5 was found to favor the binding site within HSA's domain II. In the case of the VLP adsorbent, a comprehensive approach combining theoretical techniques (docking and Molecular Dynamics) with experimental validation was employed to identify top-performing ligands. Among these, two synthesized ligands, A5A10 and A10A11, were particularly effective in purifying VLPs. In the context of the spike protein, an *in silico* combinatorial library was generated and screened against the RBD, providing insights in selected ligands' interactions.

Molecular modeling techniques play a pivotal role in enhancing affinity adsorbents discovery by providing insights into the molecular interactions between affinity ligands and their targets. Furthermore, they contribute to our understanding of how binding and elution buffers may influence these interactions throughout the affinity chromatography process.

Acknowledgements:

European Union's Horizon 2020 research and innovation programme under GA No899732 (PURE Project); FCT UIDP/04378/2020 and UIDB/04378/2020 UCIBIO, LA/P/0140/2020 i4HB, projects: 2022.15912.CPCA, PTDC/CTM-CTM/3389/2021; fellowships (2020.07566.BD), (2023.01023.BD); support of INCD 01/SAICT/2016 n02215.

OP7.1 - COMMENSALISM VS PATHOGENICITY IN *STAPHYLOCOCCUS EPIDERMIDIS* - UNCOVERED BY AN INTEGRATED OMICS APPROACH

Luís G Gonçalves [1], Elisabete Morais [1], Filipe Magalhães [1], Susana Santos [1], Laidson P Gomes [1], Jean Armengaud [2], Maria Zimmermann-Kogadeeva [3], Ana Gil [4], Maria Miragaia [1], Ana V Coelho [1(*)]

[1] *ITQB NOVA, Oeiras, Portugal,*

[2] *SPI, Université Paris-Saclay, CEA, INRAE, Bagnols-sur-Cèze, France,*

[3] *EMBL, Heidelberg, DE,*

[4] *Universidade de Aveiro, Portugal*

(*) e-mail: varela@itqb.unl.pt

Keywords: *Staphylococcus epidermidis*; Metabolomics and Proteomics; pH adaptation; pathogenicity.

ABSTRACT

SE is part of skin microbiota and contributes to its homeostasis and protection against pathogens. However, it is the most frequent cause of medical device-associated infections. Skin isolates belonging to clonal complex 2 (CC2) lineage are the major colonizers sharing their ecological niche with other minor genetic backgrounds (non-CC2). From genomic and proteomic data it was possible to identify relevant differences between the metabolic and biological processes of both lineages. Additionally, the intracellular metabolome and proteome associated with both lineages under pH environmental changes, mimicking the transition from the skin (pH 5.5) to blood (pH 7.4) were evaluated and showed specific responses [1]. The CC2 strain seems more prepared to survive in blood and to promote adhesion to medical-devices. The obtained results were complemented with time-course exometabolomic data during bacterial growth, which were also integrated in genome-scale metabolic models. Following a proteogenomic approach, obtained proteomics data are being used to refine the annotation of both strains genomes.

References:

[1] L G Gonçalves, S Santos, L P Gomes, J Armengaud, M Miragaia, A V Coelho "Skin-to- blood pH shift triggers metabolome and proteome global remodelling in Staphylococcus epidermidis" Front Microbiol., 2022, 13:1000737. doi: 10.3389/fmicb.2022.1000737

Acknowledgements:

This work was partially supported by project PTDC/CVTCVT/ 29510/2017 funded by Fundação para a Ciência a Tecnologia; Projects LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) and UID/Multi/04378/2019) funded by FEDER funds through COMPETE2020
- Programa Operacional Competitividade e Internacionalização (POCI); by ONEIDA project (LISBOA-01-0145-FEDER- 016417) co-funded by FEEI - "Fundos Europeus Estruturais e de Investimento" from "Programa Operacional Regional Lisboa2020"; by national funds through FCT; by Transnational Cooperation Portugal/France – Pessoa Program, supported by FCT/Campusfrance; and by SymbNet Project (IGC-UE-Horizon 2020-952537). LGG was financed by an FCT contract according to DL57/2016, (SFRH/BPD/111100/2015).

OP7.2 - PHENOTYPIC, PHYSIOLOGICAL AND GENOMIC PORTRAIT OF A "HUMAN-NAÏVE" CANDIDA GLABRATA STRAIN: A SHED OF LIGHT INTO THE PATH TOWARDS COLONIZATION OF THE HUMAN HOST

Maria Joana Pinheiro [1,2], ***Dalila Mil-Homens*** [1,2], ***Ana Mendes-Ferreira*** [3,4], ***Nuno Pereira Mira*** [1,2*]

[1] *iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal*
[2] *Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*
[3] *WM&B – Laboratory of Wine Microbiology & Biotechnology, Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, Portugal*
[4] *BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal*

(*) *e-mail: nuno.mira@tecnico.ulisboa.pt*

Keywords: *Candida glabrata*; azole resistance; OMICS analyses; colonization of human host; adaptation.

ABSTRACT

Candida glabrata is a pathobiont of humans being present as a commensal in the gastro- intestinal and genitourinary tract but, under certain conditions, turns pathogenic and causes serious disseminated infections. *C. glabrata* strains isolated in the environment (e.g. in plants or trees) is documented, but unlike clinical strains, environmental ones remain poorly studied. To fulfil this gap, we focused our attention on the genetic/genomic and physiological characterization of a *C. glabrata* strain recovered from a wine must, UTAD68. Besides contributing to understand crucial aspects about *C. glabrata*, this approach has the potential to elucidate mechanisms used by this species to successfully colonize the human host while showing how prior exposure to humans may impact relevant traits such as resistance to antifungals. Regarding genomics, we found little differences in the genomic architecture of UTAD68 and of clinical strains, being of remark a translocation on chromosomes I and L that were only observed in the environmental strain. From the phenotypic point of view, UTAD68 and clinical strains showed similar patterns of sugar consumption, stress tolerance and resistance to agricultural and clinical azoles. Infection of the model wax *Galleria mellonela* with UTAD68 induced similar death rates as those prompted by clinical strains,showing that there is virulence potential in the UTAD68. Comparative genomic analysis undertaken at higher resolution revealed prominent differences in the homologous gene alleles encoded by the two strains, with the genes differing most being those involved in adhesion. Using *in vitro* evolution, we also found that the environmental strain acquires resistance to clinical and agricultural azoles at similar rates as those observed for the clinical strains. The results of whole-genome sequencing in the evolved and non-evolved environmental/clinical strains will be discussed to clarify relevant phenotype-genotype associations determining the azole-resistance phenotype in the C. glabrata species, including the influence of genetic background.

OP8.1 - BIOCONVERSION OF PLASTICS' BUILDING BLOCKS INTO BACTERIAL CELULOSE TOWARDS CIRCULAR ECONOMY

Cátia Gil [1,2], Maria A. M. Reis [1,2], Filomena Freitas [1,2], Cristiana A. V. Torres [1,2]*

[1] Laboratory i4HB—Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal
[2] UCIBIO—Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

(*) e-mail: c.torres@fct.unl.pt

Keywords: bacterial cellulose (BC); plastic monomers; upcycling; terephthalic acid (TPA); lactic acid (LA)

ABSTRACT

The consciousness of the environmental impacts caused by plastic pollution has driven the development of plastic waste valorization approaches to enable a transition from a linear to a circular economy. Polyethylene terephthalate (PET) is one of the most utilized petroleum-based plastic, synthesized by the polymerization of terephthalic acid (TPA) and ethylene glycol (EG) monomers. Despite its practical advantages, PET overuse combined with its recalcitrant nature and inefficient disposable management approaches, makes these plastics responsible for a large environmental burden. Given the aforementioned drawbacks, biopolymers, such as poly(lactic acid) (PLA) have received great interest as sustainable alternative to the petrochemical materials that can meet the demands of product functioning. However, despite being renewable sourced, the end-of life management inefficiency of PLA still results in a high environmental impact.

Bearing in mind the prospective PET and PLA plastics upcycling and to assess the feasibility of plastic waste monomers bioconversion into a biodegradable material, such as bacterial cellulose (BC), *Komagataeibacter xylinus* DSM 2004 static cultivations were carried out using LA and TPA as feedstocks in Hestrin-Scharmm buffered medium (HS). BC production kinetics were determined, and the most relevant BC yields were attained from HS supplemented with LA (1.14 ± 0.07 g/L) and with the combination of LA and TPA (1.09 ± 0.03 g/L). BC yields obtained surpassed those observed for the standard medium used for BC production. However, the yields indicated that K. xylinus DSM 2004 utilized differently both compounds, since the great discrepancy in the BC yields on substrate basis were observed. Furthermore, the produced BC materials were characterized, and the results underlined the potential use of BC for the development of wound dressings and personal care products. The results gathered in this study constitutes a sustainable strategy to upcycle PLA in the near future.

OP8.2 - UNRAVELLING THE POTENTIAL OF ORANGE JUICE SIDE STREAMS: HESPERIDIN-RICH EXTRACTION AND ELECTROSPRAYING FOR A BIO-BASED INGREDIENT DEVELOPMENT

Ana A. Vilas-Boas [1(*)], ***Cristina Prieto*** [2], ***Débora A. Campos*** [1], ***Marta Correia*** [1], ***Jose Maria Lagaron*** [2], ***Manuela Pintado*** [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[2] *Novel Materials and Nanotechnology Group, Institute of Agrochemistry and Food Technology (IATA), Spanish Council for Scientific Research (CSIC), Paterna, Spain*

(*) *e-mail: avboas@ucp.pt*

Keywords: Orange by-products; Circular economy; Hesperidin; extraction; Electrospraying.

ABSTRACT

The upcycled ingredients have gained attention regarding their potential to enhance the food industry's circular economy and meet the demands of a health-conscious society. Orange juice production generates about 50% of side streams, including orange peels (OP). OP can provide sustainable raw material for novel bio-based and upcycled ingredients due to their flavonoid-richness, which has been linked with non-communicable diseases prevention. Moreover, hesperidin showed a potential gut microbiota modulation that inhibits pathogens and promotes probiotic bacteria growth. This study aimed to i) optimise OP hesperidin-rich extraction using the Box-Behnken design and ii) assess the impact of extract drying with electrospraying assisted by pressurised gas (EAPG). The dry extract (DE) was evaluated for its antioxidant (AOX), antimicrobial, prebiotic, antidiabetic, and antihypertensive activities.

The statistical analysis indicated that the proposed model was adequate for describing the hesperidin-rich extraction conditions and under optimal conditions, the recovery of hesperidin was 0.84 ± 0.01 mg/g, fresh basis. This sustainable extraction was scaled up and, the EAPG was used for drying the extract since the EAPG showed a competitive advantage of drying without aids (e.g. maltodextrin) and, unlike traditional techniques, EAPG works at room temperature, protecting phenolic compounds from thermal degradation. The DE showed 5.95 ± 0.11 and 3.60 ± 0.09, mg/g DE of hesperidin and narirutin, respectively. The hesperidin-rich extract has been proven to have AOX, antidiabetic and antihypertensive properties and, at 1% (m/v), it inhibited about 60% of the growth of *Echerichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Enterococcus faecalis. It also* showed prebiotic activity for the growth of *Akkermansia muciniphila*, *Lactobacillus casei*, and *Bifidobacterium animalis BB-12.* Overall, the results indicate potential for developing upcycled ingredients from OP *aligned with the SDG's and the European Green Deal.* Furthermore, the study showed that EAPG is a promising and innovative high-throughput technique for scaling up the drying process.

Acknowledgements:
The authors would like to thank the MEDISMART project (reference PRIMA/0014/2019) for financial support and the scientific collaboration of CBQF under the FCT project – UIDB/Multi/50016/2020. In addition, the author Ana A. Vilas-Boas would like to acknowledge FCT for the individual PhD grant (2020.05655.BD).

OP9.1 - EATRIS | EUROPEAN INFRASTRUCTURE FOR TRANSLATIONAL MEDICINE

Helena Paula Baião 1,2,
[1] *INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde, I.P, Portugal.*
[2] *Coordinator EATRIS Portugal*

(*) ***eatrisportugal@infarmed.pt***

Keywords: EATRIS, translational medicine

ABSTRACT

EATRIS is the European infrastructure for translational medicine. We bring together resources and services for research communities to translate scientific discoveries into benefits for patients.

EATRIS is a non-profit organisation that provides access to a vast array of expertise and facilities from over 150 top-tier academic centres across Europe. Main focus on improving and optimising preclinical and early clinical development of drugs, vaccines and diagnostics, and overcome barriers to health innovation.

The EATRIS research infrastructure offers a broad range of research services for both academia and industry across various research fields. In addition, we work with public funding agencies, charities and policy makers with tailored actions to help improve the translational research and innovation ecosystem.

Find out more about EATRIS here.

Acknowledgements:

With thanks to EATRIS CS and EATRIS PT

OP9.2 - EURESTOP- European Network for diagnosis and treatment of antibiotic-resistant bacterial infections: Opportunities and initiatives COST ACTION [CA21145]

M Amparo F Faustino [1(*)]

[1] *LAQV-REQUIMTE & University of Aveiro, Aveiro, Portugal*

(*) *faustino@ua.pt*

Keywords: COST action, antimicrobial resistance, European Network

ABSTRACT

Drug-resistant bacteria represent a real threat to global health with a significant economic impact. The lack of effective drugs and inadequate diagnostic means have a significant impact on the treatment and survival of critically ill patients, extending the risk to the world population beyond the hospital setting. The fact that research in this field is fragmented and predominantly monodisciplinary hinders the development of innovative diagnostic and therapeutic solutions.

The EURESTOP COST Action seeks to address these challenges by uniting industrial and academic European scientists in a collaborative, multidisciplinary initiative to understand the genetic and molecular foundations of bacterial drug resistance and develop innovative diagnostic tools and explore antibody-based therapies, and delivery of clinical-ready repurposed drugs for the personalized treatment of drug-resistant bacterial infections [1].

This communication will highlight the opportunities arising from the EURESTOP COST Action, emphasizing the benefits of fostering networks among European scientists, as well as the training opportunities for a new generation of young scientists in various aspects related to bacterial drug resistance.

Acknowledgements:
The author acknowledges the University of Aveiro and FCT/MCT for the financial support for the LAQV-REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020), through national funds (OE) and where applicable co-financed by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Special thanks are extended to Cost Action 21145 – European Network for the Diagnosis and Treatment of Antibiotic-Resistant Bacterial Infections.

References:

[1] www.eurestop.eu

MICROBIOTEC 23

Poster Session

Abstracts

MICRO BIOTEC 23

Poster Session

Topic 1

P1.1 - HETEROLOGOUS EXPRESSION AND CHARACTERISATION OF NOVEL CHITINASES FROM A YET UNCULTURABLE, HIGHLY ABUNDANT BACTERIAL SYMBIONT OF OCTOCORALS

João Almeida [1,2,*], Mafalda Leitão [1,2], Nuno Borges [3], Rodrigo Costa [1,2], Ângela Taipa [1,2], Tina Keller-Costa [1,2,*]

[1] Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal
[2] Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal
[3] Instituto Politécnico de Setúbal, EST Setúbal, Campus do IPS, Setúbal, Portugal
[(*)] e-mail: joao.fragoso.almeida@tecnico.ulisboa.pt | tinakellercosta@tecnico.ulisboa.pt

Keywords: Chitin, Symbiosis, *Endozoicomonadaceae,* Chitinases, Marine Biotechnology, Blue bioeconomy

ABSTRACT

Chitin is the most abundant polysaccharide in the ocean, present in phyto- and zooplankton, fungi, shells of crustaceans, and other invertebrate animals. Octocorals (*Octocorallia, Anthozoa, Cnidaria*) are found worldwide, from tropical to cold-water marine biomes. As suspension-feeders, octocorals ingest a chitin-rich diet, and their associated microbiome likely has a key role in chitin metabolization. Among the symbionts presumably involved in chitin turnover is the dominant but difficult-to-cultivate bacterial family Endozoicomonadaceae. In an earlier metagenomics study, our team identified two new species of a novel genus in this family, *Candidatus Gorgonimonas eunicellae* and *Ca. Gorgonimonas leptogorgiae* [1]. Each species harboured a functional and unique chitinase (EC 3.2.1.14) gene. Given their marine origin, we hypothesized that these chitinases may have properties distinct from those currently in the market. Since *Candidatus Gorgonimonas* remains unculturable, their chitinase genes were here de novo synthesized, cloned into pet19 plasmids, heterologously expressed in *Escherichia coli*, and purified by affinity chromatography. Analysis by SDS-PAGE revealed a single band in both cases, corresponding to MWs of 58 and 64 kDa. Enzymatic activity tests with commercial, fluorochrome-labelled substrates showed that both enzymes presented endo-chitinase activity (i.e., ability to cleave large chitin polymers) but lacked exo-chitinase activity (i.e., ability to cleave small chito-oligosaccharides). The larger chitinase was then characterised for optimum pH (5) and temperature (30-37ºC), being active in the 4-40ºC and 4-8 pH ranges. Under optimal conditions (pH 5, 35ºC), the specific activity was 1.6 U/mg. Further studies, such as co-factor (metal ion) influence on activity, pH- and thermostability and substrate specificity are ongoing. This novel marine chitinase possesses lower optimal temperatures compared with commercial chitinases from soil microorganisms (*Trichoderma* and *Streptomyces*), holding promise for future usage in the upcycling of chitin-rich seafood waste.

[1] Keller-Costa et al., 2022, Microbiome 10, 151 https://doi.org/10.1186/s40168-022-01343-7

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) through the research project grants 'ChicoBionts' - EXPL/BIA-MIC/0286/2021. The authors also acknowledge FCT funds in the scope of projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and project LA/P/0140/2020 of i4HB. João Almeida is the recipient of a doctoral student grant (2022.12932.BD) conceded by the FCT. Tina Keller-Costa. is the recipient of an investigator contract (CEECIND/00788/2017) conceded by the FCT.

P1.2 - MICROBE-ENHANCED PHYTOREMEDIATION WITH SALICORNIA EUROPAEA IN AQUAPONICS SETUPS

Maria J. Ferreira [1,2,3]***, Erika Garcia*** [3]***, Isabel N. Sierra-García*** [1]***, Diana C.G.A. Pinto*** [2]***, Javier Cremades*** [3]***, Helena Silva*** [1] ***and Ângela Cunha*** [1*]

[1] *Department of Biology & Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Portugal.*
[2] *LAQV-REQUIMTE & Department of Chemistry, University of Aveiro, Portugal.*
[3] *Centre for Advanced Scientific Research (CICA), University of A Coruña, Spain.*

[(*)] *e-mail: acunha@ua.pt*

Keywords: halophytes, bioremediation; aquaponics; biostimulants, metabolic profile

ABSTRACT

The marine aquaculture industry is expanding, but grapples with issues like nutrient control, waste management, and disease prevention. Using halophyte plants for bioremediation has proven effective in reducing environmental impacts, particularly within integrated multitrophic aquaculture (IMTA) systems. Plant growth-promoting bacteria (PGPB) are effective as biofertilizers, but their use in aquaculture wastewater phytoremediation remains unexplored. To address this, we tested whether introducing halophytes to specific PGPB would speed up the removal of surplus inorganic nutrients. The nutrient extraction capacity of the halophyte Salicornia europaea, when inoculated with PGPB, was examined, and set against non-inoculated controls for comparison. Two bacterial strains, Brevibacterium casei EB3 and Pseudomonas oryzihabitans RL18, both isolated from the rhizosphere of wild S. europaea specimens, were tested. Two experimental setups were used: a smaller scale in growth chambers and a larger pilot-scale indoors. Small-scale setups used either individual strains or a combined mix of the two, while pilot-scale setups used only the combined mix. We analyzed plant biomass, water nutrient concentration, metabolite profiles, and bacterial communities associated with roots. Plants inoculated with the bacterial strains exhibited a 2.6-fold increase in biomass and demonstrated a significantly enhanced capacity to extract nitrogen and phosphorus from the water. A notable shift was observed in the plants' metabolite profile upon inoculation, characterized by a rise in phenolic content. When analyzing the structure of bacterial communities linked to the roots of S. europaea using PCR-DGGE, distinct differences emerged between the pot and tank setups. This suggests that the operational conditions inherent to each system exert a marked influence on the microbial community dynamics.

Based on the findings, the synergistic use of halophyte plants and PGPB bacteria holds potential as an effective bioremediation strategy for marine aquaculture systems. Plant-derived bioactive molecules may enhance economic and environmental sustainability.

Acknowledgements:
This work was supported by project Rhizomis PTDC/BIA-MIC/29736/2017 financed by Fundação para a Ciência e a Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020), LAQV-REQUIMTE (UIDB/50006/2020 + UIDP/50006/2020)). We also acknowledge FCT/FSE for the financial support to Maria João Ferreira through a PhD grant (PD/BD/150363/2019).

P1.3 - INSIGHTS INTO THE CULTURABLE BACTERIAL COMMUNITY OF LONG-TERM AQUARIUM TROPICAL OCTOCORALS

Matilde Marques [1,2,*], Elsa Santos [3], Núria Baylina [3], Raquel Peixoto [4], Tina Keller-Costa [1,2¥*], Rodrigo Costa [1,2¥]

[1] Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico (IST), University of Lisbon, Lisbon, Portugal
[2] Department of Bioengineering, Instituto Superior Técnico (IST), University of Lisbon, Lisbon, Portugal
[3] Oceanário de Lisboa, Esplanada D. Carlos I, Lisbon, Portugal
[4] Red Sea Research Center, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia
[¥] These authors contributed equally

[(*)] e-mail: matildesangremanmarques@tecnico.ulisboa.pt | tinakellercosta@tecnico.ulisboa.pt

Keywords: Corals, Microbiome Stewardship, Mesocosm, *Endozoicomonas,* Symbiosis, Marine Microbiology

ABSTRACT

Coral reefs are highly complex marine ecosystems that host diverse microbial consortia, which play key roles in coral fitness. Due to climate change, coral mass mortality events have led to severe biodiversity loss. A shift from stony coral-dominated reefs towards octocorals (Subclass Octocorallia) has been registered in several geographical areas; nevertheless, octocorals are still affected by climate change. To better understand the symbiotic relationships that octocorals establish with microorganisms, in this study we determine the taxonomic composition of culturable, octocoral-associated bacteria in a long-term aquarium mesocosm. Three octocoral species, Litophyton sp., Lobophytum sp. and Sclerophytum sp., were sampled from a live coral aquarium, from Oceanário de Lisboa, and their associated bacterial community cultured on diluted marine R2A and Marine Agar media. A total of 90 bacterial strains were isolated. 16S rRNA gene-based phylogenetic analyses grouped the isolates into six bacterial classes, 14 orders and 26 classified genera. Additionally, six unclassified isolates were obtained, two of them likely representing new bacterial families in the Alteromonadales and Cellvibrionales orders, and the other four likely representing a new gammaproteobacterial order. The collection comprised multiple, 'harder-to-cultivate' genera such as Endozoicomonas, Fictibacillus, and Flammeovirga. Moreover, possible new bacterial species in the genera Amylibacter, Dietzia, Endozoicomonas, Fictibacillus, Nocardioides, and Ureibacillus were identified. The Endozoicomonas genus, in particular, has been frequently reported from multiple coral species, and suggested as a core symbiont of the healthy holobiont. The preservation of core symbionts of healthy corals in captivity highlights the possibility of using sustainable, man-made ecosystems as repositories of stable and healthy coral microbiomes. Our study showcases the phylogenetic uniqueness of culturable, bacterial symbionts of tropical octocorals maintained for long-term periods in controlled mesocosms. This emphasizes the key role that aquarium facilities can have in coral reef restoration and as source of new bacterial species worth further research.

Acknowledgements:
This work was supported by the Fundo Azul program of Direção-Geral de Política do Mar (DGPM; Ministry of the Sea, Portugal) through grant FA_05_2017_032. The authors also acknowledge Portuguese Foundation for Science and Technology (FCT) funds in the scope of projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and the project LA/P/0140/2020 of i4HB. M.M. was supported by the doctoral Grant SFRH/BD/151376/2021 financed by the FCT, under the MIT Portugal Program. T.K.C. is the recipient of an investigator contract (CEECIND/00788/2017) conceded by the FCT.

P1.4 - EFFECTS OF DEFORESTATION ON SOILS FROM GUINEA-BISSAU

Rafael António [1*], Ana João Martins [1], Inês Cordeiro [1], Eduardo Onofre Feijão [2], Ana Rita Matos [3], Filipa Monteiro [4,5], Mónica Sebastiana [3]

[1] Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
[2] Marine and Environmental Sciences Centre (MARE), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
[3] Instituto de Biosistemas e Ciências Integrativas (BioISI), Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
[4] Linking Landscape, Environment, Agriculture and Food (LEAF), Associate Laboratory TERRA, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal
[5] Centre for Ecology, Evolution and Environmental Changes (cE3c) & Global Change and Sustainability Institute (CHANGE), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[(*)] e-mail: rpantonio@fc.ul.pt

Keywords: soil biodiversity; land use; PLFA; tropical soils; West Africa.

ABSTRACT

Current studies show a decline in soil biodiversity, which puts at risk future food production since soil organisms play a key role in essential ecosystem functions such as, nutrient cycling and soil fertility, soil carbon sequestration, climate regulation, litter decomposition, erosion, water retention, interaction with plants, among others. This study aims to evaluate how the conversion of a tropical native forest into a field for agricultural production impacts the soil chemistry and its microbial communities. The soils analyzed were collected in Guinea-Bissau, at 3 locations with different land uses: a primary forest, an annual crop field (peanut) and a perennial crop field (cashew), during the wet and dry season. We analyzed several soil parameters including pH, water content, N, P and C contents, soil respiration, and soil protein index. Mycorrhizal spore density, analysis of soil PLFA (Phospholipid fatty acids), and qPCR targeting genes involved in N, C and P cycling, were selected for investigating alterations in soil microbial diversity.

Our study on a tropical ecosystem from Guinea-Bissau shows that forest soils have an overall higher values for most chemical parameters, while peanut and cashew soils have lower values, with some exceptions, such as N-NO3-. Regarding soil biodiversity, our results point to a greater soil biodiversity in forest soils, and we expect that qPCR analysis will support and enhance this finding. Our results confirm that land use changes alter soil chemistry and its microbial communities and there is a decrease in soil quality and biodiversity following the conversion of forests to crop fields.

Acknowledgements:
This study was supported by FAO (United Nations) under the project TCP/GBS/3802, and by FCT under the project PTDC/ASP-AGR/0760/2020, and the grants UIDB/04046/2020 and UIDP/04046/2020 to BioISI, and UIDB/04129/2020 to LEAF. MS was supported by the FCT grant DL57/2016/CP [12345/2018]/CT [2475]. FM was funded by the FCT grant 00392.CEECIND.

P1.5 - MINING THE BIOSYNTHETIC POTENTIAL FOR SPECIALIZED METABOLISM OF LEGE CULTURE COLLECTION

Raquel Castelo-Branco [1,2*], Adriana Rego [1], João Morais [1,2], Vitor Vasconcelos [1,2], Pedro N. Leão [1]

[1] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Matosinhos, Portugal
[2] Faculty of Sciences, University of Porto, Porto, Portugal

[(*)] e-mail: rcastelobranco@ciimar.up.pt

Keywords: *Cyanobacteria,* genome mining, natural products, biosynthetic potential

ABSTRACT

Natural products (NPs) (often referred as specialized or secondary metabolites) are an unparalleled source of bioactive compounds, many of which are applied in different fields such medicine or agriculture. Traditionally, these compounds were isolated by bioactivity-guided approaches; however, advances in genomics and bioinformatics, have paved the way for modern genomics-based discovery approaches. In microbial genomes, genes for the synthesis of NPs are physically grouped together in biosynthetic gene clusters (BGCs). Advances in sequencing technologies and bioinformatics tools have played a pivotal role in the discovery of BGCs through genome mining revealing that bacteria are a massive untapped trove of new metabolites that is reflected in a much greater biosynthetic diversity than the compounds characterized so far.

In the last years, Cyanobacteria have been pointed out as one of the most promising groups of bacteria as producers of NPs due to their potent bioactivity and structural uniqueness. However, there are few reports exploring the biosynthetic potential of these organisms. This study focuses on describing the natural product genetic potential in cyanobacterial strains from an important Culture Collection from Portugal, the Blue Biotechnology and Ecotoxicology Culture Collection (acronym LEGE), which harbor more than 700 cyanobacterial strains covering a wide range of geographical habitats. Regardless of such biodiversity, only a small fraction of the diversity present at LEGE CC was studied and chemodiversity potential of many unique strains remains unknown.

In this work, we report 65 newly-generated LEGE strains genomes in Cyanobacteria, which expand the genomic representation of the Phylum from undersampled habitats, mainly from Portugal. Using 16S rRNA gene-based phylogeny and sequence similarity BGC networking analysis we characterize the biosynthetic potential of cyanobacterial strains from LEGE Culture Collection. Our results highlight a number of highly interesting BGCs for genome mining among these cyanobacterial strains that could open new avenues for drug discovery.

P1.6 - EXPLORING THE POTENTIAL OF CO-CULTURES TO ENHANCE SECONDARY METABOLITES PRODUCTION IN THE MARINE BACTERIUM AND ANTIBIOTIC PEPTIDE PRODUCER AQUIMARINA SP. AQ135

Ana Maria Fernandes [1,2]***, Joana F. Couceiro*** [1,2]***, Tina Keller-Costa*** [1,2*]***, Rodrigo Costa*** [1, 2*]

[1] *iBB—Institute for Bioengineering and Biosciences and i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal*
[2] *Department of Bioengineering, Instituto Superior Técnico, Lisbon, Portugal*

[(*)] *e-mail: tinakellercosta@tecnico.ulisboa.pt | rodrigoscosta@tecnico.ulisboa.pt*

Keywords: Marine Bacteria, Secondary Metabolites, Co-culture, LC-MS, Bioactivity

ABSTRACT

Microbial-based production of secondary metabolites is often hindered by laboratorial growth conditions which usually do not provide cues from the natural environment, resulting in either very low or no yields since the underlying biosynthetic gene clusters (BGCs) may be silent under such conditions.

The marine-sponge derived bacterium Aquimarina sp. Aq135 (Bacteroidota, Flavobacteriaceae) possesses twelve BGCs coding for novel natural products. It produces the peptide antibiotics aquimarins, potent inhibitors of Gram-positive bacterial pathogens. However, under standard culture conditions antibiotic yields by this presumably prolific bacterium are very low.

This study investigates whether co-cultivation with another, sympatric marine bacterium – namely coral- derived Vibrio sp. strain EL41 - enhances secondary metabolite production in Aquimarina. The Aquimarina- Vibrio pair was inoculated (1) simultaneously in five different optical density (OD) ratios – Aq135/EL41: 50/50, 60/40, 70/30, 80/20 and 90/10 – and (2) with the same OD but unsynchronized with a 16h, 24h, 36h or 48h delay of Vibrio inoculations. Cell numbers of both strains in the co-cultures were quantified by viable cell counting. An equitable ratio of cells was obtained for the Aq135/EL41 pair in the unsynchronized method with a 24h Vibrio inoculation delay and chosen for further analyses. The culture supernatants of the co- culture and of both strains grown individually were subjected to solid phase extraction and analyzed by liquid chromatography-high resolution-mass spectrometry (UPLC-HR-MS). UPLC-HR-MS results showed significant differences between extracts of Aq135, EL41 and the Aq135/EL41 co-culture with chromatography peaks unique to the co-culture. Disk diffusion assays determined higher activity of the co- culture extracts against the Gram-positive human- and aquaculture pathogen Streptococcus iniae. Metabolomics and meta-transcriptomics analyses are ongoing to discern differentially expressed secondary metabolites and genes of Aquimarina sp. Aq135 under co-cultivation. Our results suggest that laboratory manipulation with naturally occurring bacterial competitors induces activation of otherwise silent BGCs in understudied, likely prolific secondary metabolite-producing bacteria.

Acknowledgements:
This study was supported by the "BLUE BIOECONOMY PACT" (Project Nº. C644915664-00000026), co-funded by Next Generation EU European Fund, under the incentive line "Agendas for Business Innovation" within Component Funding Scheme 5 - Capitalization and Business Innovation of the Portuguese Recovery

and Resilience Plan (RRP). Further support was provided by the Fundo Azul program of Direção-Geral de Política do Mar (DPGM; Ministry of the Sea, Portugal) through grant FA_05_2017_032. The authors also acknowledge FCT funds in the scope of projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and project LA/P/0140/2020 of i4HB. Joana F. Couceiro acknowledges doctoral grant PRT/BD/153503/2021 from the MIT Portugal program, financed through FCT. T.K.C. is the recipient of an investigator contract (CEECIND/00788/2017) conceded by the FCT.

P1.7 - ENTOMOPATHOGENIC FUNGI AS A POTENTIAL BIOCONTROL AGENT OF SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII* (MATSUMURA), IN BLUEBERRY PRODUCTION

Sara Peixoto [1], Maria F. Gonçalves [2], Guilhermina Marques [2*]

[1] Department of Biology, University of Aveiro and CESAM – Centre for Environmental and Marine Studies, Aveiro, Portugal;
[2] Department of Agronomy, University of Trás-os-Montes e Alto Douro and CITAB – Center for the Research and Technology of Agro-Environmental and Biological Sciences, Vila Real, Portugal.

(*) e-mail: gmarques@utad.pt

Keywords: Integrated pest management, biological control, trapping, repellent effect, oviposition.

ABSTRACT

Drosophila suzukii is a fruit fly that has become a serious economic pest of small fruits worldwide. Current pest control measures, that rely heavily on the use of broad-spectrum insecticides, are usually ineffective, essentially due to their restricted use close to harvest to protect consumers. Therefore, more efficient and sustainable strategies are needed. Studies reporting the effectiveness of entomopathogenic fungi (EPF) against *D. suzukii* are scarce and not fully understood. Thus, this work aimed to evaluate the potential of three species of Metarhizium (*M. anisopliae, M. robertsii, and M. guizuansuense*) and *Beauveria bassiana* to protect blueberries from

D. suzukii infestation. Two-choice trials were performed in arenas. Each arena was composed of two traps containing an attractive substance and two blueberries treated with EPF (4.0x106 conidia/ml) or water (control). The traps were sealed cups containing eight holes and a red line around the cup. Twenty adult flies were added in each arena. After three days, the number of flies inside the traps, the mortality, and the number of oviposition punctures in blueberries were examined. A significantly lower number of flies were found inside the traps containing blueberries treated with EPFs compared to the control, suggesting a potential repellent effect on flies. Once inside the traps, the mortality of adults was significantly higher in EPF treatments compared to the control, exhibiting a mortality rate of 62.5% in *B. bassiana* and 88.0% in *M. guizuansuense*. Only

B. bassiana (0.8 eggs vs. 6.9 eggs in control) and *M. anisopliae* (1.8 eggs vs. 6.9 eggs in control) showed a significantly reduced number of egg punctures in blueberries compared to the control. This work revealed that *Metarhizium* and *Beauveria* can be effective biocontrol agents of *D. suzukii*, highlighting their relevant inclusion in insect management programs for blueberry production.

Acknowledgements:
This research received funding from Fundação para a Ciência e Tecnologia (FCT) through the project DrosuGreen: Controlling the quarantine pest Drosophila suzukii through epidemiological studies and new Green biocontrol techniques with the Grant Number: PTDC/ASP-PLA/4477/2020. The authors also would like to thank CITAB-Center for the Research and Technology of Agro-Environmental and Biological Sciences, project UIDB/04033/2020.

P1.8 - ELIMINATION OF ANTIBIOTIC RESISTANCE GENES DURING CONVENTIONAL ACTIVATED SLUDGE WASTEWATER TREATMENT: THE EFFECT OF TEMPERATURE

Sara Ribeirinho-Soares [1,2(*)]***, Vasco Braga*** [3]***, Sofia Cabral*** [4]***, Vítor J.P. Vilar*** [2,5]***, Célia M. Manaia*** [6]***, Olga C. Nunes*** [1,2]

[1] *LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal* [2] *ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*
3 Efacec Engenharia e Sistemas SA, São Mamede de Infesta, Portugal
[4] *ME Water – Manvia Efacec Exploração Técnica Sistemas Ambientais do Minho, Maia, Portugal*
[5] *LSRE-LCM – Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Porto, Portugal*
[6] *Universidade Católica Portuguesa, CBQF - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

(*) *e-mail: saramariasoares@hotmail.com*

Keywords: anthropogenic contamination; bacterial community structure and composition; abundance of antibiotic resistance genes.

ABSTRACT

Antibiotic resistance is one of the major threats to global health. Alongside, the worldwide water crisis is increasing, being wastewater reuse one of the most promising ways to deal with the water crisis. Most urban wastewater treatment plants (UWWTP) rely on conventional activated sludge (CAS) technology, which reduces the loads of organic matter, suspended solids and microorganisms. However, CAS treated wastewater still contains high loads of antibiotic resistant bacteria and related genes (ARBs&ARGs). Thus, understanding how the operational conditions of the CAS systems influence the removal of ARBs&ARGs during wastewater treatment is crucial.

This study aimed at assessing the effect of temperature on the fate of ARGs during CAS treatment. To achieve this, two laboratory CAS installations were operated continuously in an UWWTP at temperatures of 10 °C (day 1-56); 5 °C (day 57-91); 15 °C (day 92- 113) and 28 °C (114-147) while the control CAS operated continuously at room temperature (19.6 ± 4.2 °C, day 1-147). The remaining operating parameters were set equal in both the counterparts and the abundance of fecal contamination indicators, ARGs as well as bacterial community composition were accessed weekly in the final treated wastewater and in the surplus sludge.

Regardless of the operating temperature, concentration values of chemical and biological oxygen demand and total suspended solids in the CAS effluents agreed with the European Council directive concerning urban wastewater treatment. However, higher operation temperatures resulted in effluents with lower microbiological quality. This study shows that low CAS operation temperature can deflect levels of potentially harmful bacteria of anthropogenic origin in the effluent and surplus sludge.

Acknowledgements:

This work was financially supported by: UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM) and LA/P/0045/2020 (ALiCE) funded by national funds through FCT/MCTES (PIDDAC). Sara Ribeirinho-Soares acknowledges the Portuguese Foundation for Science and Technology (FCT) for her PhD grant (PD/BD/05480/2021), financed by national funds of the Ministry of Science, Technology and Higher Education and the European Social Fund (ESF) through the Human Capital Operational Programme (POCH).

P1.9 - APPLICATION OF PLANT GROWTH PROMOTING BACTERIA AS BIOSTIMULANTS IN *OLEA EUROPAEA*

Lara Soares [1], Pedro Monteiro [1], Jyoti Chhetri [1], I. Natalia Sierra-García [1], Gloria Pinto [1], Ângela Cunha [1*]

[1] CESAM e Departamento de Biologia. Universidade de Aveiro, Portugal.

[(*)] e-mail: acunha@ua.pt

Keywords: PGPB; *Bacillus siamensis, Pseudomonas oryzihabitans,* leaf gas exchange; photosynthesis.

ABSTRACT

Indigenous Portuguese olive tree varieties are valued for their oil quality and resilience to abiotic stresses. However, they are often susceptible to various diseases, and climate change effects. This study aims to elucidate the benefits of inoculating olive trees with Plant Growth-Promoting Bacteria (PGPB) strains and to assess the most effective combination of inoculants.

Bacillus siamensis RN42, isolated from Negrinha do Freixo trees, was tested either independently or combined with the halotolerant Pseudomonas oryzihabitans RL18, isolated from Salicornia europaea.

One-year-old Galega vulgar tree roots were subjected to four distinct inoculation treatments: (1)

P. oryzihabitans RL18 - A; (2) B. siamensis RN42 - B; (3) Both – A+B; (4) No inoculation - C. Plants were cultivated under greenhouse conditions for 12 weeks. Photosynthetic performance was evaluated with an infrared gas analyser (IRGA) and biochemical markers were analysed on leaf material.

Plants treated with B. siamensis RN42 exhibited the highest stomatal conductance and net rates of CO2 assimilation and transpiration. Furthermore, there was a significant increase in the concentration of soluble sugars, consistent with cellular signalling pathways associated with stress responses.

The most promising results were seen when using B. siamensis RN42, a strain native to the rhizosphere of olive trees, as the only inoculant. P. oryzihabitans RL18, had previously been recognized for augmenting plant growth in saline stress conditions. Yet, in the absence of these specific stressors, this strain did not exhibit any significant improvement in plant condition. Interestingly, when P. oryzihabitans RL18 was combined with B. siamensis RN42, the advantageous effects of the latter were negated. This suggests that in the absence of external stressors like salinity, the potential benefits of the halotolerant strain in the inoculant mix might be overshadowed by antagonistic interactions between the bacterial strains.

Acknowledgements:
This work was supported by project Halius (PTDC/BIA-MIC/3157/2020) financed by Fundação para a Ciência e a Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020).

P1.10 - PHOTODYNAMIC INACTIVATION OF *OLEA EUROPAEA* PATHOGENS: PROSPECTS ON A NOVEL PHYTOSANITARY APPROACH

Joana Soares [1], Isabel N. Sierra-Garcia [1], M. Amparo F. Faustino [2], Ângela Cunha [1*]

[1] CESAM e Departamento de Biologia. Universidade de Aveiro, Portugal.
[2] LAQV-Requimte e Departamento de Química. Universidade de Aveiro, Portugal.

[(*)] e-mail: acunha@ua.pt

Keywords: *Photodynamic inactivation, Colletotrichum gloeosporioides, Pseudomonas savastanoi,* olive tree tuberculosis, anthracnose.

ABSTRACT

The bacterium *Pseudomonas savastanoi* that causes tuberculosis in olive trees (*Olea eueopaea*) and the fungus *Colletotrichum gloeosporioides*, that causes anthracnose, represent major sanitary threats to olive groves. In modern agriculture, the use of chemical biocides is being increasingly limited or even prohibited, reflecting the pressing need to integrate OneHealth principles into farming practices. The search for sustainable phytosanitary approaches is becoming more and more crucial.

This research tested the hypothesis that *P. savastanoi* and *C. gloeosporioides* are susceptible to photodynamic inactivation with the commercial photosensitizer Toluidine Blue O (TBO), an agent with low toxicity, contributing to the development of photodynamic plant protection products.

Complete photoinactivation (> 6 log reduction) was attained with 0.1 mM of TBO after 60 min of irradiation with white light at an irradiance of 135 mW/cm2 in cell suspensions of *P. savastanoi*, as well as in biofilms constructed in artificial substrates. On the surface of leaves, no significant reduction (< 1 log reduction) of the concentration of viable cells on the biofilms could be demonstrated. The assays conducted with the fungus, indicate that TBO is toxic to *C. gloeosporioides*, inhibiting mycelium growth even in the absence of light.

The results confirm that these two pathogens can be effectively controlled with TBO. Although the combination of antibacterial and antifungal effects can be regarded as a plus of TBO, the modes of action against bacteria and fungi are different and impose operational limitations on the efficiency of the treatments. To control of tuberculosis, a very early treatment, relative to the moment of infection, is recommended to prevent bacteria from accessing the plant's internal tissues and forming biofilms. In contrast, the growth of *C. gloeosporioides* mycelium, following spore germination, is effectively inhibited, even during alternating dark (night) and light (day) periods, as would occur under natural sunlight conditions.

Acknowledgements:
This work was supported by project Halius (PTDC/BIA-MIC/3157/2020) financed by Fundação para a Ciência e a Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020) and LAQV-REQUIMTE (UIDB/50006/2020 + UIDB/50006/2020).

P1.11 - FEATHER WASTE RECYCLING: CREATING ECO-FRIENDLY BIO-PESTICIDES

Mário Teixeira [1]*, Júlia Gomes [1], Nelson Simões [1]

[1] Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, Ponta Delgada.

(*) e-mail: mario.b.teixeira@uac.pt

Keywords: feather, waste, bio-pesticides, *Bacillus thuringiensis,* circular economy

ABSTRACT

The reuse of waste is one of the hallmarks of the circular economy. Feather waste is an additive in soils since they are a source of amino acids and nitrogen for plants and can absorb pesticides. We tested the production of *Bacillus thuringiensis* (Bt) local isolates in feather waste. The Bt cells were grown in two liquid minimal media at 30 ºC with horizontal agitation for 48 hours: the T4 medium was supplemented with yeast extract and 1% w/v of feathers, and Medium T5 was not supplemented. Bt spores and crystals were counted, and entomotoxicity against the pest *Ephestia kuehniella* larvae was used as a toxicity model to assess the Bt entomotoxicity and compare Bt isolates.

A total of 274 isolates were tested: 54.95% of the isolates were able to grow in the medium T4, producing spores and crystals, and only 39.05% of isolates presented grown in the T5 medium. The S175A isolate that presented more growth in the T5 medium presented an LC50 of 25.30 µg/g *Ephestia kuehniella*. The cry genes in S175A isolates were tested and presented cry1Aa, cry1Ab, cry1Ac, cry2Aa, and cry2Ab, all of which have been proven to be highly toxic to several pests.

Further analysis of isolates grown in feathers revealed the presence of other cry genes, including cry1Ad, cry1B, cry1Fb, cry4, cry10, cry11, cyt1, and cyt2. Interestingly, these genes are toxic to soil pests such as *Phthorimaea operculella, Tecia solanivora, and Agrotis ípsilon*.

Our data show that it is feasible to use feather waste as an alternative substrate for the growth of Bt, acting as a biopesticide for soil pests and helping Europe achieve climate neutrality by 2050.

Acknowledgements:
This research was supported by Base Funding (2020-2023) of Centro de Biotecnologia dos Açores (R&D Unity 5292), FCT (P).

P1.12 - EVALUATING OLEA EUROPAEA RHIZOSPHERE COLONIZATION USING GFP-MODIFIED PLANT-GROWTH PROMOTING BACTERIA

Eva Peixoto Martins [1], Maria Tavares [1], Jyoti Chhetri [1], Ângela Cunha [1], Tânia Caetano [1], I. Natalia Sierra-Garcia [1*]

[1] CESAM & Departamento de Biologia. Universidade de Aveiro, Aveiro, Portugal.

[(*)] e-mail: inatalia.sierra@ua.pt

Keywords: *Pseudomonas oryzihabitans,* GFP, root surface, biostimulants

ABSTRACT

Plant-growth promoting bacteria (PGPB) are a group of beneficial bacteria that associate with plant roots and promote plant growth through various mechanisms. Their beneficial effects on plants can help reduce the dependence on chemical fertilizers and pesticides, leading to sustainable agricultural practices. Yet, determining if a particular PGPB strain can be effectively inoculated in a candidate host plant is a critical step in the process of developing PGPB-based biostimulants.

This study investigated the capacity of *Pseudomonas oryzihabitans* (RL18), known for its ability to promote plant growth in saline conditions, to colonize the roots of roots of young olive trees (Olea europaea). This strain was transformed with the plasmid pMF230 (Addgene) which allows the constitutive expression of Green Fluorescent Protein (GFP). Electrocompetent cells of *P. oryzihabitans* RL18 were prepared by washing an overnight culture twice with 300 mM sucrose and resuspending in the same solution. For electroporation, 500 ng of pDNA were mixed with 100 µL of electrocompetent cells. After electroporation (2.5 kV), transformants were selected on LB-agar plates supplemented with ampicillin (100 mg/L) and carbenicillin (400 mg/L). The successful transformation (RL18_GFP strain) was confirmed by PCR targeting the gfp gene and by observing GFP fluorescence under UV light.

To assess the kinetics of colonization, roots of young olive trees (<1 year-old) were inoculated with RL18_GFP by immersion in a bacterial cell suspension. Plants immersed in saline solution were used as non-inoculated controls. Root samples were periodically collected and examined under fluorescence microscopy (Leica, with the program LAS X, filter I3). After 2-hours, patches of fluorescent cells were visible on the root surface, indicating that RL18_GFP can colonize olive tree roots.

The findings from this study are now being used in experiments assessing plant growth promotion in Olea europaea with various PGPB strains and consortia. The RL18_GFP is a valuable model for advancing plant-microbial interaction research, especially by tracing rhizosphere bacteria to the endosphere.

Acknowledgements:
This work was supported by project Halius (PTDC/BIA-MIC/3157/2020) financed by Fundação para a Ciência e a Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020).

P1.13 - WIDESPREAD OCCURRENCE OF CHITINASE-ENCODING GENES SUGGESTS THE ENDOZOICOMONADACEAE FAMILY AS A KEY PLAYER IN CHITIN PROCESSING IN THE MARINE BENTHOS

Daniela M. G. da Silva [1*], Filipa R. Pedrosa [1], M. Ângela Taipa [1,2], Rodrigo Costa [1,2], Tina Keller-Costa [1,2*]

[1] Department of Bioengineering, Instituto Superior Técnico, Av. Rovisco Pais, Lisbon, Portugal
[2] iBB - Institute for Bioengineering and Biosciences and i4HB - Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal

[(*)] e-mail: daniela.marina@tecnico.ulisboa.pt | tinakellercosta@tecnico.ulisboa.pt

Keywords: *Chitinolytic bacteria,* nutrient cycling, glycoside hydrolases, host-microbe interactions, *Oceanospirillales, Endozoicomonas.*

ABSTRACT

Chitin is the most abundant natural polymer in the oceans, where it is primarily recycled by chitin-degrading microorganisms. *Endozoicomonadaceae* (*Oceanospirillales*) bacteria are prominent symbionts of sessile marine animals, particularly corals, and presumably contribute to nutrient cycling in their hosts. To reveal the chitinolytic potential of this iconic, animal-dwelling bacterial family, we examined 42 publicly available genomes of cultured and uncultured *Endozoicomonadaceae* strains for the presence of chitinase-encoding genes. Thirty-two of 42 *Endozoicomonadaceae* genomes harbored endo-chitinase – (EC 3.2.1.14), 25 had exo-chitinase – (EC 3.2.1.52) and 23 polysaccharide deacetylase-encoding genes. Chitinases were present in cultured and uncultured *Endozoicomonadaceae* lineages associated with diverse marine animals, including the three formally described genera *Endozoicomonas*, *Paraendozoicomonas* and *Kistimonas*, the new genus *Candidatus* Gorgonimonas, and other, yet unclassified groups of the family. Most endo-chitinases belonged to the glycoside hydrolase family GH18 but five GH19 endo-chitinases were also present. Many endo-chitinases harbored an active site and a signal peptide domain, indicating the enzymes are likely functional and exported to the extracellular environment where endo-chitinases usually act. Phylogenetic analysis revealed clade-specific diversification of endo-chitinases across the family. The presence of multiple, distinct endo-chitinases on the genomes of several *Endozoicomonadaceae* species hints at functional variation to secure effective chitin processing in diverse micro-niches and changing environmental conditions. We demonstrate that endo-chitinases and other genes involved in chitin degradation are widespread in the *Endozoicomonadaceae* family and posit that these symbionts play important roles in chitin turnover in filter- and suspension-feeding animals and in benthic, marine ecosystems at large.

Acknowledgements:
The authors are grateful to João Almeida for initial help with KBase and iTOL and to Sandra Silva for help with COG and Pfam annotations using the Melange pipeline. This work was supported by the Portuguese Foundation for Science and Technology (FCT) through the research project grants 'ChicoBionts' - EXPL/BIA-MIC/0286/2021. The authors also acknowledge FCT funds in the scope of projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and project LA/P/0140/2020 of i4HB. T.K.C. is the recipient of an investigator contract (CEECIND/00788/2017) conceded by the FCT.

P1.14 - IMPROVING BIOMETHANE PRODUCTION BY ADDING CONDUCTIVE NANOMATERIALS

Ana Catarina Silva [1], Cátia S. N. Braga [1], Inês Oliveira [1], O. Salomé G. Soares [3,4], M. Fernando R. Pereira [3,4], Luciana Pereira [1,2], Andreia F. Salvador [1,2], Gilberto Martins [1,2*]

[1] CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal.
[2] LABBELS – Associate Laboratory, Braga/Guimarães, Portugal.
[3] 1 LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Material, Faculty of Engineering, University of Porto, Porto, Portugal.
[4] ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal.

(*) e-mail: gilberto.martins@deb.uminho.pt

Keywords: Anaerobic digestion; conductive materials, carbon nanomaterials, pure cultures of methanogens.

ABSTRACT

The search for renewable energy sources is one of the top priorities of humanity. Biomethane production through anaerobic digestion (AD) could help to reduce the dependence on fossil energy. The addition of conductive nanomaterials (CM) to AD systems results in the acceleration of methane production (MP) and in the improvement of the resilience of these systems. However, the mechanisms underlying this phenomenon are still unclear, particularly regarding the effects of materials on the activity of methanogens. The present work aimed to evaluate the effect of adding different carbon nanomaterials to a hydrogenotrophic and an acetoclastic culture.

For this purpose, experiments were conducted with pure cultures of *Methanobacterium formicicum* and *Methanosaeta harundinacea*, in the presence and absence of 0.5 g/L of activated carbon (AC), carbon nanotubes (CNT), carbon black, graphite, and graphene. The experiments were conducted under strictly anaerobic conditions, and MP was monitored over time.

In the assay with *M. formicicum*, the lag phase was shorter in all conditions with the presence of CM, compared to the control without CM. The initial MP rates reached values 11.6x, 9.4x, 6.4x, 6.3x, 5.3x, 3.0x and 2.1x higher than the control, with AC_300-500µm, AC_100-300µm, carbon black, AC_<100µm, graphite, graphene and CNT, respectively. Regarding the assay with *M. harundinacea*, the lag phases were also reduced with all the CM tested, and the MP rates were higher than the control in the presence of graphite and glassy carbon. In the presence of the other CM, the MP rate was also higher than the control, but in less extent.

In conclusion, the presence of CM had a positive influence on both MP rates and the duration of the lag phase, and time of incubation.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology under the scope of the CM4Methane project (Ref:PTDC/BTA-BTA/2249/2021).

P1.15 - PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE BIOACTIVE PROPERTIES OF AYAHUASCA BEVERAGES

Joana Gonçalves [1,2 *], Ângelo Luís [1,2], Ana Gradillas [3], Antonia García [3], José Restolho [1], Nicolás Fernández [4], Fernanda Domingues [1], Eugenia Gallardo [1,2], and Ana Paula Duarte [1,2]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.
[2] Laboratório de Fármaco-Toxicologia, UBI Medical, Universidade da Beira Interior, Covilhã, Portugal
[3] CEMBIO, Center for Metabolomics and Bioanalysis, Facultad de Farmacia, Universidad San Pablo CEU, Madrid, Spain;
[4] Cátedra de Toxicología y Química Legal, Laboratorio de Asesoramiento Toxicológico Analítico (CENATOXA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

(*) e-mail: joanadgoncalves13@gmail.com

Keywords: Ayahuasca; antioxidant; anti-inflammatory; antimicrobial; phytochemical characterization

ABSTRACT

Ayahuasca is an indigenous psychoactive beverage, traditionally consumed by tribes in South America. In recent years, recreational consumption of this beverage has expanded worldwide. Thus, this work focuses on the study of antimicrobial, antioxidant and anti-inflammatory properties, as well as the phytochemical profile of decoctions of a commercial mixture, four individual plants and four mixtures of the same plants, used in the Ayahuasca beverages. For that, the presence of phenolic compounds was determined by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q/TOF-MS). Additionally, the phenolic profile was analyzed, having determined the content of flavonoids and total phenolic compounds. The anti-inflammatory activity was evaluated by a protein denaturation method, and the antioxidant activity was determined by DPPH free radical scavenging assay and β- carotene bleaching test. Lastly, the disc diffusion assay, resazurin microtiter method, anti-quorum sensing and anti-biofilm activity assays, were performed, in order to evaluate the antimicrobial properties. The obtained results demonstrated that the samples presented a high content of phenolic compounds, reflecting in a significant anti-inflammatory and antioxidant activity. Important antimicrobial properties were also demonstrated, with emphasis on the effect of *P. harmala* and *B. caapi* on strain of *A. baumannii*, inhibiting the production of violacein pigment and the biofilm formation.

Acknowledgements:
Joana Gonçalves acknowledges the PhD fellowship from FCT (Reference: SFRH/BD/149360/2019). Ângelo Luís acknowledges the contract of Scientific Employment in the scientific area of microbiology financed by FCT under the scope of DL 57/2016. This work was partially supported by CICS-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT) and by Fundo Europeu de Desenvolvimento Regional (FEDER) under the scope of PORTUGAL 2020 and Programa Operacional do Centro (CENTRO 2020), with the project references UIDB/00709/2020 and UIDP/00709/2020.

P1.16 - *ACACIA LONGIFOLIA* AND THE MICROBIAL CROWD IN ROOT-NODULES: WHAT HAPPENS AFTER A FIRE EVENT?

Joana Jesus [1*], Cristina Máguas [1], Ricardo Dias [1,2], Mónica Nunes [3], Pedro Pascoal [3], Marcelo Pereira [3], Helena Trindade [1]

[1] Centre for Ecology, Evolution and Environmental Change (cE3c), Faculty of Sciences, University of Lisbon (FCUL), Global Change and Sustainability Institute (CHANGE), Lisboa, Portugal
[2] Biosystems and Integrative Sciences Institute (BioISI), Lisboa, Portugal
[3] Centro de Testes de Ciências, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[(*)] e-mail: jgjesus@fc.ul.pt

Keywords: *Bradyrhizobium, Coniochaeta,* histology, infected cells, NGS, starch, symbiosis

ABSTRACT

Biological invasions provide an opportunity to study adaptation, especially under climate change scenarios, with extreme events like wildfires becoming more frequent and posing major challenges. Plant-microbe interactions can trigger invasion, and the identification of the microbial partners involved is important. *Acacia longifolia* is one of the most aggressive invaders worldwide; as a legume, nitrogen-fixing bacteria live inside root-nodules and promote growth, but overall microbial diversity involved remains unclear. Acacias have been described as promiscuous concerning its microbial partners, and in this study, we wanted to focus on after fire events. For that, we addressed root nodules' structure and microbial diversity through histology and Next-Generation Sequencing, targeting 16S and 25S-28S rDNA genes for bacteria and fungi, respectively, in root nodules from 1-year-old saplings, comparing unburnt and burnt sites. Our results show that nodules share a similar structure, but after fire nodules had a higher number of infected cells and greater starch accumulation in comparison to nodules form unburnt sites. Starch can be a possible carbon source for the microbiota. Regarding diversity, *Bradyrhizobium* was the preferential partner given its dominance in both sites, followed by *Tardiphaga*, a non-rhizobial Alphaproteobacteria, and *Synechococcus*, a cyanobacteria. Additional nitrogen-fixing bacteria were described (i.e., more diversity) in burnt site, highlighting the importance of this process for plant establishment. We describe for the first time the presence of a mycobiome inside root-nodules. Major differences were identified in this group between burnt and unburnt sites, and include genera previously described as plant endophytes. *Coniochaeta* was dominant in the burnt site, suggesting its role as a facilitator for symbiotic associations. This tripartite symbiosis (i.e., bacteria–fungi–plant), along with the ability to behave as a promiscuous host, could ease *A. longifolia* establishment, constituting an important trait that can explain its dispersal and invasive success.

Acknowledgements:
We thank the Centre for Ecology, Evolution and Environmental Changes (cE3c) for the financial funds through FCT (Fundação para a Ciência e a Tecnologia) by Portuguese National Funds attributed to cE3c within the strategic project UIDB/00329/2020 and through the Project R3forest—Using exotic biomass for post-fire recovery: reuse, regenerate and reforest (PCIF/GVB/0202/2017). We thank also Biosystems and Integrative Sciences Institute (BioISI) for the financial support to Marcelo Pereira (UIDB/04046/2020 and UIDP/04046/2020). Joana Jesus was funded by the PhD research fellowship 2021.08482.BD through Fundação para a Ciência e a Tecnologia (FCT, Portugal).

P1.17 - CHARACTERIZATION OF THE MICROBIOME OF BIOCHAR-SUPPLEMENTED RECYCLED MANURE SOLIDS

Joana Fernandes Guerreiro [1,2*], Ana Esteves [1,2], Ana José Pires [1,2], Lélia Chambel [3], Mónica Nunes [4], Pedro Pascoal [4], Marcelo Pereira [4], David Fangueiro [5], Luís Tavares [1,2], Ricardo Dias [3,6], Ricardo Bexiga [1,2], Manuela Oliveira [1,2,6]

[1] CIISA—Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal
[3] Biosystems and Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[4] Centro de Testes de Ciências, University of Lisbon, Lisbon, Portugal
[5] LEAF Research Center, Terra Associate Laboratory, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal
[6] cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

[(*)] e-mail: jguerreiro@fmv.ulisboa.pt

Keywords: Biochar, Dairy cows, Microbiome, Recycled Manure Solids

ABSTRACT

An attractive alternative that has emerged for recycling the substantial amounts of animal manure produced daily in dairy farms is the use of Recycled Manure Solids (RMS) as bedding material. This ecological strategy has been associated with increased cow wellbeing, but also has its risks, since it can contribute to the dissemination of pathogenic and resistant bacteria to farm personnel, animals, and the environment. In that context, the use of biochar, an inorganic char coproduct obtained from thermochemical processing of biomass, seems promising since its application in animal waste may eliminate relevant bacterial species. Considering that, we aimed to evaluate the potential of RMS supplemented with a pine biochar produced in Portugal as a new cow bedding material.

RMS obtained from a dairy farm were transferred to ventilated containers and incubated in conditions that mimicked regular farm husbandry during two periods: April-May (humid season) and June-July (dry season). For each of those periods, 2.5%, 5% and 10% of biochar were added to RMS, in triplicate containers. Non-supplemented RMS containers were kept as a negative control. Composite RMS samples (10 g) from each condition were then collected at four timepoints (days 0, 5, 15, 30) and their microbiome profile determined by performing complete 16S rDNA gene sequencing using Nanopore next-generation sequencing.

Biochar supplementation altered the microbiome of RMS, affecting its diversity and pathogenic bacterial distribution. The most beneficial effect was obtained for long-term storage (30 days), particularly for samples supplemented with 2.5% of biochar, as those conditions led to a decrease in microbiome diversity and reduced abundance of some known agents of disease in dairy cattle, including species of the Enterobacteriaceae and Staphylococcaceae families.

Our results suggest that application of biochar to RMS might encourage the safe and sustainable use of this environmental-friendly resource in animal production.

Acknowledgements:
Authors would like to acknowledge: CIISA—Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal (Project UIDB/00276/2020); Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal (LA/P/0059/2020); Biosystems and Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, Lisbon, Portugal (M.P. was funded by UIDB/04046/2020 and UIDP/04046/2020).

P1.18 - ANTIDERMATOPHYTIC ACTIVITY OF THE AZOREAN BLACK TEA (*CAMELLIA SINENSIS)*

José Sousa-Baptista [1,2†], Ana Filipa Lenha-Silva [1†], Rita Domingues [1*], Daniela Calheiros [1], Edmilson Correia [1], Chantal Fernandes [1], Teresa Gonçalves [1,2]

[1] CNC—Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, Coimbra, Portugal
[2] FMUC—Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[†] The first and second authors contributed equally for the work

[(*)] e-mail: ritasjpd1997@gmail.com

Keywords: *Camellia sinensis,* Azorean black tea, antifungal, dermatophytes, cell wall, chitin, glucan, ergosterol

ABSTRACT

Background: The treatment of dermatophytosis, the most common human fungal infection throughout the world, is difficult, not free of toxicity for the patients, and contributes for the development of resistance to antifungals. Consequently, new drug alternatives are required and extracts obtained from natural sources, have been explored due to their antimicrobial activities. The aim of this study was to determine the antidermatophytic activity of the aqueous Azorean black tea extract (ABT), and to evaluate its synergy with clinically used antifungals, together with an approach to the mechanisms of action.
Methods: The antifungal susceptibility was assessed according to the broth microdilution assay (EUCAST E.DEF 9.3.1 standards). The potential synergy effect of ABT with terbinafine and griseofulvin was evaluated by the checkerboard assay. The mechanism of action was appraised by the quantification of the fungal chitin, ß-1,3-glucan and ergosterol contents in response to ABT.
Results: The MICs of the ABT were 250 µg/mL for *Trichophyton mentagrophytes*, 125 µg/mL for *Trichophyton rubrum* and 500 µg/mL for *Microsporum canis* and the extract was fungicidal at these concentrations. It was also observed an addictive effect of ABT in association to terbinafine on these three dermatophytes. The ABT extract caused a significant reduction on β-1,3-glucan content in these dermatophytes revealing the synthesis of this cell wall component as a possible target of the extract.
Conclusions: ABT is an alternative approach to improve the effectiveness of the conventional antidermatophytic treatment.

Acknowledgements:
This work was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER- 000012- HealthyAging2020 and CENTRO-01-0145-FEDER- 022095: ViraVector; the COMPETE 2020 – Operational Programme for Competitiveness and Internationalisation, and the Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia,I.P.:UIDB/04539/2020 and UIDP/04539/2020. JS-B was granted an Internship by the Direção Regional do Emprego e Qualificação Profissional, da Região Autónoma dos Açores.

P1.19 - SPENT COFFEE GROUNDS: A POTENTIAL ANTIFUNGAL AGENT?

Daniela Calheiros [1,4*], Maria Inês Dias [2,3], Ricardo C. Calhelha [2,3], Lillian Barros [2,3], Isabel C. F. R. Ferreira [2], Chantal Fernandes [1,+], Teresa Gonçalves [1,4,+]

[1] CNC—Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[2] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
[3] Laboratório Associado Para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal
[4] FMUC—Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[+] These authors contributed equally to this work.

[(*)] e-mail: danielamcalheiros@gmail.com

Keywords: Spent coffee grounds - SCG, phenolic compounds, antifungal activity, *Candida spp., Trichophyton spp..*

ABSTRACT

Coffee generates tons of solid waste, known as spent coffee grounds (SCG), a coffee by- product containing bioactive compounds. Fungal infections are increasing together with resistance to antifungals, turning essential the discovery of novel antifungal drugs. SCG re-use and valorisation reduces large amounts of waste, contributing to sustainability and circular economy.
Ethanolic caffeinated and decaffeinated SCG extracts, from commercial coffee capsules were prepared and its phenolic profile was determined. We evaluated the antifungal activity against filamentous fungi and yeasts and modifications of cell membrane and cell wall components. Ultrastructural changes were studied by TEM. The anti-inflammatory activity was evaluated on RAW 264.7 macrophages and cytotoxicity on tumor (AGS, $CaCo_2$, MCF-7, NCI-H460), and non-tumor (PLP2) cell lines.
The extracts analysis showed caffeoylquinic acid, feruloylquinic acid, and caffeoylshikimic acid derivatives. SCG ethanolic extracts showed antifungal activity against, as follows: caffeinated SCG extract MIC was 137.5 µg/mL for *Candida krusei*, *Trichophyton mentagrophytes*, *Trichophyton rubrum,* for *Candida parapsilosis* was 275.0 µg/mL.; decaffeinated SCG extract MIC values were 150.0 µg/mL, for *C. krusei*, *C. parapsilosis*, *T. rubrum,* and 300.0 µg/mL for *T. mentagrophytes*. Caffeinated SCG caused a significant reduction ($p<0.01$), on ergosterol content while decaffeinated SCG significantly reduced ($p<0.01$) chitin levels of *C. parapsilosis*, indicating the synthesis of ergosterol and of chitin as possible targets of caffeinated and decaffeinated SCG, respectively. Ultrastructural modifications were observed in *C. parapsilosis* and *T. rubrum*. Both extracts were cytotoxic for the tumoral cell lines; the AGS cell line was more susceptible to the antiproliferative effects of SCG extracts (GI50 of 55 for caffeinated and 52 g/mL for decaffeinated SCG extracts). In PLP2 cell line, SCG had low cytotoxicity (GI50 value > 400 g/mL).
These results indicate that an SCG-based phytotherapeutic for topic administration could be an economic and eco-friendly approach to treat skin fungal infections.

P1.20 - UNVEILING MICROBIAL ROLE IN STONE PINK DISCOLORATION AT BATALHA MONASTERY

Inês Silva [1], Cátia Salvador [1], Ana Z. Miller [1,2], António Candeias [1,3,4], Ana Teresa Caldeira [1,3,4*]

[1] HERCULES Laboratory, Institute for Advanced Studies and Research, University of Évora, Évora, Portugal
[2] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain
[3] Chemistry and Biochemistry Department, School of Sciences and Technology, University of Évora, Évora, Portugal
[4] City University Macau Chair in Sustainable Heritage, Institute for Advanced Studies and Research, University of Évora, Évora, Portugal.

[(*)] e-mail: atc@uevora.pt

Keywords: Batalha Monastery; biodeterioration; pink discoloration; metagenomics; culture-dependent methods.

ABSTRACT

The microbial colonization of built heritage and monuments by fungi, algae, and bacteria is a well-known phenomenon. This colonization frequently triggers degradation processes that result in aesthetic or structural damage. The Batalha Monastery (Portugal), a UNESCO World Heritage Site, currently exhibits a high degree of surface alterations of the stone architectural elements both inside the Founder's Chapel (Fig. 1a) and the church (Fig. 1b), including an extensive pink coloration in the walls and columns. The main aim of this study was to characterize the biological colonization and understand the role of microbial communities in the biodeterioration of the architectural stone material (Ançã limestone), to help conservators and restorers and determine the most effective treatment approach to use for the preservation of the monastery.

The microbial population was characterized using both High-throughput DNA sequencing and culture-dependent methods and several orange-pink pigment-producing bacteria were identified including *Halalkalicoccus tibetensis, Bacillus aryabhattai, Bacillus firmus, Bacillus megaterium* and *Methylorubrum extorquens*. The pink discoloration observed may result from biofilms formed by bacteria that produce pigments, probably carotenoids [1]. Biocolonization tests are being performed in which stone mock-ups were prepared and inoculated with the bacteria isolated in the study, in order to simulate the natural conditions of the monastery and monitor the colonization process, to better understand the discoloration phenomenon.

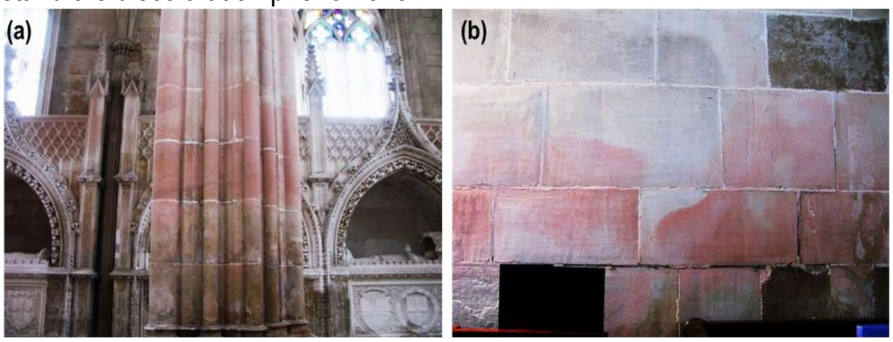


Figure 1: Surface alterations of the stone architectural elements both inside the Founder's Chapel (Fig. 1a) and the church (Fig. 1b) at Batalha Monastery.

References:

[1] J. D. Ettenauer *et al.*, "Halophilic microorganisms are responsible for the rosy discolouration of saline environments in three historical buildings with mural paintings," *PLoS One*, vol. 9, no. 8, pp. 1–8, 2014, doi: 10.1371/journal.pone.0103844.

Acknowledgements:
The authors acknowledge to FCT – Foundation for Science and Technology, I.P., within the scope of the projects: UIDB/04449/2020, MICROCENO (PTDC/CTA-AMB/0608/2020), ART3mis (2022.07303.PTDC) and I. Silva PhD Grant (UI/BD/153582/2022); and Directorate-General for Cultural Heritage (DGPC).

P1.21 - PORTUGUESE MARINE ECOSYSTEMS ARE A SOURCE OF NEW FUNGI

Alberto C. Abreu*, Ana Cristina Esteves, Artur Alves

CESAM – Centro de Estudos do Ambiente e do Mar, Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal

(*) e-mail: artur.alves@ua.pt

Keywords: Marine Fungi, Estuary; Costal Ecosystems; *Lignicolous fungi;* Fungal Diversity; Novel Species

ABSTRACT

Lignicolous marine fungi are the main wood decomposers in salt marshes, mangroves, and estuaries. But, despite their ecological importance, their diversity is still poorly known, especially in Portuguese ecosystems. Assessing the diversity of these organisms is a crucial step in discovering their biological and ecological relevance.

Samples of driftwood and wood pilings were collected at several sites in marine and estuarine ecosystems in Portugal. Additionally, *Pinus pinaster* and *Fagus sylvatica* wood baits were submerged in a coastal marine environment (Viana de Castelo) and estuarine environment (Ria de Aveiro), for 6 months. Culture-dependent methods were used to assess the fungal diversity on the wood samples and 750 lignicolous fungal isolates were obtained. Using genetic fingerprinting (MSP-PCR) for molecular typing, we selected, 224 representative isolates whose ITS (internal transcribed spacer) ribosomal DNA region was sequenced and analysed. From this collection, 124 species and 57 genera were successfully identified. The most frequent species isolated was *Lulworthia atlantica* (6.25%), a common lignicolous marine fungus, however *Fusarium* sp. was the most predominant, representing 13.40% of all species identified.

Seventy isolates potentially represent novel species and have been selected for subsequent amplification of additional molecular markers, phylogenetic analysis and morphological studies to confirm their taxonomic identification. The high variety of species may be explained by the diversification of sampling sites, both coastal marine and estuarine, as well as the different wood substrates associated with different fungal communities. Around 31% of the isolates identified could represent undescribed fungal *taxa*. This result confirms that lignicolous marine fungi are an under explored group and these ecosystems conceal a large unknown diversity. Uncovering this knowledge gap could be the solution to better understand the ecological and biological role of these microorganisms, as well as enabling access to their biotechnological applications.

Acknowledgements:
The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) and Alberto Abreu (SFRH/BD/08713/2021).

P1.22 - IMPACT OF SALINITY STRESS IN PLANT GROWTH, BIOCHEMISTRY AND MICROBIAL INTERACTIONS IN *OLEA EUROPAEA* CULTIVAR GALEGA VULGAR

sabel N. Sierra-Garcia [1]*, Mónica Marques [1], Pedro Monteiro [1], Frederico Leitão [2], Glória Pinto [1]; Ângela Cunha [1]

[1] CESAM & Department of Biology University of Aveiro, Campus de Santiago, Aveiro, Portugal
[2] Department of Life Sciences, Centre for Functional Ecology, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal.

(*) e-mail: inatalia.sierra@ua.pt

Keywords: soil salinity, salt tolerance, rhizosphere, metabarcoding, sustainable agriculture, physiological performance, leaf-gas exchanges

ABSTRACT

Soil salinity is a major threat to agriculture, soil-plant microbial communities and the economy in impacted regions. It's understood that plants' resilience to salinity is due to a combination of biochemical and physiological adaptations, as well as beneficial interactions with microorganisms that mitigate the effects of salt stress. The olive tree, *Olea europaea*, is a is a major tree crop in the Mediterranean. While generally seen as moderately salt-tolerant, there is noticeable variation in salt tolerance across its cultivars. The impact of salinity on traditional Portuguese varieties, such as Galega vulgar, remains largely unexplored.

This study examined how salinity affects the growth, leaf gas-exchanges, biochemistry, and microbiome of the olive variety Galega vulgar. Through biochemical and metabarcoding techniques, we aimed to gain a thorough insight into the influence of salt on this particular cultivar. Two-year old plants were placed in 400 mL plastic pots filled with a mixture of soil and perlite (3:2 w/w). After 2 months of acclimation, salinity was manipulated irrigating twice a week with saline solutions containing 0, 50, 100 or 150 mM NaCl. At the end of the experiment (8 weeks), shoot length and leaf-gas exchange related parameters were measured. The microbial communities associated with the rhizosphere were characterized using high-throughput sequencing.

The shoot length of the plants was significantly reduced in the 150 mM NaCl treatment. The photosynthetic rate also decreased significantly with salinities above 100 mM NaCl. Microbial analysis showed that at the family taxonomic level, at least 30 taxa identified in relative abundances higher than 1%. There was a significant increase of taxa identified as *Pseudolabrys* and *Streptomyces* in the condition of high salinity (150mM NaCl) which may be related with the effect of *Streptomyces* of relieving salinity stress in crop plants.

This study offers fresh perspectives on how *O. europaea* cultivar Galega vulgar reacts to different salinities. These findings support the idea that plant preferentially favors bacterial symbionts that can reduce the impact of salt stress. Furthermore, *Streptomyces* emerges as a potential biostimulant that could protect olive trees from the negative effects of soil salinization.

Acknowledgements:
This work was funded by FCT through project Halius (PTDC/BIA-MIC/3157/2020) and CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020)

P1.23 - INOCULATION OF BACTERIAL CONSORTIA AS A STRATEGY FOR OPTIMISATION OF LEGUME SEED MICROBIOME

Ana Santos [1], Ricardo Soares [1,2*], Helena Machado [1], Ana Barradas [3], Paula Fareleira [1,4], Isabel Videira e Castro [1,4]

[1] Instituto Nacional de Investigação Agrária e Veterinária, I.P., INIAV, I.P., Oeiras, Portugal.
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.
[3] Fertiprado–Sementes e Nutrientes Lda, Herdade dos Esquerdos, Vaiamonte, Portugal.
[4] GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal.

[(*)] e-mail: ricardo.soares@iniav.pt

Keywords: Nitrogen-fixing bacteria, Annual clovers, Seed inoculation, Biofertilizers

ABSTRACT

Inoculation of legume seeds included in biodiverse mixtures is an efficient strategy for introducing nitrogen-fixing bacteria (rhizobia) into the soil and rhizosphere of legumes. Selecting the best combination of nitrogen-fixing bacteria and host legume is essential to achieve more effective rhizobia-legume symbioses. Innovative inoculants can contain rhizobia strains selected for their ability to respond to biotic and abiotic stresses, reducing the negative impacts of these factors in agro-silvo-pastoral systems.

In this work, we studied autochthonous nitrogen-fixing bacteria isolated from the root nodules of annual clovers grown in the South of Portugal, according to their effectiveness and antagonism against phytopathogenic oomycetes. We also evaluated the viability over time of the bacterial inoculant on seed surface. Plant assays were performed under controlled environmental conditions to evaluate the symbiotic efficiency of rhizobia strains (individually and in consortium) with different *Trifolium* spp..

Inoculation experiments with annual clovers yielded the selection of five *Rhizobium* strains highly effective in nitrogen fixation. Since the *Rhizobium* strains did not show antagonistic activity against *Phytophthora cinnamomi* and *Phytopythium vexans*, a *Lysobacter* sp. strain was included in the consortium, remaining the symbiotic effectiveness very high (> 75%).

Assays with clover seeds (*T. subterraneum*) inoculated with liquid cultures of *Rhizobium* containing Arabic gum as an additive (to improve stickiness and adherence to seeds) showed a decrease in the bacterial cell viability of seed surface, from 10^6 to 10^4, per gram of seed, at the end of 18 weeks. However, the effectiveness remained high (65%). This consortium was also used to inoculate seeds of annual clovers (field assay) and all the *Rhizobium* strains were recovered from the plant root nodules, showing good performance in the nodulation process. Our results represent an important step in the implementation of more profitable and sustainable legume-rich biodiverse pastures using biofertilizers.

Acknowledgements:
Financial support was provided by the project ECOSEED (LISBOA-01-0247-FEDER-072228).

P1.24 – THE CONNECTION BETWEEN ANIMAL ORIGIN OF HISTORICAL PARCHMENT AND MICROBIOME

Maria João Penetra [1]***, António Candeias*** [1,2,3] ***, Catarina Miguel*** [1,3]***, Ana Teresa Caldeira*** [1,2,3] ******

[1] *HERCULES Laboratory, Évora University, Palácio do Vimioso, Évora, Portugal*
[2] *Chemistry Department, School of Sciences and Technology, Évora University, Évora, Portugal*
[3] *City University of Macau Chair in Sustainable Heritage, University of Évora, Casa Cordovil, Évora, Portugal*

(*) *e-mail: atc@uevora.pt*

Keywords: Parchment, Species Identification, Molecular analysis, Metagenomic analysis

ABSTRACT

Made from the hides of sheep, goat, or calf skins, parchment is a valuable record of biological, historical, and cultural knowledge [1, 2]. A key point for the characterization of illuminated manuscripts, scriptoria, and ateliers, as well as for the preservation of the historic documents, involves the discovering the provenance of the animals used to manufacture the parchment. However, this may be a very arduous task due to the document's state of conservation [1, 3–5].
This study aimed to perceive if there is or is not an association between the parchment microbiome and a specific type of animal skin, that could add additional insight into the animal origin of the manuscripts. To help decipher this, a set of several parchment samples was analyzed, either in terms of animal origin, by targeting mitochondrial DNA, or metabarcoding analysis, by targeting either the16S and ITS regions. The results were analysed using Principal Coordinate Analysis (PCoA) to reveal the biological signatures.
Overall, this study allowed us to elucidate the association between a particular microbiome pattern and a specific type of parchment. It was possible to demonstrate that in the set of the studied parchments, the results of metagenomic analysis from prokaryotic communities differed in the tested animal species. Parchments manufactured from goat and sheep skins presented significant differences in the distribution of the taxonomic groups. Prokaryotic communities seem to be better markers at making the distinction between different animals whereas the eukaryotic microorganisms do not, seeming to be more related to different storage conditions.

References

[1] S. Fiddyment, M. D. Teasdale, J. Vnouček, É. Lévêque, A. Binois, and M. J. Collins, "So you want to do biocodicology? A field guide to the biological analysis of parchment," Herit. Sci., vol. 7, no. 1, pp. 1–10, 2019.
[2] M. G. Campana et al., "A flock of sheep, goats and cattle: Ancient DNA analysis reveals complexities of historical parchment manufacture," J. Archaeol. Sci., vol. 37, no. 6, pp. 1317–1325, 2010.
[3] N. Poulakakis, A. Tselikas, I. Bitsakis, M. Mylonas, and P. Lymberakis, "Ancient DNA and the genetic signature of ancient Greek manuscripts," J. Archaeol. Sci., vol. 34, no. 5, pp. 675–680, 2007.
[4] G. Piñar, F. Cappa, W. Vetter, M. Schreiner, H. Miklas, and K. Sterflinger, "Complementary Strategies for Deciphering the Information Contained in Ancient Parchment Documentary Materials," Appl. Sci., vol. 12, no. 20, 2022.
[5] D. Pangallo, K. Chovanova, and A. Makova, "Identification of animal skin of historical parchments by polymerase chain reaction (PCR)-based methods," J. Archaeol. Sci., vol. 37, no. 6, pp. 1202–1206, 2010.

Acknowledgements:
The authors wish to acknowledge the project "ROADMAP - Research On Antonio De Holanda Miniatures Artistic Production" (PTDC/ART-HIS/0985/2021), financed by national funds through Fundação para a Ciência e a Tecnologia (FCT/MCTES), the HERCULES Lab (UIDB/04449/2020) and City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science.

P1.25 - RESISTANCE OF *ACETOBACTER ACETI* TO THE MAJOR CHEMICALS FOUND IN WINERY WASTEWATERS

Nuno Ramos [1], Ana Baía [1], Alonso Escoto [2], Maria C. Fernandes [2]*, Ana Lopes [1], Annabel Fernandes [1]

[1] FibEnTech-UBI and Department of Chemistry, Universidade da Beira Interior, Covilhã, Portugal
[2] Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja) and Mediterranean Institute for Agriculture, Environment and Development—MED, CEBAL, Beja, Portugal

(*) e-mail: maria.fernandes@cebal.pt

Keywords: Winery wastewater*; Acetobacter Aceti;* Microbial growth inhibitors; Bioremediation

ABSTRACT

The wine industry is an important economic pillar and "engine" for tourism and rural revitalization in Portugal, Spain, Italy, and France, with the wine sector being one of the most dynamic among Portuguese agricultural exports [1]. Like any other agro-industrial activity, it has environmental impacts, such as the production of wastes and wastewaters. Typically, wine industry generates 0.2-4 L of wastewater per liter of wine produced, although this figure can exceed 14 L, depending on the size of the facilities, the type of wine produced, and the winemaking and cleaning technologies used [2]. Winery wastewater (WW) is usually characterized by high salinity and organic load with many different organic compounds, such as organic acids, sugars, alcohols, and recalcitrant high-molecular weight compounds, which can inhibit microbial growth [1]. The aim of the present study was to assess the ability of the microorganism *Acetobacter aceti* to resist WW constituents and to investigate the feasibility of using this microorganism, known for its capability to oxidize a variety of alcohols and sugars to organic acids, for WW biodegradation. Using a model growth medium enriched with WW constituents, at different combinations and concentrations, the growth of *Acetobacter aceti* and the degradation of the compounds were evaluated. The obtained results showed the feasibility of using the microorganism *Acetobacter aceti* for WW biodegradation and allowed the identification of the WW constituents and concentrations that are toxic to the *Acetobacter aceti* growth.

References:

[1] A. Baía, A. Lopes, M.J. Nunes, L. Ciríaco, M.J. Pacheco, A. Fernandes. Removal of recalcitrant compounds from winery wastewater by electrochemical oxidation. Water 2022, 14, 750.
[2] A. Giacobbo, M. Oliveira, A.M. Bernardes, M.N. de Pinho. Winery wastewater treatment for biomolecules recovery and water reuse purposes. A. Basile, A. Cassano, C. Conidi (Eds). Advanced Technologies in Wastewater Treatment, Elsevier, 2023, Pages 311-354.

Acknowledgements:

Fundação para a Ciência e Tecnologia for the project Wine4H2- Exploratory Research Project 2022.02566.PTDC, FibEnTech & MED Research Unit UIDB/00195/2020 and UIDB/05183/2020, respectively. PhD grants (Ana Baía, 2022.11077.BD; Alonso Escoto, UI/BD/153579/2022), Research grant (Nuno Ramos) and Contract (Annabel Fernandes). Alentejo2020 Program through the European Social Fund by project Procurement of Highly Qualified Human Resources (ALT20-05-3559-ESF-000076) Contract (Maria C Fernandes).

P1.26 - TACKLING CO_2 EXCESS WITH BIOELECTRODES

Nuno Machado [1*], Inês A. C.Pereira [1], Américo G. Duarte [1*], Felipe Conzuelo [1*]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

[(*)] e-mail: nuno.machado@itqb.unl.pt | americoduarte@itqb.unl.pt | felipe.conzuelo@itqb.unl.pt

ABSTRACT

The development of sustainable and environmentally friendly systems that can convert CO_2 into added value products is a practical and economically viable solution to revert the high levels of atmospheric CO_2 and support a sustainable future. Biohybrid systems, combine the chemical properties of synthetic materials with biological catalysts to drive chemical reactions. To reduce atmospheric CO_2, we are developing bioelectrochemical systems that use bacterial cells as catalysts to effectively convert CO_2 into formate.

Bioelectrodes were constructed using glassy carbon electrodes modified with *Desulfovibrio vulgaris* Hildenborough (*DvH*) cells. *DvH* is a Gram-negative bacterium that carries three formate dehydrogenases (Fdh) on the periplasm, one of them being a tungsten (W)-containing enzyme with a high turnover for CO_2 reduction to formate[1].

Wild type and knocked out strains were used for electrode modification and their electrochemical characterization was performed through cyclic voltammetry. Results show that the NiFeSe-hydrogenase (Hase) also present in the periplasm can highjack electrons for hydrogen production and compromise CO_2 reduction. To overcome this, we investigate the use of *DvH ΔNiFeSe-Hase* and genetically manipulate this strain to express W-Fdh, and consequently, promote CO_2 reduction.

Our work is setting the ground for the use of genetically engineered *DvH* cells in new bioelectrochemical systems that will allow the sustainable production of biofuels and energy carriers produced from the conversion of CO_2.

References:

1. Oliveira, A.R. *et al.* (2020) 'Toward the Mechanistic Understanding of Enzymatic CO_2 Reduction', *ACS Catalysis*, 10 (6) 3844-56

Acknowledgements:

This work is funded by Fundação para a Ciência e Tecnologia (Portugal) through the project 2022.07024.PTDC, MOSTMICRO-ITQB R&D Unit [UIDB/04612/2020, UIDP/04612/2020], and LS4FUTURE Associated Laboratory [LA/P/0087/2020].

P1.27 - ONE-YEAR SURVEILLANCE OF PATHOGENS IN A FULL-SCALE MUNICIPAL WASTEWATER TREATMENT PLANT

Rafael D. S. Tavares [1,2]*, Elsa T. Rodrigues [1], Marta Tacão [2], Isabel Henriques [1]

[1] University of Coimbra, Centre for Functional Ecology (CFE), Associate Laboratory TERRA, Department of Life Sciences, Faculty of Sciences and Technology, Coimbra, Portugal
[2] Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Aveiro, Portugal

(*) e-mail: rafael.tavares@ua.pt

Keywords: wastewater, pathogens, WWTP

ABSTRACT

Wastewater Treatment Plants (WWTPs) constitute relevant pathogen reservoirs, but few studies monitor shifts in wastewater pathogens content over long periods. This study aim to characterize the profile of putative pathogens in 11 influent and 25 effluent samples collected from a municipal WWTP during 2020, identify treatment effects and seasonal trends in the relative abundance of pathogens, and explore the use of integrase genes as indicators of the occurrence of putative pathogen groups. Therefore, total DNA was extracted for 16S-targeted metabarcoding (Illumina), and for qPCR targeting the putative pathogenic groups *Enterococci*, *Klebsiella pneumoniae*, *Bacteroidota* and *E. coli* and the integrase-encoding genes *intI1*, *intI2* and *intI3*.

Illumina sequencing identified 27 putative pathogenic genera, 9 of which were present in all samples. The relative abundance of 5 genera (e.g., *Streptococcus*) was significantly reduced from influent to effluent, while 8 genera were relatively enriched (e.g., *Legionella*; DESeq2, p-value<0.05). The wastewater pathogen content was significantly different between influent and effluent (PERMANOVA, p-value<0.05), but not between seasons. However, six genera (e.g., *Bacteroides*) were enriched (higher relative abundance) in the effluent samples in at least one of the seasons, and qPCR quantification confirmed the relative enrichment for *Bacteroidota*'s genera while *E. coli* abundance significantly decreased. *Enterococci* and *K. pneumoniae* were below the detection limit. The relative abundance of integrase genes was correlated with the abundance of a minority (8 genera) of the detected pathogens (e.g., *Streptococcus*).

Several putative pathogenic genera were identified both in influent and effluent, and the pathogen profile was stable across seasons. Wastewater treatment led to an increase in the relative abundance of some genera. Although an absolute quantification was not performed, data suggest that these effluents constitute a health threat, and, thus, WWTP treatments should be improved. Integrase genes aren't reliable for estimating wastewaters' pathogen abundance; they indicate only a few specific groups.

Acknowledgements:
This study was supported by funds from FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), CFE (UIDB/04004/2020 + LA/P/0092/2020) and grants for Rafael Tavares (SFRH/BD/145147/2019), Elsa T. Rodrigues (CEECIND/03543/2017) and Marta Tacão (CEECIND/00977/2020).

P1.28 - BIOTECHNOLOGICAL PROSPECTS OF MARINE BACTERIA ISOLATED FROM THE SADO ESTUARY

Rodrigo Martins [1], Constança Bertrand [1], Francisco Quintas-Nunes [1,2], Pedro Reynolds-Brandão [1,3], Teresa Crespo [1,2], Francisco X. Nascimento [1(*)]

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal.
[2] ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.
[3] LAQV-REQUIMTE, Chemistry Dept., FCT, Universidade Nova de Lisboa, Caparica, Portugal

(*) e-mail: francisco.nascimento@ibet.pt

Keywords: bacteria; marine; microalgae; secondary metabolites; biotechnology.

ABSTRACT

Marine ecosystems stand out as one of the richest and most diverse sources of microbial communities. To thrive in the marine environment, microorganisms have developed unique adaptations, including the ability to biosynthesize a broad spectrum of bioactive molecules and the potential to establish multiple mutualistic relations with other organisms, all of which present biotechnological potential.

In this study, the phenotypic and genotypic characterization of sixteen marine bacteria isolated from seawater collected in diverse locations across the Sado estuary in Portugal is presented. In addition, the isolated bacteria were tested for their ability to promote the growth and the accumulation of valuable compounds in the microalgae, *Phaeodactylum tricornutum*.

The results obtained unveiled that multiple bacterial isolates demonstrated the production of a variety of functional and stable extracellular lytic enzymes. Some also exhibited the capacity to synthesize phytohormones like auxins (indole-3-acetic acid), produce ammonia, polyhydroxyalkanoates, and carotenoids. A thorough genomic analysis further revealed the existence of gene clusters responsible for the biosynthesis of a broad spectrum of secondary metabolites, including extracellular enzymes, carotenoids, polyunsaturated fatty acids, and polyhydroxy acids, within the diverse bacterial isolates. When cultured alongside *Phaeodactylum tricornutum*, four isolates stimulated a significant rise in microalgae cell count, cell size, auto-fluorescence, and fucoxanthin content, demonstrating their capacity to enhance microalgae growth. These discoveries shed light on the underexplored potential of marine bacteria, their applicability in a wide range of biotechnological applications, and possible interactions with microalgae.

Acknowledgements:
This work was supported by the PHYCO-µ-BIOME project (PTDC/BAABIO/1262/2020) funded by Fundação para a Ciência e a Tecnologia, Portugal (FCT) and the MULTI-STR3AM project funded by the Bio-Based Industries Joint Undertaking (JU) under grant agreement No. 511887227—MULTI-STR3AM. The JU receives support from the European Union's Horizon 2020 research and innovation 512 programme and the Bio-Based Industries Consortium.

P1.29 – PHARMACEUTICAL AND BIOTECHNOLOGICAL APPLICATIONS OF THE CORAL MICROBIOME

Joana F. Couceiro [1,2]***, Rodrigo Costa*** [1, 2*]***, Tina Keller-Costa*** [1,2*]

[1] *iBB—Institute for Bioengineering and Biosciences and i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon.*
[2] *Bioengeneering Department, Instituto Superior Técnico, Lisbon*

[(*)] *e-mail: tinakellercosta@tecnico.ulisboa.pt | rodrigoscosta@tecnico.ulisboa.pt*

Keywords: Secondary Metabolism, Natural Products, Blue Bioeconomy, Bioactivity, Antibiotics, Antifungal, Antifouling, Anticancer, Anti-inflammatory, Octocorals

ABSTRACT

Coral-associated microorganisms harbour an impressive and diverse secondary metabolite biosynthesis capacity with novel bioactive compounds being reported every year. Coevolution with their sessile coral host and the need to combat predation, overgrowth and fouling presumably led to their ability to produce different classes of compounds with a broad spectrum of activities. Although the precise ecological functions of most elucidated compounds from coral-associated microbes remain unknown or unproven, the biotechnological applications and prospective benefits of their exploitation are at hand. Bioactivities of pharmaceutical and industrial interest of coral symbiont-derived compounds include antitumoral, antibacterial, antifungal, antifouling, anti-inflammatory and antidiabetic properties among many others, indicating vast potential for blue biotechnology and blue pharma. This study reviews new natural products from coral symbionts reported between 2018 and 2022, highlighting the versatility and economic potential of this unique chemical reservoir. More than 385 novel compounds were described from coral-associated microbes in the past five years, 75% from octocoral (Octocorallia) symbionts. Over 87% of the compounds derive from coral-associated fungi of the Ascomycota phylum while only about 12% come from bacterial associates in the phyla *Actinomycetota*, *Pseudomonadota*, *Bacillota* and *Cyanobacteria*. Terpenes, alkaloids, peptides, and polyketides are the most prominent compound classes, many of which show anticancer, antibacterial, antifungal and antidiabetic activities. Despite the wide compound range described in coral symbionts, this study unveils that most of the efforts made recently target only certain microbial groups, such as actinomycetes and fungi, or specific geographical locations (e.g., South China Sea) and coral species. It hints at a yet untapped reservoir of novel natural products from coral-associated microbes that needs to be unlocked in future biodiscovery programs. This study calls on the scientific community to expand the scope of their coming research, directing it towards underexplored groups such as cold-water coral hosts and non-actinomycete bacterial symbionts.

P1.30 - PROTEIN HYDROLYSATES FROM SALMON HEADS AND CAPE HAKE BY- PRODUCTS: A POTENTIAL SOURCE OF BIOACTIVE PEPTIDES

Matilde Leitão [1,2], Amparo Gonçalves [2,3], António Marques [2,3], Helena Oliveira [2,3], Maria Leonor Nunes [3], Bárbara Teixeira [2,3], Rogério Mendes [2,3], Romina Gomes [2,4], Carla Pires [2,3]*

[1] Departamento de Química Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal
[2] IPMA,IP, Departamento do Mar e dos Recursos Marinhos, Divisão de Aquacultura, Valorização e Biosprospeção, Algés, Portugal
[3] CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal
[4] MEtRICs/DCTB/NOVA, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

(*) e-mail: cpires@ipma.pt

Keywords: salmon heads, Cape hake by-products, fish protein hydrolysates, bioactive peptides.

ABSTRACT

Fish by-products can be enzymatically transformed into high-value-added products such as protein hydrolysates (FPH), containing bioactive peptides. In this work, FPH were prepared using Alcalase, fresh salmon (*Salmo salar*) heads and frozen Cape hake (*Merluccius capensis*) by- products ("sawdust" and cut offs) from the portioning in a fish processing plant.
FPH presented high yield (68.2-75.4%) and high degree of hydrolysis (20.3-26.2%). The gel permeation profile of these FPH indicated hydrolysis of proteins into peptides with small molecular weights (Mw<1500 Da) and free amino acids. FPH from salmon heads contained higher percentage of peptides with lower Mw.
Both hydrolysates had high protein content (ca 76%, dry weight) and a quite similar amino acids profile. Glutamic and aspartic acids, glycine, alanine and proline were the most abundant AA. The composition of FPH in terms of essential amino acids was nutritionally relevant.
Both FPH exhibited antioxidant activity and those prepared from Cape hake by-products showed the highest levels of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-di(3-ethyl-benzathiazoline-sulphonic acid-(6) (ABTS) radical scavenging activities, reducing power and Fe^{2+} chelating activity. Regarding Angiotensin Converting Enzyme (ACE) inhibitory activity, the IC_{50} values were relatively low (2.23 mg/mL and 0.86 mg/mL for Cape hake by-products and salmon heads, respectively). In both FPH the α-amylase inhibitory activity was relatively low, with inhibition percentages below 30% at a FPH concentration of 150 mg/mL.
The fatty acids profile of the oil released (around 95% of lipid content found in raw material) during the preparation of FPH from salmon heads revealed a prevalence of monounsaturated fatty acids (MUFA) (51%), being oleic acid (18:1 ω9) being the most abundant (38%). Additionally, eicosapentaenoic plus docosahexaenoic acids content was around 7%.

The study reveals FPH valuable interest as potential food/feed ingredient or nutraceutical, thus enabling the valorization of salmon heads and Cape hake by-products under a circular economy context.

Acknowledgements:

This work has been developed within the scope of "BLUE BIOECONOMY INNOVATION PACT" (Project Nº. C644915664-00000026) financed by NextGenerationEU, under the incentive line “Agendas for Business Innovation” of the Recovery and Resilience Plan (PRR).

P1.31 - UNWELCOME ENTHUSIASTS OF ALMADA NEGREIROS MURAL PAINTINGS – A METAGENOMIC APPROACH TO THE MURALS SITUATED IN ALCÂNTARA MARITIME STATION, LISBON (PORTUGAL)

L. Dias [1,2]***, M. Gil*** [1,3]***, I. Silva*** [1]***, A. Candeias*** [1,3]***, A.T. Caldeira*** [1,3]*

[1] *HERCULES Laboratory and IN2PAST Associate Laboratory, Institute for Advanced Studies and Research, University of Évora, Évora, Portugal*
[2] *Geosciences Department, University of Évora, Évora, Portugal*
[3] *City University of Macau Chair in Sustainable Heritage and Institute for Advanced Studies and Research, University of Évora, Évora, Portugal*

(*) *e-mail: atc@uevora.pt*

ABSTRACT

Previously underestimated, biodeterioration is currently assumed as a key contributor to the overall deterioration processes of materials that typically compose Cultural Heritage [1]. Microbial proliferation on these surfaces can induce chemical and/or physical deterioration. Chemical deterioration primarily involves the release of deleterious compounds resulting from their metabolism [2], while physical deterioration mainly encompasses the endogenous growth of cellular structures like hyphae development of filamentous fungi [3].

The Alcântara Maritime Station, a port building located near the estuary of the Tejo River in Lisbon, Portugal, hosts important mural paintings created and designed by the renowned Portuguese multidisciplinary artist Almada Negreiros – an exceptional figure in the Portuguese artistic landscape of the 20th century. These murals, situated in the expansive central vestibule, long served as a hallmark for visitors to the country. Presently, the artworks exhibit clear signs of deterioration, highlighting the importance of conducting a multidisciplinary study to comprehend, preserve, and valorize the artwork.

This study focuses on assessing the contribution of biological agents to the overall deterioration rate. In this way, a metagenomic analysis was performed, coupled with an evaluation of microorganism-substrate interactions through high-resolution scanning electron microscopy. The results demonstrate that microorganisms predominantly colonize surfaces enriched with organic material used originally as coating and/or as adhesives in past interventions. The metagenomic study has revealed that prokaryotic communities majorly belong to the Phyla *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Cyanobacteria*/*Chloroplast*, and *Bacteroidetes*. The eukaryotic communities are predominantly composed of the Phyla *Ascomycota* and *Basidiomycota*.

References

[1] T. Branysova, K. Demnerova, M. Durovic, H. Stiborova (2022). *Journal of Cultural Heritage* 55: 245-260.
[2] L. Dias, T. Rosado, A. Candeias, J. Mirão, A.T. Caldeira (2020). *Journal of Cultural Heritage* 42: 255:262.
[3] L. Dias, V. Pires, F. Sitzia, C. Lisci, A. Candeias, A.T. Caldeira, J.Mirão (2023). *The European Physical Journal Plus* 138: 570.

Acknowledgements:
Fundação para a Ciência e Tecnologia (FCT) for their support through contract program of Ref.ª CEECINST/00069/2021 and project PTDC/ART-HIS/1370/2020: Unveiling the Mural Painting Art of Almada Negreiros (1938-1956).

P1.32 - FROM EFFLUENTS TO ASSETS: WINERY WASTEWATER UTILIZATION FOR MICROALGAE FARMING

Catarina Dias [1(*)], Ana Rita Martins [1], Ana Cláudia Sousa [1], Ana Gabriela Gomes [1], Miguel Cachã [2], Carla Santos [1]

[1] *Instituto Politécnico de Setúbal, Escola Superior de Tecnologia do Barreiro, Barreiro, Portugal*
[2] *AVIPE – Associação de Viticultores do Concelho de Palmela, Palmela, Portugal.*

[(*)] *email: catarina.r.dias@estudantes.ips.pt*

Keywords: *Chlorella vulgaris*; Environmental microbiology; Photobioreactor; Winery wastewaters.

ABSTRACT

Wine production is a significant global industry, with vineyards and wineries scattered across numerous regions worldwide. Portugal is a noteworthy contributor to this wine production landscape, with a growing emphasis on incorporating environmental microbiology and biotechnology practices to enhance its quality and sustainability. With a central focus on promoting sustainable production methods and addressing environmental challenges, the use of winery wastewaters can be a valid option for both traditional and new processes, with microalgae cultivation emerging as a very promising solution as a nutrient source. This practice showed important advancements in wastewater treatment efficiency, as it can remove compounds such as organic matter and polyphenols, as well as underscoring the potential for fostering sustainable practices within the industry, assuring the cycling of resources. The REDWine project was created, to explore and develop this concept of valorization, among other sustainable practices related with red wine production. The wastewater from wine production was studied and characterized for sugars, polyphenols, and solid contents, and supplemented the culture media for microalgae, in photobioreactor growth assays with *Chlorella vulgaris*. These experiments yielded compelling results, as the microalgae demonstrated robust growth even in media with effluent concentrations as high as 30% (v/v), despite experiencing some growth inhibition and contamination at this concentration level. The most favorable growth rate and adaptability were observed at an optimal concentration of around 10% (v/v), resulting in a growth rate 67% higher than that achieved with only 1% (v/v) of effluent incorporation. Overall, the integration of winery effluents into the cultivation medium has proven to be a viable approach to value these otherwise wastes, positively impacting microalgae growth rates and protein content, thereby contributing to the overarching goal of sustainable wine production.

Acknowledgements:
The research developed was financed by the REDWINE project under the progamme H2020 and the BBJ, with grant agreement Nº 101023567

P1.33 - MYCELIA INACTIVATION PROCESSES –MAINTAINING THE FLEXIBILITY AND STRENGTH OF MYCELIUM-BASED BIOCOMPOSITES

Ana T. Oliveira [1(*)], Miguel A. Ramos [1,2], Paula M. L. Castro [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*
[2] *Pipe Masters, Póvoa de Varzim, Portugal.*

[(*)] *email: atoliveira@ucp.pt*

Keywords: mycomaterials; mycelia inactivation; circular economy; MTT assay.

ABSTRACT

The current trend towards a sustainable and circular economy proposes the development and use of bio-based materials from renewable resources. Mycelium-based biocomposites (MBB), which consists of defragmented lignocellulosic particles linked by dense mycelium, are an ecological and innovative solution to replace petroleum-based products. MBB have shown advantageous properties, such as acoustic insulation, fire resistance, and the absence of harmful synthetic chemical components. These properties are the basis for the production and use of MBB for a wide range of applications, including paper, textiles, foams for packaging material, vehicle parts, and electronic equipment packaging materials. Briefly, MBB production is achieved through the sterilization the biomass, inoculation and incubation with selected fungi, homogenization, and interruption of fungal growth (or inactivation). Most MBB go through a heating treatment to inactivate the mycelia. However, that treatment results in rigid biocomposites with low flexibility. This work investigated the performance of alternative inactivation methods aiming to achieve flexible but sturdy MBB. Low temperature treatments and $CuSO_4$ were tested in biocomposites obtained from two fungi strains. The inactivation efficiency was evaluated through a cell viability assay, MTT assay. The physical properties of the resulting biocomposites were also assessed. Spraying MBB with a $CuSO_4$ solution did not efficiently inactivate the fungi. Although the low temperatures seemed to have inactivated the fungi two days after treatment (ca. 1% cell viability), 15 days later the mycelia resumed growth again (34% cell viability). Therefore, those treatments did not efficiently inactivate the fungi but left them in a latent dormancy state. None of the tested methods compromised the biocomposites' flexibility features. Further studies need to be conducted to identify inactivation methods that allow the production of MBB with a more diverse range of physical characteristics to expand their application potential.

Acknowledgements:
This work was financed by the project BioShoes4All – Inovação e capacitação da fileira do calçado para a bioeconomia sustentável, Footwear, within the scope of the Recovery and Resilience Plan of Portugal, in its component 12 – Sustainable Bioeconomy, Investment TC-C12-i01. Authors thank the CBQF scientific collaboration under the FCT project UIDB/50016/2020.

P1.34 - SUSTAINABLE PRODUCTION OF YEAST-DERIVED CAROTENOIDS USING RICE BRAN HYDROLYSATES

Thercia Rocha Balbino [1(*)], Salvador Sánchez-Muñoz [1], Stephanie Custódio Inácio [1], Júlio César Santos [1], Jorge Fernando Brandão Pereira [2], Silvio Silvério da Silva [1]

[1] University of São Paulo, Engineering School of Lorena, Department of Biotechnology, Lorena, SP, Brazil

[2] University of Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal.

[(*)] email: tbalbino@usp.br

Keywords: carotenoids; yeasts; pigments; hydrolysate; agro-industrial byproducts.

ABSTRACT

Biopigment production has received attention in industrial and research field, owing to its versatile applications in pharmaceutical (antimicrobial), feed/food (dietary supplement), textil (dyeing), cosmetics (UV-protection and antioxidants), and others sectors. Carotenoids are part of a diverse group of pigments with special properties (e.g., antioxidants and antimicrobials), highly valued on the market. The current high cost large-scale microbial carotenoids production underscore need for cost-effective strategies to produce these high value-added products. The economic viability of bioprocess can be improved using low-cost raw materials, such as agro-industrial byproducts. This study evaluates the potential of the yeast *Rhodotorula mucilaginosa* to produce biopigments using rice bran hydrolysate (RBH) as a growth medium. Rice bran was hydrolyzed using sulfuric acid (H_2SO_4) in a stirred tank reactor and the RBH was used as growth media to cultivate *R. mucilaginosa* for carotenoids production. The yeast was also cultured in YM medium (containing glucose, peptone, yeast extract, malt extract) as a control. The final glucose concentration for both media was adjusted to 30 g/L. After 72 h of growth, cells were harvested and biopigments were extracted and analyzed by spectrophotometry (350-700 nm). The findings demonstrate the ability of *R. mucilaginosa* to grow and produce biopigments in both YM and RBH medium. While the maximum growth rate was higher in YM (0.59) compared to RBH medium (0.35), the final cell biomass concentration was similar in both conditions (YM: 19.24 g/L and RBH: 19.61 g/L). The yeast was able to produce biopigments from RBH, with a spectrophotometric pattern resembling that of the colored extracts from YM, suggesting that similar types of biopigments were produced from both culture media. *R. mucilaginosa* shows the potential to produce biopigments from RBH without need for medium supplementation or detoxification, reducing production costs and enhancing the feasibility of incorporating this bioprocess into a biorefinery concept.

Acknowledgements:
This study was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior- Brazil (CAPES) Finance Code 001, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) process number 16/10636-8 and 2020/12059-3, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), process number 304166/2022-7. This work is part of the project "INCT Yeasts: Biodiversity, preservation and biotechnological innovation" funded by CNPq, Brasília, Brazil, grant #406564/2022-1. CIEPQPF is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UIDB/ EQU/00102/2020 and UIDP/EQU/00102/2020). J.F.B. Pereira acknowledges FCT for funding the project DRI/India/0044/2020

P1.35 - ANTI-*CANDIDA* ACTIVITY OF *CYMBOPOGON* SPP. ESSENTIAL OILS: A PROMISING NEW TREATMENT STRATEGY FOR SUPERFICIAL INFECTIONS

Sandra Simões Tomás [1,2] (*), Daniela Marques Almeida [1,2], Ana Sofia Oliveira [1,2], Joana Domingues [3,4], Rita Palmeira-de-Oliveira [1,2,5], José Martinez-de-Oliveira [1], Fernanda Delgado [3,4,6], Ana Palmeira-de-Oliveira [1,2,5], Joana Rolo [1,2]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[2] *Faculdade de Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal*

[3] *Escola Superior Agrária do Instituto Politécnico de Castelo Branco, Portugal*

[4] *Centro de Biotecnologia de Plantas da Beira Interior, Castelo Branco, Portuga.*

[5] *Labfit-HPRD: Health Products Research and Development Lda, Covilhã, Portuga.*

[6] *CERNAS: Research Center for Natural Resources, Environment and Society, Agriculture Science Research Group, Castelo Branco, Portugal.*

(*) *email: sandra.tomas@ubi.pt*

Keywords: *Candida albicans*; Infection; Natural products; Lemongrass; Vaginal.

ABSTRACT

Treatment of superficial infections caused by *Candida albicans* is often achieved with the use of azole compounds, but resistance rates are increasing alarmingly. Natural compounds such as those present in essential oils have been described as excellent antifungal agents, constituting a sustainable alternative for the development of new antifungals. In this study, we intend to explore the potential of essential oils obtained from *Cymbopogon* spp. Three lemongrass essential oils were included in the study (*Cympobogon citratus*, *Cymbopogon winterianus* and *Cymbopogon flexuosus*). The phytochemical analysis was determined by GC-MS. The minimum inhibitory concentrations (MIC) and minimum lethal concentrations (MLC) were determined against *Candida albicans* ATCC100231 using the broth microdilution method. In addition, the effect of the essential oils on the destruction of biofilms and in the inhibition of germ tube formation, has been assessed by standard techniques. Finally, the possible synergy with clotrimazole has been assessed by the checkerboard assay. Major compounds identified were geranial (*C. citratus*: 50%; *C. flexuosus*: 37%) and citronellal (*C. winterianus*: 50%). MIC ranged from 0.12% (V/V, *C. citratus* and *C. flexuosus*) to 0.25% (V/V, *C. winterianus*); and MLC ranged from 0.12% (V/V, *C. citratus*) to >1% (V/V, *C. winterianus*). Total inhibition of germ tube growth and 50% of destruction of biofilm biomass was achieved for all essential oils after treatment with 10x MIC. Synergy with clotrimazole was observed in the inhibition of growth of planktonic cells for *C. citratus* essential oil. *C. citratus's* essential oils have significant *anti-Candida* activity, probably related to their main compound, geranial. There is great potential in the applicability as a co-adjuvant constituting a new treatment strategy for superficial infections.

Acknowledgements:
The work was developed within the scope of the CICS-UBI projects [UIDB/00709/2020] and [UIDP/00709/2020], financed by national funds through the Portuguese Foundation for Science and Technology/MCTES (FCT).

P1.36 - UNLOCKING THE BIOTECHNOLOGICAL POTENTIAL OF MARINE MICROORGANISMS: THE RIVER2OCEAN PROJECT'S CONTRIBUTION TO THE BLUE BIOECONOMY

Ana Rita Bragança [1,2], João Gomes [1,2], Ana Rita Marques Silva [1,2], Tony Collins [1,2], Margarida Casal [1,2(*)], Isabel Soares-Silva [1,2], Raul Machado [1,2]

[1] *Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal*

[2] *Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal.*

[(*)] *email: mcasal@bio.uminho.pt*

Keywords: Microbial biodiversity; Biobank; Sustainable biotechnology.

ABSTRACT

The marine environment, characterized by extreme environmental conditions, including extremes of salinity, temperature, and pressure, leads microorganisms to accumulate remarkable physiological and functional heterogeneity. This feature makes them exciting targets for the exploration of new bioactive compounds and sustainable microbial capabilities. However, the marine environment is still an underexplored source of biodiversity. Within the scope of project River2Ocean, we have established a biobank of marine microorganisms that is being explored for the production of value-added compounds and processes with high biotechnological potential. A top-down screening approach was designed to assess the potential of microbial isolates obtained from marine samples collected on Portugal's northern coast. Selective media were used to isolate bacteria, yeasts, and filamentous fungi. Four ribosomal RNA regions were sequenced using Sanger Sequencing, allowing to distinguish 101 unique isolates from the original pool of 264, comprising 83 bacteria (74 Gram-negative and 9 Gram-positive), 9 yeasts, and 9 filamentous fungi. Microbial isolates were screened for the production of antimicrobial compounds and various enzymes of industrial interest, including bioremediation approaches. We have identified 26 bacteria and 7 yeast strains active against at least one of the six tested pathogens of clinical relevance. Remarkably, three isolates from the *Photobacterium* and *Paracoccus* genera have shown activity against all the tested microorganisms, indicating potential importance in addressing antimicrobial resistance. Enzyme activities were assayed using a variety of organic and polymeric substrates and, a wide range of microorganisms have been identified as producers of esterases, proteases, pectinases, xylanases and cellulases. Species from the *Vibrio*, *Aeromonas*, and *Acinetobacter* genera showed activity on 7 of the 9 substrates tested, suggesting potential for bioremediation and industrial applications. Establishing a marine microbial biobank provides a biotechnological platform with significant innovation potential to promote bilateral collaboration between the academy and industry, contributing to advancing the transition towards a blue bioeconomy.

Acknowledgements:
This work was supported by project River2Ocean – Socio-ecological and biotechnological solutions for the conservation and valorization of aquatic biodiversity in the Minho Region, with reference NORTE-01-0145-FEDER-000068, co-financed by the European Regional Development Fund (ERDF), through Programa Operacional Regional do Norte (NORTE 2020).

P1.37 - WATER DISINFECTION USING UVC DIODES THAT EMIT LIGHT AT DIFFERENT WAVELENGTHS

João Sério [1,2], Maria Eduarda Martins [1], Carolina Santos [1, 3], Ana Paula Marques [1], Vânia Pobre[2], Cecília Arraiano [2], Mónica Serrano [2], Adriano Henriques [2], Maria Teresa Crespo [2], Vanessa, Jorge Pereira [1,2]

[1] *iBET, Instituto de Biologia Experimental e Tecnológica, Portugal*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal*

[3] *Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.*

[(*)] *email: joao.serio@ibet.pt*

Keywords: Water disinfection; UV-C LEDs; inactivation mechanisms.

ABSTRACT

Ultraviolet (UV) treatment using low pressure mercury lamps that emit monochromatic light at 254nm is widely applied to achieve disinfection of water due to the proven effectiveness of this treatment process against a wide range of waterborne pathogens. Some studies have reported the use of different LED systems for the inactivation of different microorganisms in water sources. These effective disinfection systems could still be improved using a combination of wavelengths that ensure damages in the microbial DNA and RNA, affect proteins, the enzymatic activity, membrane permeability, cell wall and biological processes of the different microorganisms with no or minimum dark and light repair possibilities. The study of this disinfection method would allow the development of alternative effective water disinfection systems. In this work, LEDs that emit light at different wavelengths 255nm, 265nm, 260nm, 270nm, 280nm and different combinations of wavelengths were tested in terms of their potential to inactivate gram-negative (*Escherichia coli*) and gram-positive (*Enterococcus faecium*) bacteria that are routinely monitored as faecal contamination indicators of water quality. Inactivation assays were conducted in phosphate buffer and real water matrices using culture collection strains and bacteria isolated from real surface water matrices using selective media. The results obtained show that high levels of inactivation were achieved (higher than 3-log) after 1 minute exposure to three small LEDs that emit light at the different wavelengths. After determining the UV fluences of LEDs that emit at different wavelengths, the mechanisms of inactivation were addressed (by monitoring changes in cell morphology using fluorescence microscopy, membrane permeability, enzymatic activity as well as DNA damage and transcriptomic analysis) and the potential photoreactivation and dark repair was studied to define the best inactivation conditions that prevent reactivation.

Acknowledgements:
This research was funded by Fundação para a Ciência e a Tecnologia through the project PTDC/EAM-AMB/1561/2021. iNOVA4Health (UIDB/04462/2020, UIDP/04462/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020), a program financially supported by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior through national funds, is also acknowledged.

P1.38 - ANTIPROLIFERATIVE ACTIVITY OF BIOACTIVE COMPOUNDS PRODUCED BY BACTERIAL ISOLATES FROM PRISTINE ENVIRONMENTS.

Patrícia Gatinho [1,2]***, A. Teresa Caldeira*** [2,5]***, Daniela Grilo*** [2]***, Ana Z. Miller*** [2,4]***, Amélia M. Silva*** [3]***, Cátia Salvador*** [2(*)]

[1] *Department of Engineer, School of Science and Technology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[2] *HERCULES Laboratory & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Évora, Portugal*

[3] *Center for Research and Technology of Agro-Environmental and Biological Sciences & Department of Biology and Environment, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[4] *Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain*

[5] *Department of Chemistry and Biochemistry, School of Sciences and Technology & City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science University of Évora, Évora, Portugal.*

(*) *email: cscs@uevora.pt*

Keywords: Antiproliferative activity; Biotechnology; Caves; Tumor cells; Sustainability.

ABSTRACT

Pristine environments, such as caves, represent distinctive ecosystems that remain untouched by human influence and are exposed to extreme environmental conditions [1]. These habitats serve as bountiful reservoirs of microbial diversity, with the microorganisms inhabiting them evolving specialized traits and metabolic pathways in response to the unique selective pressures exerted by their surroundings. To survive, these microorganisms usually have the ability to produce metabolites that prevent the growth of other organisms, which can have an effect on different cells, including tumor cells. This study aims to search for new bioactive compounds with antitumoral activity produced by bacterial strains belonging to the phyla *Actinomycetota*, *Bacillota*, *Bacteroidota* and *Pseudomonadota*, which were isolated in marine, *Paleolithic*, and volcanic caves. The antitumor potential of culture supernatants of bacterial strains was tested against a breast cancer epithelial cell line MDA-MB-231 at different concentrations, and very promising results were obtained for some of the strains studied. These compounds produced by the bacteria could potentially be used as nutraceuticals or complementary agents in future cancer therapies. Bioprospection and discovery of new compounds represent an opportunity for the study of these natural habitats, allowing new products obtained by fast and low-cost biotechnological processes to be implemented as novel green-safe and sustainable solutions.

References:

[1] P. Gatinho, C. Salvador, A. M. Silva, and A. T. Caldeira, “Prokaryotic Communities from Pristine Cave Environments : Biotechnological Potential with Sustainable Production,” pp. 1–22, 2023, doi: 10.3390/su15097471.

Acknowledgements:

The authors acknowledge the FCT, Portuguese Foundation for Science and Technology, I P within the scope of the projects UIDB/ 04449 2020 (HERCULES Lab), MICROCENO (PTDC/CTA AMB/ 0608 2020)ART3mis (2022 07303 PTDC), C Salvador (DL 57 2016 /CP 1372 /CT 0019) to individual support, and the City University of Macau endowment to the Sustainable Heritage Chair Sino Portugal Joint Laboratory of Cultural Heritage Conservation Science supported by the Belt and Road Initiative.

P1.39 - TWO NOVEL *FLAVOBACTERIUM* SPECIES ISOLATED FROM ALKALINE MAGNESITE RESIDUES

Leonor Matos [1(*)], Diogo Neves Proença [1], Romeu Francisco [1], Ana Paula Chung [1], Lorrie Maccario [2], Søren J Sørensen [2], Paula V Morais [1]

[1] *University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, Coimbra, Portugal*

[2] *Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark*

[(*)] *email: leonormatos98@gmail.com*

Keywords: genome; taxonomy; *Flavobacterium*.

ABSTRACT

Bacterial strains FBOR7N2.3^T and FZUC8N2.13^T were isolated from magnesite residues from Spain, and characterized. The 16S rRNA gene sequences indicated that the strains belonged to the *Flavobacterium* genus. The 16S rRNA gene sequence similarities of FBOR7N2.3^T to that of *Flavobacterium cellulosilyticum* were 97.1% and of FZUC8N2.13^T to that of *Flavobacterium lacustre* were 97.6%. FBOR7N2.3^T and FZUC8N2.13^T stained Gram-negative, grew optimally between 25-30 ◦C and at pH of 7. The G+C content of DNA was 33.2 and 33.4 mol%, respectively. The respiratory quinone was Menaquinone 6. The major fatty acids were Summed feature 3 (16:1 7c/16:1 6c) and iso- C15:0, representing 55.0% and 58.3% of the total fatty acids, respectively. The polar lipids consisted of two aminolipids, two aminophospholipids and one glycolipid. The draft genome sequences of FBOR7N2.3^T and FZUC8N2.13^T comprise 3,851,759 and 3,549,495 bases, respectively. The assembled genomes consist of 81 and 55 contigs, respectively. The genomes encode 3,316 and 3,165 putative coding sequences, respectively. The ANI and DDH values of FBOR7N2.3^T, FZUC8N2.13^T against *Flavobacterium undicola* were of 86.2% and 85.9%; 30.4 and 30.1%; respectively. The phylogenetic, phylogenomic, phenotypic and chemotaxonomic data showed that FBOR7N2.3^T and FZUC8N2.13^T should be classified as novel species of the genus *Flavobacterium*, for which we propose the names *Flavobacterium borobia* FBOR7N2.3^T (= UCCCB 178^T) and *Flavobacterium zubiri* FZUC8N2.13 ^{T} (= UCCCB 179^T), respectively.

Acknowledgements:
This research was supported by EU Horizon 2020 R&I through project “Biorecover” (Grant nº821096), EU project “Reviving” (ERA-MIN/0007/2019) (Grant nº 67), and by national funds through FCT –Fundação para a Ciência e a Tecnologia, under the project UIDB/00285/2020 and LA/P/0112/2020.

P1.40 - BACTERIAL INTERACTIONS WITH VANADIUM: INSIGHTS OF TOLERANCE AND RESISTANCE MECHANISMS

Joana Bonito Caldeira [1(*)], Rita Branco [1], Paula Vasconcelos Morais [1]

[1] *University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, Coimbra, Portugal.*

[(*)] *email: joanaritacaldeira@gmail.com*

Keywords: vanadium; metal toxicity; bacterial resistance; critical metal.

ABSTRACT

Vanadium (V) is a versatile metal with wide applications in several industries and clinical settings, leading to a recent surge in V-consumption. Therefore, the European Union has classified V as a Critical Raw Material by the European Union. However, V also constitutes a pollutant that, in the near future, can be a considerable environmental issue. This work focuses on the study of V-resistant bacterial strains to improve our understanding of V-toxicity in the cellular environment and to elucidate the mechanisms in cells in response to V-stress. The V-resistance of several bacteria from the University of Coimbra Bacteria Culture Collection (UCBCC) was initially tested on R2A solid media supplemented with increasing Vanadate concentrations (0 to 20 mM V(V)). Strains capable of growing at the highest V(V) concentrations (20 mM V(V)) were selected to evaluate their minimum inhibitory concentration (MIC) by standard broth dilution assays. Additionally, different conditions (presence and absence of metal) were also explored to evaluate bacterial growth. Out of the tested 350 bacterial strains, approximately half grew on R2A solid media containing 20mM V(V). Among these resistant strains, 35 were selected to determine the MIC of V(V), but only a third of them were able to grow in R2A broth media with 20mM V(V). This preliminary work shows the diversity of V resistance profile among a large collection of bacterial strains isolated from different sources, which can indicate the existence of different interactions between microorganisms and V. More work should be done to access the main bacterial mechanisms involved in the V resistance processes and to expand the collection of characterized V-resistant bacteria with diverse metabolisms.

Acknowledgements:
The work was funded by Project ERA-MIN-2019_67-Reviving; Project 821096 Biorecovery-H2020-SC5-2018-2019-2020; UIDB/00285/2020 and Joana Bonito Caldeira was supported by a PhD research grant (2022.13542.BD) from FCT/MCTES through national funds.

P1.41 - IDENTIFICATION OF POTENTIAL INHIBITION OF CARBONIC ANHYDRASE IN PRESENCE OF AMINO ACID-BASED IONIC LIQUIDS (AA-ILS) USING MOLECULAR DOCKING

Michele Fraga [1], Mara G. Freire [1], Matheus M. Pereira [2(*)]

[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal*
[2] *University of Coimbra, CIEPQPF, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, Coimbra, Portugal.*

[(*)] *email: matheus@eq.uc.pt*

Keywords: carbonic anhydrase; molecular docking; amino acids based-ionic liquids.

ABSTRACT

Carbonic anhydrase (CA; EC 4.2.1.1) is a metalloenzyme that catalyzes the conversion of carbon dioxide (CO_2) into bicarbonate (HCO_3^-). CA has been widely employed in several biotechnological applications, from the treatment of gaseous effluents to the development of artificial lungs. Amino acid-based ionic liquids (AA-ILs) have already proven to be an attractive alternative to traditional organic solvents, not only due to their physical and chemical properties and biocompatibility but also due to their ability to promote a favorable environment for enzyme-based reactions. Therefore, the objective of this study was to perform a computational trial using molecular docking to evaluate the effect of AA-ILs on the CA activity. For this, molecular docking analysis was conducted using AutoDock Vina 1.1.2. The receptor employed was the bovine CA-II (PDB ID: 4cnx), while the ligands consisted of AA-IL cations and anions. All input files were generated using AutoDockTools. According to the results obtained, AA-IL cations displayed lower binding affinity (ranging from -4.8 to -2.7 kcal/mol) in comparison to AA-IL anions (ranging from -6.2 to -3.1 kcal/mol). All AA-IL ions tested showed a binding site on the CA-II structure at the active site, with the exception of [Ch]+ and $[P4444]^+$. The binding affinity to the CA-II active site allows to predict a competitive inhibition behavior, as substrates do not have access to the CA-II catalytic site. In addition, electrostatic, hydrogen bonds, and Van der Waals interactions were identified between AA-IL ions and CA-II amino acid residues. Thus, despite the biocompatible nature of AA-ILs, the results of the molecular docking analysis suggest possible competitive inhibition by AA-IL ions, resulting in a loss of enzyme activity.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 and LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). CIEPQPF is supported by the FCT through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. Fraga acknowledge FCT for the PhD grant BD/12877/2022.

P1.42 - ASSESSMENT OF IONIC LIQUIDS IN MODULATING CARBONIC ANHYDRASE ACTIVITY USING MOLECULAR DOCKING

Michele Fraga [1], Mara G. Freire [1], Matheus M. Pereira [2(*)]

[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[2] University of Coimbra, CIEPQPF, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, Coimbra, Portugal.

(*) email: matheus@eq.uc.pt

Keywords: enzyme modulators; carbonic anhydrase; ionic liquids; molecular docking.

ABSTRACT

Enzyme modulators are molecules that can either enhance or reduce the activity of enzymes. Non-covalent modulators interact reversibly with enzymes, influencing their activity through binding interactions. Ionic liquids (ILs) are promising enzyme modulators due to their unique physicochemical properties, such as tunable polarity and solubility, which enable precise control over enzyme activity and stability. Thus, the aim of this work was to identify the modulation ability of ILs on Carbonic Anhydrase type II (CA-II) using molecular docking. For this, enzymatic activity assays (colorimetric tests) were performed in the presence of several ILs at 0.1M. The effect of cation alkyl chain length and anion hydrogen bond ability was evaluated. In addition, the identification of IL ions binding sites and binding affinity on CA-II structure was obtained through molecular docking analysis, using AutoDock Vina 1.1.2. The results obtained showed that CA-II activity was higher in the presence of ILs with cations with long alkyl chains. Non-cyclic cations displayed an inhibitory effect on enzyme activity. Among the anions tested, CA-II activity increased in the presence of anions with higher hydrogen-bond basicity. Molecular docking results showed that IL ions that bind to CA-II active site decrease the enzyme activity. On the other hand, IL ions that bind to CA-II allosteric site improve the enzyme catalytic performance. Therefore, a new approach for the identification of enzyme modulation in the presence of ILs is proposed here, opening up the possibility to design specific ion combinations to improve the enzyme catalytic performance.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 and LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). CIEPQPF is supported by the FCT through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. Fraga acknowledge FCT for the PhD grant BD/12877/2022.

P1.43 - GENOME MINING REVEALS POTENTIAL OF *PSEUDOMONAS SP* STRAINS FOR IMPROVED MICROBIAL-INDUCED CALCIUM PRECIPITATION

Pedro Farias [1(*)]***, Amanda Mendonça*** [2]***, Paulo da Venda*** [2]***, Paula V Morais*** [1]

[1] *Universidade de Coimbra, CEMMPRE, Departamento de Ciências da Vida, Coimbra, Portugal*

[2] *Universidade de Coimbra, ISISE, Departamento de Engenharia Civil, Coimbra, Portugal.*

[(*)] *email: pfarias@uc.pt*

Keywords: MICP; *Pseudomonas;* urease.

ABSTRACT

Microbial-induced carbonate precipitation (MICP) is a technique that can fulfil the requirements for a green transition in the construction industry by providing alternatives to current types of cement. One way bacteria can perform MICP is by metabolizing urea into ammonia, creating an environment (high pH) that favours the conversion of carbon dioxide and soluble calcium into calcium carbonate and water, with this last reaction mediated by carbonic anhydrase. Calcium carbonate acts as a cement, binding loose aggregates or mending cracks. The most common bacteria used in this process belong to the genera *Sporosarcina, Bacillus* and *Lysinibacillus*. Our objective is to find bacterial strains, with distinct genomic mechanisms encoding urease activity, that can perform MICP. In our study, we evaluated the use of two *Pseudomonas* strains, M47T1 and HST_244P, in MICP, in comparison to each other and to reference bacteria. These strains, when compared to each other, have distinct genetic operons for the expression of agents of ureolytic activity, as well as carbonic anhydrase. Both strains possess the same arrangement of *ure* genes (required and accessory). Nevertheless, strain HST_244P has an additional *ure* gene encoding a pH-dependent urea transporter and strain M47T1 possesses an alternative route for ureolytic activity other than via urease, the urea amidolyase complex. While HST_244P maintains high pH, *circa* pH8, and low ureolytic activity, M47T1 achieves 4.2 times higher ureolytic activity with acidic pH, pH of 4. Additionally, strain M47T1 produces 4.5 g of CaCO3 per liter of inocullum, 22 times the amount produced by the reference organism, *Sporosarcina pasteurii*, 0.2 g/l. Despite the advantages of using *Sporosarcina* or *Bacillus* in MICP, other organisms with unique genetic mechanisms can offer an alternative for the implementation of biocementation. With further exploration and optimization of the genetic machinery of strain M47T1 this strain could be one of those candidates.

Acknowledgements:
This research is sponsored by national funds through FCT – Fundação para a Ciência e a Tecnologia, under the project UIDB/00285/2020 and LA/P/0112/2020; by national funds through FCT, under the project 2022.03631.PTDC and by the Next Generation EU Funds, following NOTICE N.º 02/C05-i01/2022, Component 5 – Capitalization and Business Innovation - Mobilizing Agendas for Business Innovation in the Two Wheels sector. Amanda Mendonça was supported by national funds through FCT and POCH 2021.07819.BD.

P1.44 - WINE LIQUID EFFLUENTS AS NUTRITIOUS CULTIVATION MEDIA TO PRODUCE MICROALGAE BIOMASS

Ana Rita Martins [1(*)], Catarina Dias[1(*1)], Ana Cláudia Sousa [1], Ana Gabriela Gomes [1], Miguel Cachão [2], Carla Amarelo Santos [1(*2)]

[1] *Instituto Politécnico de Setúbal, Escola Superior de Tecnologia do Barreiro, Barreiro, Portugal*
[2] *AVIPE – Associação de Viticultores do Concelho de Palmela, Palmela, Portugal.*

*(*)email: ana.rita.martins@estudantes.ips.pt(*) | catarina.r.dias@estudantes.ips.pt(*1) | carla.santos@estbarreiro.ips.pt(*2)*

Keywords: *Chlorella vulgaris*; Biotechnology; Sustainability; Wine Liquid Effluents.

ABSTRACT

Portugal has been renowned for its wine production, and in 2022 was ranked as the tenth-largest wine producer globally, boasting a rich history and notable economic influence [1]. However, the wine industry faces sustainability challenges with increased environmental awareness. It's imperative the transition to a circular economy that incorporates by-product valorization [2,3]. Unfortunately, the wine industry is associated with key areas of environmental concern including high water usage and greenhouse effect gas emissions, with a need to adequately manage the wastes generated during wine production [4]. Winery liquid effluents are rich in organic carbon and their improper discharge can pose a health risk; therefore, they must be treated appropriately resulting in an additional cost for the wineries [5,6]. Redwine is an international research project aiming to value winery liquid effluent for Chlorella biomass production. In 2022, effluent samples were collected from a winery in Setubal during the harvest season for a physicochemical analysis. The composition showed a good source of organic carbon compounds, important minerals, and trace elements, although lacking some essential growth nutrients such as nitrogen and phosphorus. Bench scale assays were carried out with two different effluents: high polyphenol content (HPh) and low (LPh) along with a reference culture medium assay. The effluent concentrations in a medium were varied from 1, 5, 10 to 30% (v/v) and growth productivities were calculated based on the optical density (590 nm). The highest productivity registered was 200 mg/(L.d) with 30% LPh compared to the 39.79 mg/(L.d) registered by the reference culture medium. After analysing the total polyphenolic concentration, the effluent (HPh) had a concentration 5 times higher compared to the LPh effluent and approximately 3 times higher biomass productivity, indicating that polyphenols may be potentially growth inhibitors. Nevertheless, these preliminary results show an interesting potential of wine liquid effluents in the growth of *Chlorella vulgaris*.

References:

[1] International Organisation of Vine and Wine, Country Statistics, 2022. Information on: https://www.oiv.int/what-we-do/country-report?oiv]
[2] M. C. Gaspar et al., "Assessment of Agroforestry Residues: Their Potential within the Biorefinery Context," ACS Sustain. Chem. Eng., vol. 7, no. 20, pp. 17154–17165, 2019, doi: 10.1021/acssuschemeng.9b03532.
[3] P. Chowdhary, A. Gupta, E. Gnansounou, A. Pandey, and P. Chaturvedi, "Current trends and possibilities for exploitation of Grape pomace as a potential source for value addition," Environ. Pollut., vol. 278, p. 116796, 2021, doi: 10.1016/j.envpol.2021.116796.
[4] G. F. Ferreira, L. F. Ríos Pinto, R. Maciel Filho, and L. V. Fregolente, "A review on lipid production from microalgae: Association between cultivation using waste streams and fatty acid profiles," Renewable and Sustainable Energy Reviews, vol. 109. Elsevier Ltd, pp. 448– 466, Jul. 01, 2019, doi: 10.1016/j.rser.2019.04.052.

[5] R. P. Rodrigues, L. M. Gando-Ferreira, and M. J. Quina, "Increasing Value of Winery Residues through Integrated Biorefinery Processes: A Review," Molecules, vol. 27, no. 15, 2022, doi: 10.3390/molecules27154709.
[6] V. P. B. de Medeiros et al., "Exploiting the use of agro-industrial residues from fruit and vegetables as alternative microalgae culture medium," Food Res. Int., vol. 137, no. August, 2020, doi: 10.1016/j.foodres.2020.109722.

Acknowledgements:
The research developed was financed by the REDWINE project under the programme H2020 and the BBJ, with grant agreement Nº 101023567.

P1.45 - ASSESSING THE BIOTECHNOLOGICAL POTENTIAL OF MARINE-DERIVED *TRICHODERMA* ISOLATES

Dmitry Martynov [1], Alberto Abreu [1], Carina Félix [2], Artur Alves [1], Ana Cristina Esteves [1, (*)]

[1] *CESAM and Department of Biology, Campus Universitário de Santiago, Aveiro, Portugal*

[2] *MARE-Marine and Environmental Sciences Centre & ARNET—Aquatic Research Network Associated Laboratory, ESTM, Polytechnic of Leiria, Peniche, Portugal.*

[(*)] *email: acesteves@ua.pt*

Keywords: marine *Trichoderma*; biotechnological potential; antimicrobial activity.

ABSTRACT

Marine-derived fungi have garnered attention for their enzymatic capabilities and their potential as sources of antimicrobial compounds and other compounds with applications in industrial, pharmaceutical, and environmental sectors. Their adaptability to the challenging conditions of the marine environment adds to their appeal in the realm of biotechnology. Therefore, the effect of marine salts on the biotechnological potential of marine fungi was assessed in this study. We focused our research on marine-derived *Trichoderma* isolates. While isolates of this genus from terrestrial environments have been recognized for their effective biocontrol attributes, their counterparts from marine environments remain poorly explored. Thus, a comprehensive assessment of enzymatic, antibacterial, and antifungal activities was carried out. Most *Trichoderma* isolates were able to express extracellular enzymes: amylases, proteases, chitinases, and cellulases. Differences in enzyme production were detected when fungi were cultivated with or without sea salts, suggesting the prospect of optimizing these activities for enhanced production yields. Regarding antibacterial activity, results demonstrated that the marine *Trichoderma* strains tested revealed higher efficacy against Gram-positive bacteria. However, *Trichoderma hamatum* and *T. atroviride* displayed antibacterial activities against several Gram-negative strains, including *Pseudomonas aeruginosa*, *P. putida*, and *Aeromonas hydrophila*. Considering the overall antifungal evaluation, the *Trichoderma* collection tested revealed the ability to inhibit the growth of several plant and human pathogenic species. Both *T. hamatum* and *T. atroviride* demonstrated notable effectiveness against all the six phytopathogenic fungi tested. In the presence of *Trichoderma* strains, *Glomerella cingulata* experienced significant growth inhibition. Additionally, a considerable number of our isolates demonstrated a suppression of mycelium proliferation and parasitism on the tested fungi, including *Botrytis cinerea*, *Diplodia corticola*, and *Lasiodiplodia hormozganensis*, highlighting their potential as biocontrol agents against these fungal species. The current study glances on the need for further exploration in marine mycology to identify valuable compounds boosting blue biotechnology and environmental sustainability.

Acknowledgements:
The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020).

P1.46 - MICROBIOME ANALYSIS AND BACTERIAL CONSORTIUM: KEYS TO HEALTHY AND SUSTAINABLE POTATO SOIL MANAGEMENT

Paula V. Morais [1], Adriana Mata [1], Carlos Cruz [2], Marta Acin-Albiac [3], Beatriz Garcia-Jimenez [3], Alberto Acedo [3], Isabel Conceição [2], Diogo Neves Proença [1(*)]

[1] *University of Coimbra, Department of Life Sciences and CEMMPRE, ARISE, Coimbra, Portugal*

[2] *University of Coimbra, Department of Life Sciences and CFE, Coimbra, Portugal*

[3] *Biome Makers Inc., West Sacramento, California, US.*

[(*)] *email: diogo.proenca@uc.pt*

Keywords: microbiome; bacterial consortium; sustainable agriculture; healthy soils; biological control agents; plant-parasitic nematodes.

ABSTRACT

Production of potato (*Solanum tuberosum*) represents a vital component of global agriculture, providing a staple food source for millions of people. However, the low potato yield average in Portugal may be attributed to plant-parasitic nematodes (PPN). Global challenges such as food security, climate change, environmental degradation, and agriculture practices highlight the need to discuss the role of beneficial bacteria and their impact on potato crop. This work aims i) to assess the microbiome of potato fields of healthy soils (without PPN) and diseased soils (with PPN) to understand the effect of PPN on the soil microbial diversity and ii) to find the beneficial bacterial consortium for effective management of PPN, and plant growth promotion. Forty-five potato producing fields, from North to South of Portugal, were evaluated and 450 soil samples were collected. The microbiomes and the identification of nematodes were performed before(T0) and after(T1) potato cultivations. Through the bioinformatic pipeline, the taxonomy was assigned from ASVs. Moreover, 30 bacterial strains were isolated and characterized regarding plant growth-promoting(PGP) traits, antibiotic resistance, nematicidal activity, and plant phytosanitary. The α-diversity was significantly higher in T1 and the presence of PPN significantly increased diversity indices for bacterial and fungal communities. Organic management(OM) had a significantly higher median Soil Quality Index than conventional management. *Bacillus subtilis* was the most abundant species in OM. The saprophytic genus *Humicola* was found as part of the OM core microbiome. The bacterial consortium was established as a biological and sustainable alternative for chemical reduction practices, and it was composed by one *Bacillus* and two *Pseudomonas* strains showing complementary PGP traits and 100% efficacy to kill the nematodes. In conclusion, this study contributes to a better overview of organic and conventional management and shows that the use of beneficial microorganisms can be a real opportunity for a more sustainable agriculture.

P1.47 - TAPPING INTO THE BIOTECHNOLOGICAL POTENTIAL OF ENVIRONMENTAL BIODIVERSITY: THE EXAMPLE OF A PLATINUM HIGH-AFFINITY BIOPOLYMER

Romeu Francisco [1(*)], Leonor Matos [1], Diogo Margato [1], Beatriz Rito [1], Ana Paula Chung [1], Ana Paula Piedade [1], Paula V. Morais [1]

[1] *University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Coimbra.*

(*) *email: rmfrancisco@uc.pt*

Keywords: Biopolymer; Platinum Group Metals; Critical Raw Materials.

ABSTRACT

Platinum-group metals (PGM) constitute a group of valuable metals of industrial and economic importance and are considered Critical Raw Materials by the European Union. The current supply of those elements by the mining activities is limited to very few sources, and therefore, the risks of disruption of the normal flux of tradable processed resources are considerable when taking into account global instability. Moreover, the extraction processes are costly and prone to cause environmental impacts. For these reasons, the need to increase the recycling efficiency of these elements is of the utmost importance to achieve a higher global sustainability. The objective of this study was to produce a high affinity biopolymer able to extract PGM from aqueous solutions. Two strains were used to produce the main building blocks of this biopolymer, which were subsequently cross-linked: a metallophore with high affinity towards Pt, purified by FPLC from strain FBOM7R9A, isolated from an alkaline residue, and a polymer produced by strain 20M4. The genomes of both strains were analysed for the genes involved in metallophore and polymer synthesis. The metallophore binding ability was tested by fluorescence quenching. The modified biopolymer was analysed by FTIR and tested for PGM binding and extraction from mobile phase (quantifications by ICP-MS). The metallophore showed PGM specific and concentration-dependent interactions, and the modified biopolymer demonstrated a higher binding affinity towards PGM than for Fe. The biopolymer excelled in performance when compared to synthetic analogs, with 85.5-93.7% of the soluble Pt bound in 60 minutes, with no signs of saturation up to 4.8 mM, a relevant concentration when considering operational mining leachates. This work demonstrates the importance of tapping biodiversity and the biotechnological potential of bacterial isolates in order to obtain new biomaterials able to answer the challenges of economic and environmental sustainability.

Acknowledgements:
This research was supported by the mobilizing agendas AM2R (nº7253) and Microelectronics (nº8000) (EU funds and PRR), EU Horizon 2020 R&I through project “Biorecover” (Grant nº821096), EU project “Reviving” (ERA-MIN/0007/2019) (Grant nº 67), UID/EMS/00285/2020 and LA/P/0112/2020.

P1.48 - CHEMICAL-MEDIATED INTERACTIONS BETWEEN CYANOBACTERIA AND AMOEBAE

Francisco Camacho [1,2(*)], **Pedro Leão** [2], **Ana Vieira** [2]

[1] *FCUP – Faculty of Sciences of the University of Porto, Porto, Portugal*

[2] *Cyanobacterial Natural Products Team, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal.*

[(*)] *email: fcamacho@ciimar.up.pt*

Keywords: Chemical ecology; cyanobacteria; amoebae; grazing; natural products.

ABSTRACT

Cyanobacteria are photosynthetic organisms found in both aquatic and terrestrial ecosystems, and are known to be rich sources of potentially valuable secondary metabolites. Cultivation of individual species in laboratory conditions, with little or no competition/stress, hinders the expression of biosynthetic gene clusters (BGCs) and only reveals a small fraction of the metabolites synthesized in nature. Free-living amoebae are unicellular organisms ubiquitously present in the environment, known to co-exist and graze on cyanobacteria. Several factors seem to influence the susceptibility to predation, but the chemical mediators behind this process are still to be identified. Morphology or surface signatures may confer grazing resistance, but the production and release of cyanobacterial metabolites are hypothesized to be the most significant contributors to the resistance of these organisms against amoebae grazing. The aim of this project is to identify new interactions between cyanobacteria and amoebae, focusing on the discovery of new chemical metabolites produced as a defense mechanism towards grazing. Therefore, a variety of cyanobacterial strains from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) of CIIMAR are being co-cultured with different species of amoebae and screened through grazing plaque assays. This screening allows us to select the cyanobacterial strains that are resistant to amoebae grazing. So far, we have screened approximately 130 cyanobacterial strains, and observed grazing resistance in 21 of those strains. This number will be further narrowed through bioactivity assays, through the addition of the crude extracts of the resistant cyanobacterial strains directly to the amoeba cultures. Crude extracts showing activity towards amoebae will lead to selection of cyanobacterial strains for large-scale growth, compound isolation and structural elucidation. In conclusion, based on the ecological interactions that cyanobacteria experience in the environment, we expect to develop new workflows for natural product discovery, while discovering novel compounds with potential pharmacological interest.

Acknowledgements:
This project received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 952374. This study was also partially funded by national funds - Fundação para a Ciência e a Tecnologia, I.P., through strategic funding grants UIDB/04423/2020 and UIDP/04423/2020 and through the FCT exploratory project CYAmoeba (2022.04572.PTDC).

P1.49 - LEACHING TESTS AS PREDICTION MODELS IN MINE RESIDUE VALORISATION

Rita Branco [1(*)], Diogo Margato [1], Joana B. Caldeira [1], António Correia [2], Paula V. Morais [1]

[1]*University of Coimbra, Centre for Mechanical Engineering Material and Processes, ARISE, Department of Life Sciences, Coimbra Portugal*

[2]*University of Coimbra, CIEPQPF—Chemical Process Engineering and Forest Products Research Centre, Department of Civil Engineering, Coimbra, Portugal.*

[(*)] *email: rbranco@uc.pt*

Keywords: valuable metals; organic acids; batch assays; columns assays.

ABSTRACT

The metal reservoirs in the Earth are limited and have declined due to the continuous increase in society's needs. Mining activities have generated large amounts of tailings, which can constitute an environmental issue that can be, on the other hand, seen as a promising source of valuable metals. Leaching tests using different strategies are relevant to predicting the best approach to apply in metal recovery in a real scenario. Tailings from a tungsten exploration mine (Panasqueira mine, Portugal) were subjected to leaching tests in batch and columns to evaluate the leachability of several valuable metals (e.g. Fe, Zn, Al, Cu, Mn, Ga, W). Batch assays were conducted using different strategies: without organic acid supplementation, with supplementation with several concentrations of different organic acids (e.g. oxalate, citrate, malate, formate), and with augmentation of different microbial suspensions. Column assays were performed using two configurations: total recirculation of leachate without media changing and total recirculation of leachate with media renovation every 48 h over 2 weeks. The metal content of the leachates, pH, conductivity, and microbial population (CFU/ml) were evaluated. In general, the highest metal mobilization in the batch assays was achieved using oxalate at 0.5 or 1%. Thus, column tests were performed using 0.5% of oxalic acid, which promoted better results than the control column. Results also indicated that consecutive steps of medium renovation increased the mobilization of Fe, Al and Mn. However, Cu and Zn leaching occurred mostly in the first stage of medium recirculation. Assays conducted with residues and suspensions of *Aspergillus niger* or 3 strains of *Burkholderia* sp. showed a significant decrease in pH, indicating that strains might be promising organic acid producers. In conclusion, this study provides assessment methodologies, at a lab scale, for the effect of different conditions in metal leaching optimization processes.

Acknowledgements:
The work was supported by Project ERA-MIN-2019_67- Reviving; Project 821096 - Biorecovery - H2020- SC5-2018-2019-2020; UID/EMS/00285/2020 (CEMMPRE) LA/P/0112/2020; mobilizing agendas AM2R (nº7253) and Microelectronics (nº8000) (EU funds and PRR). Joana Caldeira was supported by a PhD research grant (2022.13542.BD) from FCT/MCTES through national funds.

P1.50 - DEVELOPMENT OF ANTIGEN-DISPLAYING *BACILLUS* SPORES FOR ORAL VACCINATION AGAINST VIBRIOSIS AND MYCOBACTERIOSIS IN FISH

Gabriela C. Gonçalves [1,2], Rafaela A. Santos [1,2], José Dias [2], António P. Carvalho [1,2], Patricia Díaz-Rosales[3], Aires Oliva-Teles [1,2], Ana Couto [1,2], Cláudia R. Serra [1,2(*)]

[1] *Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal*

[2] *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal*

[3] *Fish Immunology and Pathology Group, Animal Health Research Centre, Valdeolmos-Alalpardo, Madrid, Spain.*

[(*)] *email: cserra@ciimar.up.pt*

Keywords: *Bacillus* spores; antigen-displaying vehicles; oral vaccines; aquaculture.

ABSTRACT

As the global consumption of fish food continues to increase, aquaculture is continuously growing to fulfill this demand. The sustainable growth of aquaculture is threatened by the occurrence of fish diseases that cause massive economic and production losses. Vaccination is the most appropriate method of preventing the negative impact of diseases outbreaks, but most of the vaccines available are injectable, being expensive, labor-intensive and stressful to fish. Aquaculture is in urgent need of novel vaccination strategies, such as immersion and oral vaccination. In this work, we aimed to develop oral vaccines using *Bacillus subtilis* spores - biological structures that resist the conditions of the gastrointestinal tract, can be used as antigen-display systems, and can be incorporated into the fish feed for oral vaccination. We focused on using spores from probiotic *Bacillus* strains, previously isolated from fish, to develop oral vaccines against two major bacterial diseases affecting aquaculture: vibriosis and mycobacteriosis. Genes encoding four immunogenic proteins (VAA and TolC from *Vibrio anguillarum* and Esx-1 and Ag85A from *Mycobacterium marinum*) were fused to the gene encoding the spores' crust protein CotY. The resulting plasmids were used to transform the laboratory *B. subtilis* strain 168 and the probiotic strains FI314, FI330 and FI442, resulting in a successful chromosomal integration. The biocompatibility of the recombinant spores was tested by exposing 3 days-post-fertilization zebrafish larvae to a spore's suspension of 10^8 CFU mL^{-1} with mortality followed for 72h. Survival of all exposed zebrafish larvae indicates that the recombinant spores tested as vaccine candidates are biocompatible with fish larvae, being currently under testing for assessing their potential to protect zebrafish larvae upon infection with *V. anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus* and *M. marinum*, following previously established infection models.

Acknowledgements:
Funding: FEDER, POCI, PT2020, and FCT/MCTES through project PTDC/CVT-CVT/2477/2021, PhD fellowship 2021.07724.BD (GG), CIIMAR Strategic Funding UIDB/04423/2020 and UIDP/04423/2020.

P1.51 - USING AGRO-INDUSTRIAL BYPRODUCTS FOR A MORE SUSTAINABLE PRODUCTION OF NATURAL PIGMENTS

Bruna Basto [1,2 (*)], Nuno Ribeiro da Silva [1,2], José António Teixeira [1,2], Sara Cruz Silvério [1,2]

[1] *CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal.*

[(*)] *email: bb_basto@hotmail.com*

Keywords: Cheese whey; Corn steep liquor; Filamentous Fungi; Immobilization; Natural Pigments.

ABSTRACT

Pigments have a vast record of enhancing product appeal in industries like food, cosmetics, textiles, pharmaceuticals, and tanneries. A shift towards eco-consciousness has fueled the demand for biocompatible, natural pigments, prompting interest in microbial fermentation. Filamentous fungi, particularly *Penicillium* species, stand out as promising pigment producers. Recently, we have demonstrated that a *Penicillium* strain is able to produce different pigment mixtures under different fermentation conditions and culture media [1], [2]. Using agro-industrial byproducts, such as cheese whey and corn steep liquor, as substrates for microbial growth can be a sustainable approach to reduce production costs and byproduct accumulation. Both cheese whey and corn steep liquor are rich in valuable nutrients and were shown to enhance pigment production when used as medium supplements [1]. In this work, we present a comparative study involving three fermentation types (submerged, submerged with biomass immobilization, and solid-state fermentation) and employing two distinct culture media (synthetic medium composed of commercial substrates (A), and an alternative medium only composed of cheese whey and corn steep liquor (B)). Notably, we found that, under submerged fermentation either with free or immobilized biomass, the alternative medium (B) provides similar results in terms of pigment production to the reference synthetic medium (A). These results show that it is possible to obtain a value-added product exclusively using agro- industrial byproducts, which not only decreases the associated production costs but also contributes to the circular economy. Moreover, we describe a more sustainable approach to obtain natural pigments, which can also help to address environmental concerns, ethical issues, and/or consumer demands raised against synthetic pigments.

References:

[1] B. Basto, N. R. da Silva, J. A. Teixeira, and S. C. Silvério, "Production of Natural Pigments by Penicillium brevicompactum Using Agro-Industrial Byproducts," *Fermentation*, vol. 8, no. 10, p. 536, Oct. 2022, doi: 10.3390/FERMENTATION8100536.

[2] C. S. Fonseca *et al.*, "*Penicillium brevicompactum* as a novel source of natural pigments with potential for food applications," *Food Bioprod. Process.*, vol. 132, pp. 188–199, Mar. 2022, doi: 10.1016/j.fbp.2022.01.007.

Acknowledgements:

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

P1.52 - IN-VITRO ASSESSMENT OF BIOCIDES EFFECT ON TWO ACTIVATED-SLUDGE FILAMENTOUS BACTERIAL STRAINS

Vânia Ferreira [1,2], Nicolina Dias [1,2], Manuel Mota [1,2], Olívia Pereira [1,2], Ana Nicolau [1,2(*)]

[1]*CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2]*LABBELS – Associate Laboratory, Braga/Guimarães, Portugal.*

[(*)] *email: protozoa@deb.uminho.pt*

Keywords: wastewater treatment; activated-sludge; filamentous overgrowth; biocides.

ABSTRACT

Wastewater treatment is the world largest biotechnology in the world and stands for the defense of public health on one side and the protection of the natural aquatic ecosystems on the other. Activated-sludge process (AS) is the most widely used technique for wastewater treatment. The AS bacterial communities include floc-forming and non-floc-forming organisms, the latter mostly filamentous bacteria. AS goals often fail due to the overgrowth of filamentous bacteria, in a phenomenon known as filamentous bulking. Filamentous bacteria can also lead to the formation of foams compromising the treatment. The in-vitro effect of three biocides - triclosan, cetyl trimethyl ammonium bromide (CTAB), and glutaraldehyde - on two filamentous bacteria of usual occurrence in activate-sludge, *Gordonia amarae* and *Sphaerotillus natans*, was assessed. The XTT reduction assay showed a dose- dependent effect of all the three compounds on bacterial viability. Differences in the susceptibility to the exposure to each biocide also suggested strain-dependent effects. CTAB was the most toxic compound to G. amarae and the intermediate toxic when considering the assays with S. natans. Regarding the recovery assay (using cytometry), the results showed that both strains had not recovered from it in any of the three cases 48 h after biocide removal. In both strains, filaments were fragmented to single cells and, in the three cases, the ability to produce new filaments was lost at least until 48 h after the biocide removal. Glutaraldehyde was the biocide with the most prolonged toxicity in the case of N amarae, but CTAB showed the more pronounced acute effects on both strains. The differences observed in the toxicity effects corroborate the existence of different mechanisms of action of the three biocides and must be taken in consideration when the control of the overgrowth of a particular strain in activated-sludge is being considered.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology(FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

P1.53 - BIOTECHNOLOGICAL PRODUCTION OF NEW GREEN BIOCIDES FOR APPLICATION IN CULTURAL HERITAGE

Cátia Salvador [1(*)], Patrícia Gatinho [1,2], Inês Caldeira [1], A. Teresa Caldeira [1,3]

[1] *HERCULES Laboratory & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Évora, Portugal*

[2] *Department of Engineer, School of Science and Technology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[3] *Department of Chemistry and Biochemistry, School of Sciences and Technology & City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science University of Évora, Évora, Portugal.*

(*) *e-mail: cscs@uevora.pt*

Keywords: Patrimony Biodeterioration; Killer Toxins; Green Biocides; Antimicrobial Potential; Sustainable Biotechnology.

ABSTRACT

Biodeterioration phenomenon has been considered a high priority issue in the context of Cultural Heritage safeguarding, highlighting the need for innovation in this area. In many cases, synthetic polymers are used in an attempt to control the biodeterioration of heritage, but these have high toxicity, their effect is not long-lasting, and many microorganisms quickly develop resistance to these products [1]. The biotechnological production of ecological biocides was an alternative found to the use of these products, these bioactive molecules synthesized by microorganisms as a defense mechanism, also known as killer toxins, which, after being produced and purified, can be used to control microbiological proliferation in heritage assets. These biocides should offer more effective and sustainable alternatives while being safe for human health and the environment without negative impact on assets [2]. This work was carried out within the scope of the ART3*mis* Project (2022.07303.PTDC) with the aim of producing killer toxins from yeast strains and evaluating their antimicrobial activity against different species of Microorganisms isolated from Cultural Heritage. The antimicrobial spectrum of metabolites produced by selected killer yeasts strains (*Sacharomyces* sp., *Pichia* sp., *Zygoascus* sp., *Torulaspora* sp., and Hanseniaspora sp.) was evaluated in solid medium and liquid medium against bacterial strains (*Methylobacterium extorquens, Gordonia alkanivorans, Microbacterium foliorum* and *Bacillus firmus*), and in solid medium against biodeteriogenic fungi (*Cladosporium* sp., *Penicilium* sp. *Aspergillus* sp. and *Fusarium* sp.). Indeed, there were several encouraging results regarding the development of new ecological biocides that can effectively suppress the biodeteriogenic action of a wide range of microorganisms commonly found in different Cultural Heritage materials.

References:

[1] P. Gatinho, C. Salvador, A.M. Silva, A.T. Caldeira (2023). Prokaryotic Communities from Pristine Cave Environments : Biotechnological Potential with Sustainable Production, *Sustainability*, 15(9), 7471, DOI: 10.3390/su15097471.

[2] C. Salvador, A. Candeias, A.T.Caldeira (2016) Biodeterioração de pinturas de cavalete: Desenvolvimento de novas estratégias de mitigação. *Conservar Património*, *23*, 119–124. DOI: 10.14568/cp2015032.

Acknowledgements:
The authors acknowledge the FCT, Portuguese Foundation for Science and Technology, I P within the scope of the projects UIDB/ 04449 2020 (HERCULES Lab), ART3mis (2022 07303 PTDC), C Salvador individual support (DL 57 2016 /CP 1372 /CT 0019).

P1.54 - PLYLOGENOMICS AS BASELINE FOR TAXONOMY DESCRIPTION: *AMPHIBACTER PEREZI* GEN. NOV., SP. NOV.

Sara Costa [1(*)], Diogo Neves Proença [2], Isabel Lopes [1], Paula Vasconcelos Morais [2]

[1] *CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal*

[2] *Department of Life Sciences, CEMMPRE and ARISE, University of Coimbra, Coimbra, Portugal.*

[(*)] *email: sdcosta@ua.pt*

Keywords: Amphibian skin microbiome; new bacterium species; *Burkholderiales*; genome.

ABSTRACT

The *Burkholderiales* order comprises common bacteria isolated from soil, freshwater, drinking water, and it is frequently reported as one of dominant orders represented on the amphibian skin microbiome studies. In addition, comprises bacteria with high importance on their resistance against pathogens. Bacterial strain SL12-8^T was isolated from the skin microbiota of the common green frog *Pelophylax perezi* from different populations and characterized in a polyphasic approach and describe a new genus and new species. Strain SL12-8 ^{T} stained Gram-negative and formed rod-shaped cells that grew optimally at 25°C and at pH 7.0 – 7.5. The G+C content of the DNA was 66.0 mol% determined by HPLC. The Ubiquinone 8 (UK-8) was the respiratory quinone identified on strain studied, as well as in the most closely related taxon. The major fatty acids were Summed in Feature 3, Summed in Feature 8 and C16:0, representing 84% of the total fatty acids. Phylogenetic analyses based on the 16S rRNA gene sequences placed strain SL12-8 ^{T} within the order *Burkholderiales* in a distinct lineage. The 16S rRNA gene sequence similarities of strain SL12-8 ^{T} to that of, *Piscinibacter aquaticus* DSM 19915 ^{T} (94.52%), *Rubrivivax albus* (94.45%), *Ideonella benzenivorans* (94.43%), *Scleromatobacter humisilvae* (94.43%), *Aquincola rivuli* (94.29%). The draft genome sequence of strain SL12-8 ^{T} comprises 3,115,197 bases. The assembled genome consists of 53 large contigs with more than 500 bp and the genome encodes 2,839 putative coding sequences. The analysis of the available genomes from the closest genera showed 227 core genes that reveals a novel genus-level clade including the strain SL12-8 ^{T}. Analysis of SL12-8 ^{T} genome revealed the betalactone and terpene biosynthetic gene clusters. The phylogenomic, phenotypic and chemotaxonomic data showed that strain SL12-8 ^{T} (= UCCCB 131 ^{T}) represents the type of a novel species and genus, for which we propose the name *Amphibacter perezi* gen. nov., sp. nov.

P1.55 - THE IMPACT OF WILDFIRES ON AMPHIBIAN SKIN MICROBIOME - A CASE STUDY BEFORE AND AFTER HELL

Sara Costa [1(*)], Lorrie Maccario [2], Diogo Neves Proença [3], Søren J Sørensen [2], Isabel Lopes [1], Paula V. Morais [3]

[1] *CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal*

[2] *Department of Biology, Section of Microbiology, University of Copenhagen, Copenhagen, Denmark*

[3] *Department of Life Sciences, CEMMPRE and ARISE, University of Coimbra, Coimbra, Portugal.*

[(*)] *email: sdcosta@ua.pt*

Keywords: amphibian skin microbiome; wildfire; Illumina; 16s.

ABSTRACT

Wildfires have been identified as a significant contributor to diffuse contamination in aquatic ecosystems, namely through the input of toxic compounds such as polycyclic aromatic hydrocarbons and metals that are adsorbed to the produced ashes. The effect of this events should be evaluated because they cause effects on amphibians' development and potentially in their skin-microbiome, as showed in works of with bacteria associated to the skin of Iberian frog (*Rana iberica*) and fire salamander (*Salamandra salamandra*). Moreover, due to climate change, these events could increase its frequency and its severity. Aqueous ashes extracts can also influence the growth of bacteria with antimicrobial activity against amphibian pathogens, and as it is known, any change in the composition of the skin microbiome (dysbiosis) can affect the amphibians' health. The present study aimed at assessing the skin microbiome from adults of the species *Pelophylax perezi*, before and after a wildfire. The study was conducted in a recent burnt coastal area called Lagoa das Braças, in the centre-west region of Portugal, part of Natura 2000 Site of Community Importance. Adult individuals of *P. perezi* frogs were previously sampled before and after wildfire. Then we used Illumina Miseq sequencing to evaluate the genetic diversity and abundance of the samples. The wildfire was evaluated as having moderate severity, and the burned area characterized as a coastline of continuous sand dunes with resinous (mainly maritime pine *Pinus pinaster* Ait. and the invasive *Acacia* spp.) forest and scrubs as major land cover. Results from microbiome analysis showed a decrease in bacterial species richness and α-diversity in the post-fire scenario. Similarity analysis of species composition showed two distinct clusters of before and after wildfires. *Acidobacter* relative abundance is significantly increased in samples before fire, while *Paenibacillus*, *Streptococcus*, Order *Rhizobiales* (unclassified at the genus or family level) are increased after.

P1.56 - NATURAL PRODUCT BIOSYNTHETIC POTENTIAL REFLECTS MACROEVOLUTIONARY DIVERSIFICATION WITHIN A WIDELY DISTRIBUTED BACTERIAL TAXON

Sandra Godinho Silva [1,2], ***Masun Nabhan Homsi*** [3], ***Tina Keller-Costa*** [1,2], ***Ulisses Nunes da Rocha*** [4], ***Rodrigo Costa*** [1,2(*)]

[1] *iBB- Institute for Bioengineering and Biosciences, Bioengineering Department, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*

[2] *Associate Laboratory i4HB, Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*

[3] *Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany*

[4] *Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany.*

(*) *email: rodrigoscosta@tecnico.ulisboa.pt*

Keywords: Polyhydroxyalkanoates; Marine Bacteria; Functional Annotation.

ABSTRACT

Flavobacteriaceae spp. are key players in global biogeochemical cycling and known for their versatile carbohydrate and peptide degradation capacities. Yet knowledge of their secondary metabolic traits as possible adaptive features underlying their broad range occurrence in terrestrial and marine ecosystems is still narrow. Here, we analysed 2,680 genomes to determine whether natural product biosynthesis potential reflects the taxonomic diversity of *Flavobacteriaceae* species. We uncovered 12,493 secondary metabolite biosynthetic gene clusters (SM-BGCs), with 9,330 SM-BGCs detected from 1,923 *Flavobacteriaceae* genomes, and 3,163 SM-BGCs retrieved from 757 genomes of the closely related *Weeksellaceae* family. Noticeably, 88.6% of the observed SM-BGCs were inferred to lead the biosynthesis of likely novel natural products. We found an unanticipated, large diversity of SM-BGCs encoding carotenoid (*n* = 2,225) and flexirubin pigments (*n* = 1,256), the vast majority of which awaiting formal description. A previously unknown phylogenetic signal reflecting natural product biosynthesis diversification within the studied taxa was unveiled, as the distribution of closely related SM-BGCs across genomes usually followed family- and genus-specific patterns. *Aquimarina*, *Kordia* and *Tenacibaculum* spp. possessed large genomes and a wide repertoire of SM-BGCs and peptidases, likely underpinning their broad host range and opportunistic-to-pathogenic behaviour. Using a machine learning approach (Feature Selection), we reveal that marine and non-marine *Flavobacteriaceae* genomes are differentially enriched in CAZymes and peptidases with distinct functionalities and molecular targets. Our findings reveal tightly intertwined taxonomic and natural product biosynthesis diversification in the *Flavobacteriaceae* family. We posit that the carbohydrate, peptide, and secondary metabolism triad synergistically shape the evolution of this keystone bacterial taxon and break new ground for the study of *Flavobacteriaceae* spp. as sources of novel drug leads.

Acknowledgements:

This study was supported by the by the Fundo Azul program of Direção-Geral de Política do Mar (DGPM; Ministry of the Sea, Portugal) through grant FA_05_2017_032. It was further supported by the mobilizing agenda "BLUE BIOECONOMY PACT" (Project Nº. C644915664-00000026, work-package 9), co-funded by Next Generation EU European Fund, under the incentive line "Agendas for Business Innovation" within Component Funding Scheme 5 - Capitalization and Business Innovation of the Portuguese Recovery and Resilience Plan (RRP). Further support was provided by the Portuguese Foundation for Science and Technology (FCT) in the scope of the projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and the project LA/P/0140/2020 of i4HB.

P1.57 - INFLUENCE OF HARVESTING LOCATION AND PERIOD ON BIOACTIVE COMPOUNDS IN PORTUGUESE MACROALGAE: A NUTRITIONAL AND ENVIRONMENTAL PERSPECTIVE

Marta Coelho [1(*)], Débora Borges [2], Isabel Costa [2], Ana M. Gomes [1], Manuela E. Pintado [1(**)]

[1]*CBQF–Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto, Portugal*

[2]*Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, Matosinhos, Portugal.*

(*) email: mcoelho@ucp.pt | mpintado@ucp.pt

Keywords: macroalgae; carotenoids; bioactive compounds; harvesting.

ABSTRACT

In recent times, algae have become the focus of significant attention owing to their exceptional nutritional content, adaptability, and potential to alleviate the environmental impact of conventional food production systems. Algae are rich in proteins, essential fatty acids, vitamins, and minerals, providing a distinctive and nourishing food source. The current study sought to examine the correlation between the harvesting location and period and bioactivity of Portuguese macroalgae. *Codium* spp. and *Osmundea pinnatifida* were collected from various beaches between January and September. After drying, the macroalgae were analyzed for their bioactive components, including fatty acids, phenolic compounds, carotenoids, and chlorophylls. *Codium* spp. exhibited a higher concentration of polyunsaturated fatty acids compared to *Osmundea pinnatifida* (0.093 ± 0.0012 to 0.068 ± 0.0001 mg / 100 g DW, respectively). The fatty acid composition was influenced by both the location and the harvesting period, with seaweeds collected in May displaying a 35% higher total fatty acid content than those harvested in January. Similar patterns were observed for phenolic compounds and carotenoids. *Osmundea pinnatifida* contained three times more phenols than *Codium* spp., primarily anthocyanins, especially cyanidin. Additionally, *Osmundea pinnatifida* exhibited 20% higher antioxidant activity in May compared to January. *Codium* spp. showed higher levels of natural pigments, including zeaxanthin, astaxanthin, and chlorophylls (a and b). The total carotenoid content varied with the harvesting months; for instance, January displayed 184.55 ± 2.92 µg β-carotene eq. / g DW, while July showed 151.24 ± 3.54 µg β-carotene eq. /g DW. The harvesting location also played a role; macroalgae from Agudela Beach exhibited 123.34 ± 13.65 µg β- carotene eq. / g DW, whereas those from Viana Beach showed 49.29 ± 12.06 µg β-carotene eq. / g DW. These findings underscore the significance of considering both the harvesting period and location to ensure macroalgae with enhanced health-promoting properties.

Acknowledgements:
The authors would like to thank to the project Pacto da Bioeconomia Azul - Projeto Nº. C644915664-00000026 financiado pelo Plano de Recuperação e residência Nacional "PRR ". We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.

P1.58 - *BACILLUS* SPP. NATURAL ANTIMICROBIAL COMPOUNDS ARE PROMISING ANTIMICROBIAL AGENTS FOR AQUACULTURE

Rafaela A. Santos [1,2(*)], Mariana Reis [1], Rodrigo Oliveira [2], Gabriela C. Gonçalves [1,2], Russell Jerusik [3], Aires Oliva-Teles [1,2], Cláudia R. Serra [1,2]

[1] *CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, Matosinhos, Portugal*

[2] *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, Porto, Portugal.*

[3]*Epicore Networks Inc., 4 Lina Lane, Eastampton, New Jersey, United States of America.*

[(*)] *email: rafaela.santos@ciimar.up.pt*

Keywords: Natural Antimicrobial Compounds; Fish-pathogens; Zebrafish; Aquaculture.

ABSTRACT

Aquaculture is an indispensable industry to satisfy the world's fish demand. However, bacterial disease outbreaks are responsible for huge economic losses worldwide, remaining a major challenge for sustainable aquaculture expansion. Although antibiotics are an important tool for disease treatment, their damaging effects on the environment and public health have led to increased restrictions on their use in aquaculture. In a post-antibiotic era, it is urgent to find new sustainable strategies to control aquaculture bacterial outbreaks, assuring advanced and integrated health care for humans, animals, and the environment - One Health. Over the last years, about 57% of the approved antimicrobial drugs were based on natural products or their derivates, and microbes are considered the most important source of bioactive compounds. In comparison with other beneficial microorganisms, *Bacillus* spp. are a valuable source of Natural Antimicrobial Compounds (NACs) that may be a promising solution to the challenges faced by modern aquaculture. NACs-producing *Bacillus* spp. as probiotic organisms in aquaculture are well- studied and characterized. However, the application of purified NACs in aquaculture was never attempted. To fulfil this gap, we isolated and characterized several bioactive *Bacillus* spp. Because two of these *Bacillus* isolates have exceptional and broad antimicrobial and immunostimulatory characteristics, we decided to evaluate the potential of using their NACs as disease-preventive molecules for fish. For that, we used zebrafish as model organism and discovered that when larvae were treated with the crude extracellular NACs of the two *Bacillus* spp., their survival increased up to 50% upon challenge with *Edwardsiella tarda*, one important and devastating fish pathogen. Now, we are using different biochemical techniques to purify the bioactive compounds of the two *Bacillus* spp. through a bioactivity-guided approach. Bioactive compounds will then be evaluated *in vivo* for their efficacy in preventing fish bacterial infections.

Acknowledgements:
This work was financed by Fundo Europeu de Desenvolvimento Regional (FEDER) funds through the COMPETE 2020 Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through Fundação para a Ciência e Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project PTDC/CVT-CVT/2477/2021.

P1.59 - PREDICTION OF NEUROTOXIC POTENTIAL OF AMINO ACID-DERIVED DBPS USING MOLECULAR DOCKING

Matheus M. Pereira [1 (*)], **Rui C. Martins** [1]

[1] *University of Coimbra, CIEPQPF – Chemical Engineering Processes and Forest Products Research Center, Department of Chemical Engineering, Faculty of Sciences and Technology, Rua Sílvio Lima, Polo II, Coimbra, Portugal.*

[(*)] *email: matheus@eq.uc.pt*

Keywords: Amino acid-derived DBPs; neurotoxicity; human acetylcholinesterase; molecular docking.

ABSTRACT

Disinfection By-Products (DBPs) are a class of chemical compounds formed as a result of disinfection processes in water treatment, can represent potential health and environmental risks. The most common and extensively studied DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs), are regulated due to their known health concerns. Amino acid-derived DBPs (AA-DBPs) are chemical compounds that are formed by the oxidation and halogenation of amino acids present in natural organic matter in water sources during water disinfection processes. Due to their potential reactivity and structural complexity, amino acid-derived DBPs are of particular interest in water quality research and identification of their potential health risks remain relatively understudied. Therefore, this work aims to investigate the potential neurotoxicity of AA-DBPs. For this, the binding mechanism of several AA-DBPs on human acetylcholinesterase (AChE) were evaluated trough molecular docking. The effect of halogenation degree was evaluated for mono- and di-halogenated AAs. In addition, the mechanism of AA-DBPs binding trough AChE tunnels and binding affinity on enzyme active site, were calculated using Caver Web 1.0. Molecular docking results showed that most of AA-DBPs display higher bind affinity inside the access tunnels of AChE, in comparison with enzyme active site, indicating that AA-DBPs are promising AChE inhibitors, blocking AChE mechanism of breaks down the neurotransmitter acetylcholine. Thus, the identification of AA-DBPs potential neurotoxicity is here presented, highlighting the importance of further evaluation these compounds with experimental assays to obtain their level of toxicity.

Acknowledgements:
The work was developed within the scope of the project H2OforAll financed by the European Union, under the Grant Agreement GA101081953. The authors acknowledge the funding for CIEPQPF supported by the FCT through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020).

P1.60 - EXPLORING BIOACTIVE COMPOUNDS: UNVEILING THE HIDDEN TREASURES OF BACTERIAL ISOLATES FROM ALGARVE COAST UNDERSEA CAVES

Cátia Salvador [1(*)], Patrícia Gatinho [1,2], Sílvia Macedo-Arantes [1], M. Rosário Martins [1,3], A. Teresa Caldeira [1,4]

[1] *HERCULES Laboratory & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Évora, Portugal*

[2] *Department of Engineer, School of Science and Technology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[3] *Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal*

[4] *Department of Chemistry and Biochemistry, School of Sciences and Technology & City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science University of Évora, Évora, Portugal.*

[(*)] *email: cscs@uevora.pt*

Keywords: Marine Caves; Bacteria; Bioactive Compounds; Antioxidant Properties; Anti-tumoral Potential; Sustainable Products.

ABSTRACT

Microorganisms thriving in unique hypogenic environments, such as undersea caves, present a promising frontier for bioactive compounds discovery. These environments constitute ecosystems that provide an unparalleled canvas for the evolution of a wide range of microorganisms, resulting in unexplored biodiversity wealth. Within these cryptic realms, microorganisms have adapted to oligotrophic conditions by weaving complex metabolic networks, thus unlocking an untapped treasure trove of novel bioactive compounds. The quest for undiscovered microorganisms is driven by the significant potential to harness these biocompounds produced through their secondary metabolism, which can exhibit various biological functions, including antioxidant and antitumor activities [1]. Cancer remains among the top leading causes of death worldwide. Given this global challenge, there is an urgent need to discover innovative drugs that are more effective and have fewer side effects. Exploring the bioactive compounds produced by hypogean microorganisms may hold the key to developing groundbreaking pharmaceuticals. This study aims to prospect for new bioactive compounds produced by bacterial cultures, isolated from undersea caves (Sagres, Algarve-Portugal) with an emphasis on assessing their antioxidant and antitumor potential. Antioxidant properties were assessed through DPPH radical scavenging and lipidic peroxidation using the β-carotene linoleate system methods. Antiproliferative potential was evaluated against a breast cancer epithelial cell line MDA-MB-231 [2]. Free-cells culture broths, particularly those produced by isolates of genera *Sulfitobacter*, *Cobetia*, and *Pseudoalteromonas* exhibited high antioxidant activities and robust antiproliferative capacity. Our results are promising in the quest for new bioactive compounds from bacterial isolates found in undersea caves, creating potential for sustainable solutions with broader medicinal applications. This also represents an opportunity to preserve and value Natural, Genetics and Cultural Heritages.

References:
[1] P. Gatinho, C. Salvador, A.M. Silva, A.T. Caldeira (2023). Prokaryotic Communities from Pristine Cave Environments: Biotechnological Potential with Sustainable Production, *Sustainability*, 15(9), 7471, DOI: 10.3390/su15097471.
[2] S. Arantes, …, C. Salvador, A.T. Caldeira , M.R. Martins (2019). Toxicological and pharmacological properties of essential oils of Calamintha nepeta, Origanum virens and Thymus mastichina of Alentejo (Portugal), *Food and Chemical Toxicology*, 133, 110747, DOI: 10.1016/j.fct.2019.110747.

Acknowledgements:
The authors acknowledge the FCT- Portuguese Foundation for Science and Technology-IP within the scope of the projects UIDB/04449 2020 (HERCULES Lab), ART3*mis* (2022 07303 PTDC), C. Salvador individual support (DL57 2016 /CP 1372 /CT 0019).

P1.61 - BIOPOLYMERS PRODUCED BY BACTERIA AS SUSTAINABLE BIOTOOLS FOR METAL REMOVAL

Ana Paula Chung [1(*)], ***Paula V.Morais*** [1]

[1]*University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Coimbra, Portugal.*

[(*)] *email: ana.chung@uc.pt*

Keywords: Biopolymers; metals; biosorption.

ABSTRACT

An increasing interest has been directed to bacterial biopolymers as sustainable biotools to remove metal contaminants from wastewaters. The use of bacterial biopolymers is attractive to the industry since it is aligned with the environmental policies and societal demands focused on the development of greener technologies. The presence of different functional groups, such as amino, carboxyl, hydroxyl, and phosphate, confers to biopolymers excellent metal-binding properties with varying degrees of specificity and affinity. In this work the biopolymers produced by *Ochrobactrum tritici* 5bvl-1 and *Mesorhizobium qingshengii* Jales-19 were investigated as biosorbents to remove iron and nickel ions from aqueous solutions. The metal biosorption assays were performed in 20 ml of aqueous metal solution (1, 2 and 3 mM of Fe^{3+} and Ni^{2+}) and two different concentrations of biopolymer (1mg/ml and 2mg/ml). SEM-EDS was used to analyze the morphological structure and elements. The sugar composition of biopolymers was analyzed by TLC, after hydrolysis of biopolymers with 2M of trifluoroacetic acid at 105 ◦C for 5h. Results: The biosorption efficiency of both biopolymers was always higher at the concentration of 2 mg/ml, ranging between 95.1% and 100% after 60 minutes. The biosorption capacity also increased with the increased initial concentration of Fe^{3+} (1 to 3 mM), reaching the maximum values of 46.27 and 54.24 mg/g, for 5 bvl-1 and Jales-19 biopolymer, respectively. The SEM-EDS images of both biopolymers showed the presence of Fe and Ni on their surfaces, corroborating the results of biosorption assays. The TLC analysis identified glucose as the sugar that composes both biopolymers. Galactose and arabinose are the two other sugars that are part of the biopolymer of Jales-19 strain. In the case of 5bvl-1 strain, rhamnose and fructose or mannose are the other sugars that compose this biopolymer.

Acknowledgements:
This research is financed by PRR - Recovery and Resilience Plan and by the Next Generation EU Funds, following NOTICE N.º 02/C05-i01/2022, Component 5 – Capitalization and Business Innovation - Mobilizing Agendas for Business Innovation under the AM2R project "Mobilizing Agenda for business innovation in the Two Wheels sector" (reference: 7253). This work is also supported by the projects Biorecover from the European Union's Horizon 2020 research and innovation program under grant agreement No 821096 and ERA-MIN 2 REVIVING ERA-MIN-2019_67 from the Fundação para a Ciência e Tecnologia (FCT) and sponsored by the FEDER funds through the program COMPETE – Programa Operacional Factores de Competividade – and by national funds through FCT, under the project UIDB/00285/2020 and LA/P/0112/2020.

P1.62 - UNCOVERING THE CULTURABLE ENDOPHYTIC FUNGI FROM HALIMIONE PORTULACOIDES

Cristina Torcato [1], Sara Alves [1], Liliana Santos [1], Cátia Fidalgo [1], Artur Alves [1(*)]

[1] CESAM – Centro de Estudos do Ambiente e do Mar, Departamento de Biologia, Universidade da Aveiro, Aveiro, Portugal

[(*)] e-mail: artur.alves@ua.pt

Keywords: Halophytes; Endophytic fungi; Taxonomic affiliation.

ABSTRACT

Halimione portulacoides is a halophyte plant that is commonly found in European saltmarshes and estuarine banks, being one of the most abundant and productive species. Endophytic fungi colonize the intercellular and/or intracellular spaces of the host plant tissues. These fungi are relevant to plant physiology due to their plant growth promoting traits and pathogen antagonism (host defence). However, there are no comprehensive surveys of culturable endophytic fungi in *H. portulacoides*. The purpose of this study was to unravel the diversity of culturable endophytic fungi from *H. portulacoides*.

In December 2021, ten healthy plants were collected at Marinha Santiago da Fonte, Aveiro. From each plant, leaves and roots were superficially disinfected using <5% sodium hypochlorite followed by 70% ethanol and water. Plant tissues were plated in two culture media: Potato Dextrose Agar and Minimal Medium both supplemented with 3% salt. Pure cultures were established, and the isolates were subjected to Microsatellite PCR fingerprinting analysis. The ITS region of the ribosomal rRNA gene cluster of isolates representative of the overall diversity in the collection was amplified and sequenced.

The collection established was composed by 425 isolates, 129 of which were considered representative and identified by analysing their ITS sequence. The isolates belong to 18 families and 26 genera. The most abundant genus was *Penicillium* with 29 isolates, followed by *Fusarium* (19), *Stemphylium* (17), *Alternaria* (13), *Neocamarosporium* (11) and *Monosporascus* and *Diaporthe* (6 each).

Data obtained show that a large diversity of fungal genera is present in *Halimione portulacoides*. Previous studies have shown that *Penicillium* spp. displayed a role in enhancing plant resistance to abiotic stress and disease suppression. *In vitro* assays will be performed to assess this potential in our collection. Additionally, other loci will be sequenced to allow a reliable species level identification of the isolates.

Acknowledgements:
The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia to Cristina Torcato (2021.06747.BD), to Cátia Fidalgo (CEECIND/01373/2018) and to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020).
Liliana Santos is funded by national funds (OE), through FCT in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

P1.63 - MICROBIOME RECRUITMENT AND ASSEMBLY DYNAMICS IN *NANNOCHLOROPSIS OCEANICA* CULTIVATIONS

Francisco Nunes [1,2], Constança Bertrand [1,2], Rodrigo Martins [1,2], Pedro Brandão [1,2], Teresa Crespo [1,2], Francisco Nascimento [1,2(*)]

[1] *Instituto de Biologia Experimental e Tecnológica (iBET), Oeiras, Portugal*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB-NOVA), Oeiras, Portugal*

[(*)] *e-mail: francisco.nascimento@ibet.pt*

Keywords: Microalgae; *Nannochloropsis oceânica*; microbiome.

ABSTRACT

The microbiome plays a vital role in regulating and potentiating the development of several eukaryotic hosts, including microalgae. Beneficial symbiotic and mutualistic members of the microbiome can promote microalgae growth and production of bioactive compounds, through a variety of beneficial syntrophic relationships. On the other hand, opportunistic pathogens can greatly impact microalgae leading to cell lysis and disruption, and ultimately cultivation collapses. Despite the known beneficial/detrimental properties of some bacterial members of the microalgae microbiome, not much is understood about the microbiome assembly of relevant microalgae such as *Nannochloropsis oceanica* nor the mechanisms involved in microalgae-bacteria interactions. In our work, a detailed characterization of the *N. oceanica* microbiome assembly dynamics was performed. The *N. oceanica* strain CCAP211/46 was cultivated in natural seawaters (estuary and oceanic) obtained from two distinct locations in Portugal, Sado and Tejo, and microbiome assembly studied throughout cultivation time. Members of the microalgae microbiome were isolated and characterized, leading to the development of a representative microbiome.

The obtained results demonstrated that the presence of the microbiome impacted *Nannochloropsis oceanica* growth. Moreover, different water sources impacted microbiome compositions in the *N. oceanica* cultivations, suggesting selection of the microbiome by the microalgae host. The microalgae microbiome assembly occurred differently throughout the cultivation time, indicating that this is a highly dynamic process. The characterization of the bacterial members of the microbiome revealed several distinct activities (e.g., production of phytohormones, lytic enzymes) between the different members, as well as dissimilar effects in microalgae growth and development. Ultimately, the obtained data brings new relevant insights into the importance of microbiome assembly and function in *N. oceanica* cultivations which may be key to the development of a wide range of industrial applications.

Acknowledgements:
This work was supported by the PHYCO-µ-BIOME project (PTDC/BAABIO/1262/2020) funded by Fundação para a Ciência e a Tecnologia, Portugal (FCT). Francisco Nunes (2022.10633.BD), Constança Bertrand (2023.02799.BD), Rodrigo Martins (2023.02007.BD) and Pedro Brandão (2021.07927.BD) acknowledge PhD fellowships funded by FCT.

P1.64 - TRACE METAL IMPACTS ON N_2O METABOLISM IN DEEP-SEA ISOLATES

Leonor Pizarro [1(*)], ***Laurine Mathé*** [1,2], ***Maria de Fátima Carvalho*** [1,3], ***C. Marisa R. Almeida*** [1,4], ***Catarina Magalhães*** [1,4], ***Miguel Semedo*** [1]

[1] *CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal*

[2] *Faculty of Science and Engineering, University Pierre et Marie Curie, Sorbonne University, Paris, France*

[3] *ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal*

[4] *Department of Chemistry and Biochemistry of the Faculty of Sciences, University of Porto, Porto, Portugal*

[(*)] *e-mail: lpizarro@ciimar.up.pt*

Keywords: Cadmium; Copper; Deep-sea bacteria; Deep-sea mining; Denitrification; Nitrous oxide.

ABSTRACT

Deep-sea bacteria have high environmental importance due to their active role on nutrient cycling, among other activities. Some of these bacteria are responsible for maintaining low levels of nitrous oxide (N_2O), a powerful greenhouse gas, by reducing it to dinitrogen gas (N_2) through the activity of the N_2O reductase enzyme.

The expansion of deep-sea mining may expose these bacteria to metals like cadmium and copper. However, we have limited knowledge about how this affects the N_2O-reducing pathway, especially in deep-sea conditions. Given N_2O's role in global warming and increasing human activities in the deep ocean, closing this knowledge gap is crucial.

The goal of this study is to understand the potential effects of cadmium and copper on the N_2O metabolism of two deep-sea bacterial strains: *Shewanella loihica PV-4* and *Thalassospira indica PB8B*.

Metal exposure experiments were performed in semi-closed bioreactors under aerobic conditions. When bacterial growth reached mid-exponential phase, anaerobic conditions were created for stimulating N_2O metabolism. Headspace gas samples were taken during anoxia to measure N_2O accumulation over time. Cell suspension was also collected for RNA extraction to measure the expression of *nirK* and *nosZ* genes, involved in N_2O production and reduction, respectively.

Our results suggest that cadmium and copper have different impacts on N_2O metabolism and that these impacts may be different between the two tested strains. Since *S. loihica* PV-4 can produce and reduce N_2O, while *T. indica* PB8B is only able to reduce it, the differential metal impacts between strains may cause an imbalance of net N_2O production, leading to ecological consequences. In this research, we also look forward to exploring the role of high hydrostatic pressure in N_2O reduction activities by these strains. We believe our findings will contribute to the global efforts of assessing the potential impacts of deep-sea mining on ecosystem functions.

Acknowledgements:
This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101038095 and is also integrated in the project DEEPRESIST (FCT grant 2022.06475.PTDC). FCT is acknowledged for UIDB/04423/2020 and UIDP/04423/2020 strategic funding.

P1.65 - ADSORPTION OF PERFLUOROOCTANESULFONIC ACID (PFOS) USING A SILICA-BASED AEROGEL

Maria Inês Roque [1(*)], ***Vittorina Rocha*** [1], ***Eva Domingues*** [1], ***Rui Martins*** [1], ***Luísa Durães*** [1]

[1] *University of Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal*

[(*)] *e-mail: inesroque@eq.uc.pt*

Keywords: per- and polyfluoroalkyl substances; perfluorooctanesulfonic acid; adsorption.

ABSTRACT

The per- and polyfluoroalkyl substances (PFAS) are emerging contaminants and can be present in many sources such as fire extinguishing foams, shampoos, food containers, to establish anti-adherence for cookware. However, the use of these substances leads to their long presence in the environment, as they are 'forever chemicals', which can also contribute to the development of carcinogenic effects in humans. Therefore, it is fundamental to remove these pollutants from water. Silica-based materials have been known to be interesting pollutant adsorbents, being a possible solution to remove the PFAS in water. With this work, a silica-based aerogel was developed, by the sol-gel method, with an affinity to adsorb the PFOS from water. The material was characterized by SEM, water contact angle, BET surface area and zeta potential. To assess its possible applicability, the adsorption isotherms and kinetics were studied. Furthermore, the variation of the L/S ratio was also conducted to establish how it would affect the adsorption of the PFOS. The removal of the PFOS in continuous was also tested to verify how the removals would be affected by a constant intake of PFOS.

The adsorption isotherm showed a great affinity between the PFOS and the adsorbent synthesized, reaching removals from 94.5% to 100% for solutions between 5 and 200 mg L^{-1}. The adsorption kinetics showed that the equilibrium was reached within the first two minutes.

Acknowledgements:
Funded by the European Union, under the Grant Agreement GA101081953 attributed to the project H2OforAll - Innovative Integrated Tools and Technologies to Protect and Treat Drinking Water from Disinfection Byproducts (DBPs). This research was co-funded by the European Regional Development Fund (ERDF). The researchers from CIEPQPF were supported the by Portuguese Foundation Science and Technology (FCT), under project UIDB/EQU/00102/2020.

P1.66 - LOW-COST AND ENVIRONMENTALLY FRIENDLY PURIFICATION OF PHENOLIC COMPOUNDS IN BIOLOGICAL FLUIDS

Cariny Polesca [1(*)], Helena Passos [2], Ana Catarina Sousa [3], Nguyen Tue [4], João Coutinho [1], Tatsuya Kunisue [4], Mara Freire [1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[2] Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal
[3] Department of Biology, School of Science and Technology, University of Évora, Évora, Portugal
[4] Center for Marine Environmental Studies (CMES), Ehime University, Ehime Prefecture, Japan

[(*)] e-mail: carinypolesca@ua.pt

Keywords: biological fluids; bisphenol A; hydrophobic eutectic solvents; triclosan.

ABSTRACT

Phenolic compounds, commonly found in industrial, agricultural, and domestic effluents, pose a significant risk to the environment and human health due to their toxicity. However, their normally low levels make them difficult to detect using conventional techniques, besides requiring long times and high process costs. This highlights the necessity to develop effective and low-cost techniques to detect and quantify these contaminants. In this study, a low-cost and environmentally friendly technique for extracting and purifying phenolic compounds was developed using hydrophobic eutectic solvents (HES) in a one-step process. Bisphenol A (BPA) and triclosan (TCS) were used as model compounds for assessing phenolic contaminant levels in water and biological fluids (blood). Systems composed of different HES (at different molar ratios), combined with potassium citrate buffer at different volume ratios, were carefully evaluated and optimized to achieve maximum extraction efficiency. Encouraging results led us to further analyze the samples using liquid chromatography-tandem mass spectrometry (LCMS/MS), a highly sensitive and selective technique, that enables the simulation of real-world scenarios with low concentration levels, which is a challenge in accurate identification and quantification of phenolic compounds. The results obtained in this study demonstrated the remarkable performance of HES in a one-step extraction and purification technique, achieving up to 95 wt % efficiency. Overall, this research proposal is an efficient, time-saving, and cost-effective approach for detecting phenolic compounds in aqueous and biological samples, contributing to a safer and healthier environment.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). C. Polesca acknowledges FCT- Fundação para a Ciência e a Tecnologia for the PhD grant with the reference UI/BD/151282/2021. H. Passos acknowledges FCT, I.P., for the researcher contract CEECIND/00831/2017, under the Scientific Employment Stimulus-Individual Call, 2017. A. C. Sousa acknowledges the support from the Comprehensive Health Research Centre (CHRC) project UIDP/04923/2020. N.M. Tue acknowledges the support from LaMer—Leading Academia in Marine and Environment Pollution Research, a project on Joint Usage/Research Center of the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

P1.67 - THE SOIL BACTERIOME OF THREE TRADITIONAL FRUIT TREES IN ALGARVE

Isabel Matos [1,2], ***José Matos*** [1,2], ***Alcinda Neves*** [1], ***Luís Cabrita*** [3], ***Leonor Faleiro*** [1,2(*)]

[1] *Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, Faro, Portugal*
[2] *Algarve Biomedical Center—Research Institute, Faro, Portugal*
[3] *Direção Regional de Agricultura e Pescas do Algarve, Faro*

[(*)] *e-mail: mfaleiro@ualg.pt*

Keywords: soil microbiota; almond; carob tree; fig tree; drought; bacteriome.

ABSTRACT

The traditional fruit trees, such as almond, carob tree and fig tree in the Algarve have been exploited on a dryland basis and are considered crops with potential to generate good economic profitability. The hydrological drought in the Algarve region tends to worsen with climate changes. The impact of climate change on the soil microbiota can be very significant. In the current study the soil bacteriome associated to the almond variety "Lourencinha" and "Boa Casta", carob tree "Galhosa" and "Mulata do Espargal", the fig tree "Lampa Preta" and "Bebera Preta" were analyzed by a meta-taxonomic approach using *16S rRNA* gene sequencing through the MinION of Oxford Nanopore system.

The results evidence that the phylum *Bacillota* and the phylum *Pseudomonodata* are the two more abundant in all varieties of the three fruit trees, and the third phylum varies between A*cidobacteriota* and *Actinomycetota*. Planctomycetota is the fourth more abundant phylum for some fruit tree varieties, such as almond "Boa Casta", carob tree "Mulata do Espargal", and fig tree "Bebera Preta", in contrast the phylum Myxococcota is the fourth phylum just observed in the fig tree "Lampa Preta". *Neobacillus niancini* is the most abundant species in all fruit tree varieties, but the remaining bacterial species patterns diverge among the varieties, namely *Metabacillus litoralis* is the second most abundant species in the almond variety "Lourencinha" and fig tree "Lampa Preta" and "Bebera Preta", whereas *Nitrospira moscoviensis* is the second most abundant species in the almond "Boa Casta" and carob tree "Mulata do Espargal". Some bacterial species are only observed in a specific fruit tree or variety, such as *Niallia nealsonii* and *Tumebacillus soli* are only present in the soil from the two fig tree varieties. Our results evidence common and specific bacterial members of the soil microbiota in the fruit trees varieties.

Acknowledgements:
The authors are grateful to the financial support through the Projects PRR-C05-i03- I-000010–"Valorização de Recursos Genéticos Tradicionais", PRR-C05-i03-I-000010-LA4.1 "Conservação e fertilidade do solo", PRR-C05-i03-I-000010-LA4.3 "Gestão dos recursos hídricos", PRR-C05-i03-I- 000010-LA4.4 "Variedades adaptadas às alterações climáticas" and PRR-C05-i03-I-000010-LA4.7 "Promoção de ações de divulgação".

P1.68 - THE BIOTECHNOLOGICAL POTENTIAL OF MARINE *PENICILLIUM* SPECIES FROM THE PORTUGUESE COAST

Maria Gouveia [1], ***Alberto Abreu*** [1], ***Carina Félix*** [2], ***Artur Alves*** [1], ***Ana Cristina Esteves*** [1(*)]

[1] *CESAM and Department of Biology, Campus Universitário de Santiago, Aveiro, Portugal*
[2] *MARE-Marine and Environmental Sciences Centre & ARNET—Aquatic Research Network Associated Laboratory, ESTM, Polytechnic of Leiria, Peniche, Portugal*

[(*)] *e-mail: acesteves@ua.pt*

Keywords: *Penicillium* spp.; antifungal activity; antimicrobial activity; *biotechnological potential.*

ABSTRACT

Marine fungi have an import role in marine and estuarine ecosystems. It is known that marine fungi are a source of compounds with a potential biotechnological interest and applicability in several industries. In Portugal, in the last few years there has been increasing interest in marine fungi, with new species being identified, but the potential that this group represents is still largely unknown. We characterised the biotechnological potential of 18 *Penicillium* isolates from Portugal coast and the estuary of Ria de Aveiro regarding enzymatic, antibacterial, and antifungal activities. We also assessed the effect of salt on these activities.

At least one *Penicillium* isolate was able to inhibit growth of several plant pathogenic fungi (*Lasiodiplodia hormozganensis, L. theobromae, Diplodia corticola, Botrytis cinerea, Alternaria infectoria, Diaporthe eres, Fusarium oxysporum* and *Glomerella cingulata*), with most of them being able to inhibit more than 50% of the fungi assayed. *Botrytis cinerea* was the test fungus with the highest count of isolates (13) able to inhibit its growth. Most *Penicillium* isolates were able to inhibit the growth of the gram-positive bacterium *Kocuria rhizophila*, and one isolate was able to inhibit gram-negative bacterium *Aeromonas hydrophila.* Around 72% of the isolates secrete at least half of the extracellular enzymes studied with industrial application, specifically cellulases and xylanases, both under saline and non-saline conditions. In the presence of salt, only 3 of the 8 enzymes studied were produced by more than 50% of the collection in study: cellulases, xylanases and caseinases. Among those, isolates showed a higher enzymatic index for cellulases and xylanases when comparing with non-saline conditions.

This study reinforced the importance of studying marine fungi and their potential applications in industries such as pharmaceutical, food and agriculture, with this *Penicillium* collection being able to inhibit important plant pathogens like *D. eres*, *B. cinerea* and *L. theobromae.*

Acknowledgements:
The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020)

P1.69 - MICROALGAE ALLOW REDUCING AERATION REQUIREMENTS AND IMPROVE THE EFFICIENCY OF GRANULAR SLUDGE TREATING COASTAL AQUACULTURE STREAMS AIMED AT RECIRCULATION

Ana T. Oliveira [1(*)], ***Paula M. L. Castro*** [1], ***Catarina L. Amorim*** [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[(*)] *e-mail: atoliveira@ucp.pt*

Keywords: microalgae-bacterial granular sludge; bioremediation; coastal aquaculture effluents; reduced aeration; recirculation.

ABSTRACT

Water pollution has become a global issue of increasing environmental concern. In the journey towards the energy and carbon neutrality of wastewater treatment processes, an increasing interest has been placed in microalgal-bacterial granular sludge (MBGS) systems. The symbiotic relationship between microalgae and bacteria in granules in terms of gas exchange could lead to energy savings and greenhouse gases emission reduction.

This study aimed to ascertain on the minimum dissolved oxygen content needed to efficiently remove pollutants and allow for water recycling in coastal aquaculture facilities. For that, a photo-sequencing batch reactor was inoculated with microalgae-bacterial granular sludge and operated under a stepwise decrease of the airflow rate from 3.0 to 1.5 L min^{-1} for 134 days. The removal efficiency of chemical oxygen demand (COD) was kept at high level (47 to 77%). Complete ammonium removal was achieved throughout operation, independently of the applied airflow rate whilst nitrite and nitrate removal improved at lower airflow rates (< 2.0 L min^{-1}). In fact, the mass balance on nitrogen species further revealed that the overall nitrogen removal improved indicating that water of quality for recirculation can be obtained at lower airflow rates (<2.0 L min^{-1}). Although the reduction of the airflow rate till ca. 1.5 L min^{-1} benefited the reactor performance, outgrowth of filamentous microorganisms started to occur, compromising the quick and efficient separation of the biomass from treated water.

The MBGS system operated under extremely low airflow rates represents a promising solution in recirculation aquaculture systems, rendering water with chemical quality that complied with marine fish toxicity limits, whilst potentially reducing the aquaculture farm operational costs. Nevertheless, an airflow rate threshold should not be surpassed to prevent the outgrowth of filamentous microbes.

Acknowledgements:
This work was financed by Norte 2020 through Project 3BOOST (POCI-01-0246- FEDER-181302). Authors thank the CBQF scientific collaboration under the FCT project UIDB/50016/2020.

P1.70 - MACROMOLECULAR CHARACTERIZATION OF DIVERSE MICROALGAE

Ana J. Alves [1], Decha Kumla [1], Alcina M.M.B. Morais [1], Rui M.S.C. Morais [1(*)]

[1] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

(*) e-mail: rcmorais@ucp.pt

Keywords: *Porphyridium; Phaeodactylum; Chlorella e Arthrospira; Tisochrysis; Tetraselmis; Odontella; Nannochlropsis; Dunaliella* proteins; lipids; fibre; ashes; water.

ABSTRACT

Microalgae are a natural resource with a unique nutritional profile of beneficial interest to humans, providing an opportunity for the development of innovative products of high value in nutraceutical, pharmaceutical and cosmetic levels. Microalgae may be processed using different methodologies and presented in the form of pastes or powder, depending on their application.

The objective of the present study was to make a comparison among the composition of several microalgae — *Arthrospira sp., Chlorella sp., Dunaliella sp., Nannochlropsis sp., Odontella sp., Phaeodactylum sp., Porphyridium sp., Tetraselmis sp, Tisochrysis sp.* — in terms of proteins, carbohydrates, fibre, lipids, ashes and water contents.

The methods used were Kjeldahl for protein, AOAC 991.43 and AOAC 985.29 for fibre, Method M1, Method F1A and Method A1 [1], for water, lipids and ashes, respectively.

The proteins were the main compounds in 20 to 60 % DW in the different microalgae. *Arthrospira sp., Chlorella sp., Phaeodactylum sp., Odontella sp.* and *Tisochrysis sp.* presented the highest protein contents, from 40 to almost 70 % AFDW. The microalgae with highest lipid content were *Nannochlropsis sp.* (40 % AFDW) and *Tisochrysis sp.* (almost 20 % AFDW).

The present study allows to conclude that the studied microalgae are a high potential source of proteins, especially *Arthrospira sp.*, but also lipids, particularly *Nannochlropsis sp.*

References:
[1] Leatherhead Food RA - Analytical Methods Manual 3rd Ed. England (1987).

Acknowledgements:
This work was supported by project EXTRATOTECA- COMPETE2020 POCI-01- 0247-FEDER-033784 and National Funds from FCT - Fundação para a Ciência e a Tecnologia through Project UID/Multi/50016/2019.

P1.71 - UNLOCKING THE BIOTECHNOLOGICAL POTENTIAL OF AN IRIDESCENT MARINE BACTERIA ASSOCIATED WITH MICROALGAE – *CELLULOPHAGA LYTICA* NFXS1

Constança D. F. Bertrand [1], ***Rodrigo Martins*** [1], ***Francisco Quintas-Nunes*** [1,2], ***Pedro Reynolds-Brandão*** [1,3], ***Maria Teresa G. Crespo*** [1,2], ***Francisco X. Nascimento*** [1(*)]

[1] *iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal.*
[2] *ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.*
[3] *LAQV-REQUIMTE, Chemistry Dept., FCT, Universidade Nova de Lisboa, Caparica, Portugal*

[(*)] *e-mail: francisco.nascimento@ibet.pt*

Keywords: bacteria; marine; microalgae; secondary metabolites; biotechnology.

ABSTRACT

Bacteroidetes play pivotal roles in marine ecosystems, participating in nutrient cycling processes and even conducting algal bloom modulation. Despite their promising potential for exploration towards biotechnological applications, the diversity and unique metabolites of Bacteroidetes remain largely unexplored. This study presents a broad characterization of *Cellulophaga lytica* NFXS1, a marine Bacteroidetes strain isolated from a coastal seawater in Setúbal, Portugal. The characterization encompassed whole genome analysis, unveiling multiple genes related to the degradation of various substrates (e.g., cellulose, agarose, alginate, and carrageenan) and to the production of carotenoids. The strain exhibited the capability to: 1) grow on several carbon sources; 2) produce phytohormones (e.g., IAA); and 3) promote the growth of commercially interesting marine microalgae. Overall, these findings expose the marine biotechnological potential of *Cellulophaga lytica* NFXS1, from the production of high-value secondary metabolites like carotenoids to its ability to act as a microalgae growth-promoting bacterium.

Acknowledgements:
This work was supported by the PHYCO-μ-BIOME project (PTDC/BAABIO/1262/2020) funded by Fundação para a Ciência e a Tecnologia, Portugal (FCT) and the MULTI-STR3AM project funded by the Bio-Based Industries Joint Undertaking (JU) under grant agreement No. 511887227—MULTI-STR3AM. The JU receives support from the European Union's Horizon 2020 research and innovation 512 programme and the Bio-Based Industries Consortium.

P1.72 - PATHOGENIC, PHYLOGENETIC, AND ANTIBIOTIC-RESISTANCE PROFILE OF *ESCHERICHIA COLI* IN AQUATIC ENVIRONMENTS

Lúcia Gomes [1,2*], ***Ana Machado*** [1,2], ***Adriano A. Bordalo*** [1,2]

[1] *ICBAS—Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal*
[2] *CIIMAR – Interdisciplinar Center of Marine and Environmental Research, University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal*

[(*)] *e-mail: luciaazevedogomes@outlook.com*

Keywords: *Escherichia coli;* phylogenetic group; pathotypes; antimicrobial resistance; recreational water; One Health

ABSTRACT

Escherichia coli is a commensal bacterium that inhabits the lower intestine tract of humans and other warm-blooded animals, and thus routinely used as a microbiological indicator of fecal contamination in the aquatic environment. Several *E. coli* strains have the ability to be pathogenic for humans and animals, causing gastrointestinal and extraintestinal infections, and thus being of public health concern. The aim of this study was to assess the *E. coli* pathogenic, phylogenetic, and antibiotic-resistance profile from aquatic environments. Surface water samples were collected from estuarine and coastal beaches, as well as from treated wastewater. Detection and isolation were performed using selective and differential culture media; and presumptive isolates were confirmed by PCR approach. The *E. coli* isolates (n = 272) were submitted to an extensive virulence gene screening to determine the dispersion of virulence-associated genes and phylogenetic group association. The results showed that 35% (96/272) of the isolates were diarrheagenic *E. coli* (DEC). A higher frequency of subgroups linked to non-human mammals and birds origin (B1 and D1) was detected, compared to the predominantly human originated ($B2_2$ and $B2_3$). The *E. coli* isolates susceptibility was evaluated against 22 antibiotics belonging to 9 classes, using the Kirby-Bauer disc diffusion method. The resistance patterns exhibited a wide variability with 72% (195/272) of the isolates showing resistance at least 3 different antibiotic classes (multidrug resistance). Among DEC isolates 75% (72/96) exhibited multidrug resistance. The isolates belonging to phylogenetic subgroup A1 (16/272) and B1 (17/272) showed highest virulence and multidrug resistance rates. The outcomes highlights the circulation of pathogenic and antibiotic-resistant *E. coli* in recreational waters. This study emphasizes the importance of additional monitoring parameters to improve public health risk management in order to guarantee the safety of recreational water users.

Acknowledgements:
This research was funded by the ATLANTIDA project (NORTE-01-0145-FEDER-000040), supported by the Northern Portugal Regional Operational Program (NORTE2020), under the Portugal 2020 Partnership Agreement and by the European Regional Development Fund (FEDER). This work was also partially supported by Project Forbath (E! 115737) under the EUREKA EUROSTARS EU Partnership Program for Innovative SMEs, and by ANI through national funds. CIIMAR is supported by FCT/UIDB/04423/2020 and UIDP/04423/2020.

P1.73 - WOOL DYEING PERFORMANCE USING CHLOROPHYLL EXTRACTED FROM *CHLORELLA VULGARIS NIVA CHL-108* MICROALGAE

Alexandre M.S. Jorge [1(*)], ***Pedro R. M. Pedroso*** [1], ***Jorge F.B. Pereira*** [1]

[1] *University of Coimbra, CIEPQPF, FCTUC, Department of Chemical Engineering, Coimbra, Portugal*

[(*)] *e-mail: alexandrej@eq.uc.pt*

Keywords: Chlorophyll; Textile; Dyeing; Wool; Aqueous two-phase systems; Alcohol/salt; Liquid-liquid extraction.

ABSTRACT

Microalgae are promising and sustainable sources for obtaining different valuable compounds such as chlorophyll, a pigment with applications in fields such as, food, feed, and cosmeceutical. Considering its interesting green colour, other industrial applications, such as textile dyeing, should be explored, in particular, aiming to reduce the dependence of non-sustainable synthetic dyes. However, a significant challenge in their industrial utilization lies on finding cost-effective and eco-friendly operations for their recovery, which are still highly dependent on using large quantities of solvents in solid-liquid and liquid-liquid extractions, resulting in high operational costs and additional environmental concerns. To overcome these issues, this work focused on the extraction of chlorophyll from *Chlorella vulgaris NIVA CHL-108* using alcohol/salt aqueous biphasic systems (ABS). The results showed high recoveries of chlorophyll using this type of systems. Subsequently, aiming to demonstrate the industrial applicability in textile dyeing processes, the chlorophyll-concentrated phase was used in wool dyeing, with and without mordants. The process circularity was demonstrated, by recycling the ABS phase-forming compounds, and the re-extraction and reuse of the unfixed chlorophyll from the previous dyeing step. Alcohol/salt ABS-based platforms have proven to be simple, circular and cost-efficient solutions for the extraction of chlorophyll, enabling the use of this biomolecule in textile wool dyeing.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. The authors would like to thank *Algaementum* for providing the microalgae used in this work. J.F.B. Pereira and A. M. S. Jorge acknowledge FCT for funding the project DRI/India/0044/2020.

P1.74 - FROM LAB TO SEEDS TO SEEDLINGS: ECO-FRIENDLY *PINUS RADIATA* PRODUCTION USING PLANT GROWTH PROMOTING BACTERIAL CONSORTIA

Frederico Leitão [1,2(*)], ***Marta Alves*** [2], ***Glória Pinto*** [2], ***Isabel Henriques*** [1]

[1] *Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal*

[2] *Biology Department, Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal*

(*) *e-mail: f.leitao@ua.pt*

Keywords: plant-growth promoting bacteria; Pine pitch canker; plant physiology.

ABSTRACT

Plant microbiome derived bacteria can exhibit plant-growth promoting (PGP) traits, having an important role in plant defense and growth. Pine pitch canker, caused by *Fusarium circinatum*, affects *Pinus radiata* severely, while *Pinus pinea* is resistant.

This work's main goal was to isolate PGP bacteria from *P. pinea* and test them in *P. radiata seeds* to evaluate plant development. Initially, pine roots were sampled from five adult trees of *P. pinea*, in triplicate. Root was processed to obtain the bacterial fraction. Subsequent plating in TSA and R2A for 7 days (28ºC). The 16S rRNA gene of resulting isolates (n=68) was partially sequenced and phylogenetically affiliated using BLAST and EZTaxon Tool. The most abundant genera were *Paenibacillus*, *Bacillus, Streptomyces, Paraburkholderia* and *Rhizobium*. The obtained isolates tested positive for the following PGP traits: phosphate solubilization (15%); IAA production (94%); siderophores (36%); ACC deaminase (13%); oxidative stress tolerance (18%). Two isolates displayed *in vitro* inhibition of *Fusarium circinatum* in antagonism essays.

For *in vivo* trials, ten bacterial consortia were made combining stains with different PGP traits. *Pinus radiata* seeds were soaked for 2 hours in bacterial consortia solutions and sown in peat:vermiculite (1:1) soil mixture. After 30 days of germination, seedlings' height, biomass and biochemical parameters (pigments, sugars, starch, free amino acids, phenolics, flavonoids, malondialdehyde) were assessed. Two consortia increased germination up to 20%, with one of them displaying significantly more adventitious roots, higher starch and free amino acid content.

Our work showed that, in controlled conditions, PGP bacteria from a resistant pine species can positively influence growth and germination of a susceptible pine species, showing their potential as an eco- friendly tool to improve pine production. The obtained consortia should be tested in further full-scale studies in nurseries and against *F. circinatum* infection *in planta*.

Acknowledgements:
This study was supported by CESAM (UIDB/50017/2020 + LA/P/0094/2020) and CFE(UIDB/04004/2020) and Associate Terra Lab (TERRA: LA/P/0092/2020). Furthermore, FCT also funded Frederico Leitão's grant (2021.06400.BD) and Marta Alves (2022.07790.CEECIND).

P1.75 - HETEROTROPHIC CULTIVATION AND MONITORIZATION STRATEGIES TO BOOST BIOPROCESSES BASED ON THE PORTUGUESE GREEN MICROALGAE *CHLORELLA* SP. NFX-ARG AND *DYDIMOGENES* SP. NFX-PL1

João Batista [1,2(*)], ***Pedro Brandão*** [3,2], ***Francisco Nascimento*** [2]

[1] *IST- Instituto Superior Tecnico, Universidade de Lisboa, Lisboa, Portugal*
[2] *IBET- Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
[3] *FCT NOVA- NOVA School of Science and Technology, Caparica, Portugal*

[(*)] *e-mail: joao.a.p.batista@tecnico.ulisboa.pt*

Keywords: Microalgae; Heterotrophic; *Chlorella*; *Dydimogenes; 2D fluorescence; Machine Learning.*

ABSTRACT

Microalgae have gained prominence as versatile and sustainable bioproduction platforms, yielding various compounds such as proteins, essential fatty acids, carotenoids, and other bioactive compounds that are particularly appealing in the food, pharmaceuticals, and cosmetic industries. Despite the known properties of microalgae, their ability to synthesize valuable compounds is often negatively impacted under uncontrolled autotrophic cultivation conditions. One way to overcome this problem is to develop robust industrial microalgae-based bioprocesses, especially those conducted under heterotrophic conditions which are tightly controlled and monitored, leading to the consistent production of relevant bioproducts.

In this work we aim to i) study and improve the heterotrophic cultivation of two novel microalgae isolated in Portugal, *Chlorella* sp. NFX-ARG and *Dydimogenes* sp. NFX-PL1 and assess their ability to synthesize several relevant compounds; and ii) develop novel 2D fluorescence spectroscopy-based models using machine learning (ML) to monitor several microalgae cultivation parameters.

The obtained results showed the different growth and cellular traits of the two microalgae even when exposed to similar cultivation conditions. The modulation of growth media formulations allowed distinct cultivation dynamics and the accumulation of valuable compounds. Moreover, nutrient consumption analysis also showed different glucose, nitrate and phosphate consumption rates further indicating that specific and tailored media formulations with adjusted C/N/P balances should be developed for each microalgae species to achieve increased growth performances and bioproduct accumulation. 2D fluorescence spectroscopy analysis allowed the monitoring of the cultivation dynamics, including several cultivation parameters. ML models to predict parameters such as cell concentration, protein, lipids, and carotenoid accumulation; and glucose and nitrate consumption were developed.

Ultimately, the findings obtained in this work presented valuable insights into the biotechnological potential of *Chlorella* sp. NFX-ARG and *Dydimogenes* sp. NFX-PL1 as heterotrophic bioproduction platforms, as well as relevant impacts in the monitoring and future microalgae bioprocess optimization using ML.

Acknowledgements:
This work was supported by the PHYCO-μ-BIOME project (PTDC/BAABIO/1262/2020) funded by Fundação para a Ciência e a Tecnologia, Portugal (FCT) and the MULTI-STR3AM project funded by the Bio-Based Industries Joint Undertaking (JU) under grant agreement No. 511887227— MULTI-STR3AM.

P1.76 - PROSPECTION OF BIOACTIVE COMPOUNDS PRODUCED BY BACTERIAL ISOLATES FROM CAVES: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES.

Patrícia Gatinho [1,2], Cátia Salvador [2], Silvia Macedo Arantes [2], M. Rosário Martins 2,3, Ana Z. Miller [2,5], Amélia M. Silva [4], A. Teresa Caldeira [2,6(*)]

[1] Department of Engineer, School of Science and Technology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[2] HERCULES Laboratory & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Évora, Évora, Portugal
[3] Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, Évora, Portugal
[4] Center for Research and Technology of Agro-Environmental and Biological Sciences & Department of Biology and Environment, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[5] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain
[6] Department of Chemistry and Biochemistry, School of Sciences and Technology & City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science University of Évora, Évora, Portugal

(*) e-mail: atc@uevora.pt

Keywords: Antibacterial; Antioxidant; Bacteria; Biotechnology; Caves; Sustainability.

ABSTRACT

Caves, natural geological formations resulting from rock cavities with adverse abiotic conditions, are considered extreme and inhospitable habitats. This further emphasizes the remarkable adaptability of microorganisms that manage to inhabit them. These unique environmental characteristics enable microorganisms to develop specific metabolisms and produce new bioactive compounds with potential activities, including antimicrobial, antifungal, antiviral, and anticancer properties [1].

This research aims to evaluate the antioxidant and antibacterial activity against Gram-negative and Gram-positive of diluted lyophilised extracts produced by strains isolated from pristine environments such as 3 caves on Selvagem Grande Island (Madeira archipelago, Portugal), 2 caves on Lanzarote Island (Canary archipelago, Spain) and the Paleolithic Escoural Cave (Montemor-o-Novo, Portugal [2]).

The results obtained suggest that the selected bacterial isolates produce biologically active compounds that have the potential to serve as viable alternatives to conventional antibiotics or as antioxidants. These findings have wide-ranging implications for health and well-being, covering areas such as nutrition, pharmacology, cosmetics, and even the culinary sector.

References:

[1] P. Gatinho, C. Salvador, A.M. Silva, A.T. Caldeira, Prokaryotic Communities from Pristine Cave Environments : Biotechnological Potential with Sustainable Production, (2023) 1–22. https://doi.org/10.3390/su15097471.
[2] A.T. Caldeira, N. Schiavon, G. Mauran, C. Salvador, T. Rosado, J. Mirão, A. Candeias, On the

biodiversity and biodeteriogenic activity of microbial communities present in the hypogenic environment of the Escoural Cave, Alentejo, Portugal, Coatings. 11 (2021) 1–17. https://doi.org/10.3390/coatings11020209.

Acknowledgements:
The authors acknowledge the FCT, Portuguese Foundation for Science and Technology, I P within the scope of the projects UIDB/ 04449 2020 (HERCULES Lab), MICROCENO (PTDC/CTA AMB/ 0608 2020) ART3mis (2022 07303 PTDC), C Salvador (DL 57 2016 /CP 1372 /CT 0019) to individual support, and the City University of Macau endowment to the Sustainable Heritage Chair Sino Portugal Joint Laboratory of Cultural Heritage Conservation Science supported by the Belt and Road Initiative.

P1.77 - HARNESSING THE QUEST FOR ECO-FRIENDLY ALTERNATIVES TO CHEMICAL SURFACTANTS BY EXPLORING EXTREME SALINITY ENVIRONMENTS

Cátia Santos-Pereira [1,2(*)], ***Joana S. Gomes*** [1], ***Joana Sousa*** [1], ***Marta F. Simões*** [3], ***Ricardo Franco- Duarte*** [4,5], ***Sara C. Silvério*** [1,2], ***André Antunes*** [3], ***Lígia R. Rodrigues*** [1,2]

[1] *CEB - Centre of Biological Engineering, Universidade do Minho, Braga, Portugal*
[2] *LABBELS - Associate Laboratory, Braga/Guimarães, Portugal*
[3] *SKL Planets - State Key Laboratory of Lunar and Planetary Sciences, MUST, Macau SAR, China*
[4] *CBMA - Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal*
[5] *IB-S - Institute of Science and Innovation for Bio Sustainability, University of Minho, Braga, Portugal*

(*) *e-mail: catiapereira@ceb.uminho.pt*

Keywords: biosurfactants; hypersaline; metagenomics.

ABSTRACT

Surfactants are tensioactive chemical compounds extensively used worldwide in a myriad of industrial sectors, being an essential part of our everyday lives. They are present in numerous products including cosmetics, detergents, fabric softeners, toothpaste, paints, among many others, and millions of tonnes of surfactants are manufactured every year. Most commercially available surfactants are non-renewable petroleum-based compounds whose extensive use may lead to profound environmental impact. The increasing environmental awareness has prompted the search for new environmentally friendly alternatives, including the so-called biosurfactants, which are surfactants produced by microorganisms that are sustainable alternatives to their chemical counterparts. Hypersaline environments are an attractive source of microbial communities that, due to their adaptation to extreme abiotic conditions, produce special secondary metabolites constituting hotspots for the discovery of new biosurfactants.
Sampling campaigns were conducted at strategic hypersaline locations holding distinct features namely Peña Hueca lagoon (hypersaline sulphated lagoon, Spain), and salinas of Pedra de Lume (salinas in an extinct volcan crater, Cape Verde), of Aveiro (solar coastal salina, Portugal) and Rio Maior (terrestrial inland salina, Portugal). Culture-dependent and metagenomic approaches were carried out to unveil the microbial diversity and identify the most promising biosurfactant-producing organisms.
Physicochemical characterization of samples showed an interesting variability in terms of salinity, pH and ionic content. Sequence-based metagenomics revealed that the isolated metagenomes are enriched in genes involved in biosurfactant production. Culture-dependent techniques allowed the identification of halophilic microbes with remarkable surfactant-like properties. Among them, a particular isolate was found to simultaneously produce a biosurfactant and a bioemulsifier, which was characterized in detail.
The bioprospection of hypersaline locations of the Iberian Peninsula and Cape Verde allowed the identification of halophilic biosurfactant producers, which can have promising industrial applications and contribute to the quest for more sustainable alternatives to chemical surfactants.

Acknowledgements:

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and the project "Bi3S - PTDC/BII-BIO/5554/2020". Joana S. Gomes (2022.12313.BD) and Joana Sousa (2022.11695.BD) acknowledge their PhD fellowships funded by FCT. Ricardo Franco-Duarte (2022.00340.CEECIND) acknowledges his CEEC funded by FCT.

P1.78 - POTENTIAL EFFECT OF HALOTOLERANT PLANT GROWTH PROMOTING BACTERIA IN PREVENTING OLIVE KNOT DISEASE UNDER SALINE STRESS

Jyoti Chhetri [1], Lara Soares [1], Maria João Ferreira [1 (*)], Pedro Monteiro [1], I. Natalia Sierra-García [1], João Tedim [2], Gloria Pinto [1], Ângela Cunha [1]

[1] CESAM e Departamento de Biologia. Universidade de Aveiro, Portugal
[2] CICECO - Departamento de Engenharia de Materiais e Cerâmica, Universidade de Aveiro, Portugal

(*) e-mail: mjoaovf@ua.pt

Keywords: *Bacillus siamensis*; Infection; *Olea europaea*; *Pseudomonas oryzihabitans* *Pseudomonas savastanoi.*

ABSTRACT

Olive trees (*Olea europaea L.*) are resilient to drought and can moderately tolerate salt. However, modern super-intensive farming practices, and restriction irrigation, can increase their susceptibility to saline stress and disease. This study sought to test the hypothesis that halotolerant plant-growth-promoting bacteria (PGPB) can attenuate the vulnerability of salt-stressed olive trees to the knot disease caused by the bacterium Pseudomonas savastanoi.

We used a fully-factorial experimental design to examine the combined effects of:
(a) Salinity: 0 (control) or 150 mM NaCl.
(b) Inoculation with plant-growth-promoting bacteria (PGPB): *Bacillus siamensis* RN42, *Pseudomonas oryzihabitans* RL18, a combination of both, or none of the inoculants (control).
(c) Infection: experimental infection with *Pseudomonas savastanoi* or no infection (control).
The study was conducted over a 9-month period using young rooted trees in a lab setting. To assess the incidence of disease, the number of infected plants in each sub-set and the average number of knots on each plant, were determined.

Higher salinity made non-infected plants more susceptible disease. This indicates that saline-stressed plants are more vulnerable to low levels of the pathogen. In these conditions, plants inoculated with RL18 showed lower incidence of disease in comparison with plants inoculated with RN42 or both PGPB. Under heavy pathogen exposure, like that imposed by experimental infection, neither salinity nor PGPB inoculation had significant effect on the incidence of the disease.

The results suggest that *Pseudomonas oryzihabitans* RL18 can boost the plant's immune responses, but this protection is overwhelmed by high pathogen doses, making it best suited as a preventive measure.

Acknowledgements:
This work was supported by project Halius (PTDC/BIA-MIC/3157/2020) financed by Fundação para a Ciência e a Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020) and CICECO (UIDB/50011/2020, UIDP/50011/2020 and LA/P/0006/2020). We also acknowledge FCT/FSE for the financial support to Maria João Ferreira through a PhD grant (PD/BD/150363/2019).

P1.79 - DEVELOPMENT OF A COST-EFFECTIVE MEDIA FOR BIOSURFACTANTS PRODUCTION BY *PSEUDOMONAS AERUGINOSA*

Adelaide Braga [1,2], ***Beatriz B. Cardoso*** [1], ***Margarida Fernandes*** [3], ***Miguel Gama*** [1,2], ***João Rainha*** [1], ***Carla Silva*** [3], ***Lígia R. Rodrigues*** [1,2]

[1] *CEB – Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[3] *CITEVE - Portuguese Technological Centre for the Textile and Clothing Industries, Vila Nova de Famalicão, Portugal*

(*) *e-mail: abraga@deb.uminho.pt*

Keywords: Biosurfactantes; Pseudomonas aeruginosa #112; Rice husk; Vine pruning; waste cooking oils.

ABSTRACT

In the last years, the textile industry has shown a growing interest in biosurfactants due to their biocompatibility , biodegradability , and versatility at various pH and temperature ranges. These compounds have found applications as softeners, wetting agents, lubricants, foam stabilizers, and even in the scouring of wool. This study aims to develop an economically efficient medium for biosurfactant production by *Pseudomonas aeruginosa* #112. Firstly, waste cooking oils after treatment (WCOT), a residue rich in lipids, was evaluated as an inducer of biosurfactants production. Different concentrations of these substrates (1, 2.5, 5, and 10 % w/v) were tested, and glucose was used as a carbon source. In the experiments with 1 % of WCOT it was observed a significant ($p \leq 0.05$) reduction in the surface tension from 48.4 mN/m to 34.8 mN/m, suggesting the biosurfactant production. Furthermore, rice husk (RH) and vine pruning (VP) residues were identified as alternative carbon sources for biosurfactants production, when combined with WCOT . Both residues are rich in cellulose, which can be broken down into free glucose. An enzymatic pre-treatment that combines xylanase and cellulase was used to hydrolyze residues and release free glucose . The obtained results demonstrate that the combination of 1 % OUAT with hydrolyzed RH or VP resulted in a substantial (53 %) reduction in surface tension. At the end of the fermentation, 1.65 g/L and 0.26 g/L of biosurfactant were recovered for the experiments with hydrolyzed PV and RH, respectively. Additionally, the critical micelle dilution results demonstrate that the two tested media allow biosurfactant production and effective reduction of the surface tension of distilled water, even at low concentrations . This is the first report of biosurfactant production using a mixture of these three agro-industrial residues, which can be very useful for the sustainable production of these promising molecules.

Acknowledgements:
The authors acknowledge the financial support from integrated project be@t – Textile Bioeconomy (TC- C 12-i01, Sustainable Bioeconomy No. 02/C12-i01/2022), promoted by the Recovery and Resilience Plan (RRP), Next Generation EU, for the period 2021 – 2026. The authors also acknowledge the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/ 2020 unit.

P1.80 - ASSESSING GRAM-NEGATIVE ANTIMICROBIAL RESISTANT BACTERIA IN RIVERS FOR DRINKING WATER: A SEASONAL ANALYSIS

Cátia Matos [1,2], António Magalhães [3], Francisca Pereira [3], Bárbara Duarte [1,2], Margarida Valente [4], Carolina Tavares [4], Juliana Rodrigues [4], Luísa Peixe [1,2], Carla Novais [1,2], Patrícia Antunes [1,2,3]

[1] UCIBIO-Applied Molecular Biosciences Unit, Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto. Porto, Portugal
[2] Associate Laboratory i4HB – Institute for Health and Bioeconomy. Faculty of Pharmacy. University of Porto. Porto, Portugal
[3] Faculty of Nutrition and Food Sciences, Porto University, Porto, Portugal
[4] Águas do Douro e Paiva, Laboratory of Microbiology

(*) e-mail: catia.v.matos@gmail.com

Keywords: Water quality indicators; *Escherichia coli; Salmonella; Klebsiella pneumoniae;* Antimicrobial resistance; Surface waters; Environment; Public health.

ABSTRACT

The dissemination of antimicrobial resistance in the environment is an emerging global health problem. Surface water systems are subjected to diverse antimicrobial micropollutants (e.g., antibiotics, disinfectants) over the year, but the temporal changes of microbial diversity and antimicrobial resistant bacteria-ARB are still poorly understood. We aimed to assess the occurrence and diversity of Gram-negative ARB in rivers utilized for drinking water supply throughout seasons.

Sixty-four samples (n=36-water/n=28-sediment/n=6-rivers) were collected in the Porto region (n=23-Winter/n=41-Spring/Summer). They were tested for *Escherichia coli*-Ec and coliforms-*Klebsiella pneumoniae*-Kp counts and for the presence of *Salmonella* by standard methods. Identification of Ec and its phylogenetic groups-PhG, Kp and *Salmonella* were performed using PCR and *Salmonella* serogroups through serological reactions and/or PCR. Antibiotic susceptibility testing (n=20) was conducted using disk diffusion (EUCAST/CLSI) and tolerance to benzalkonium chloride-BC through broth-microdilution (range:0.125- 128mg/L;aerobiosis/pH7.3/37ºC/20h;CLSI).

Most samples contained Ec (95%; 4-13.000 CFU/100ml) and coliforms (100%; 42- 256.000 CFU/100ml), which abundance was season independent (P>0,05). They also presented *Salmonella* (25%/5-rivers), season independent (P>0,05), while Kp was found in 39% of Winter and 5% of Spring/Summer samples (P<0,05). Ec (n=138; PhG A-B1-B2 the prevalent) were most resistant to ampicillin (45%-samples) or nalidixic-acid (35%-samples), with multidrug-resistant (MDR) in 33% of samples. *Salmonella* (n=42; B-Typhimurium monophasic variant, C2 and D-non-Enteritidis) were resistant to ampicillin (13%-samples) and tetracycline (13%-samples), with MDR in 13% of samples. ARB was season independent (P>0,05). Two rivers (Winter/Summer) carried Ec and Kp producing extended-spectrum-beta-lactamases. BC MIC/MBC were ≤32mg/L for different genera.

Detection of MDR bacteria, including *Salmonella* serotypes with clinical relevance and ESBL-producing *Enterobacterales*, throughout the year, substantiates the role surface water bodies play in the dissemination of ARB with public health implications if treatment barriers are not effective. Understanding bacterial and ARB occurrence by season/river underscore the importance of One

Health strategies in preserving water quality/safety.

Acknowledgements:
This work is funded by national funds from FCT – Foundation for Science and Technology, IP, under the projects 2022.02124.PTDC, UIDP/04378/2020, and UIDB/04378/2020 of the Research Unit in Applied Molecular Biosciences – UCIBIO, and project LA/P/0140/2020 from the Associated Laboratory of the Institute of Health and Bioeconomy – i4HB. Cátia Matos was supported by a scholarship (REQUIMTE 2023-02) associated with project 2022.02124.PTDC, and Andreia Rebelo by a FCT doctoral scholarship (SFRH/BD/137100/2018).

P1.81 - UNCOVERING SEASONAL VARIATIONS IN ANTIMICROBIAL-RESISTANT *ENTEROCOCCUS* DIVERSITY WITHIN PORTO REGION'S SURFACE WATERS

Cátia Matos [1,2], António Magalhães [3], Francisca Pereira [3], Bárbara Duarte [1,2], Ana R. Freitas [1,2,4], Margarida Valente [5], Carolina Tavares [5], Juliana Rodrigues [5], Luísa Peixe [1,2], Patrícia Antunes [1,2,3], Carla Novais [1,2]

[1] UCIBIO-Applied Molecular Biosciences Unit, Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto. Porto, Portugal
[2] Associate Laboratory i4HB – Institute for Health and Bioeconomy. Faculty of Pharmacy. University of Porto. Porto, Portugal
[3] Faculty of Nutrition and Food Sciences, Porto University, Porto, Portugal
[4] 1H-TOXRUN, One Health, Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL – Gandra (Portugal)
[5] Águas do Douro e Paiva, Laboratory of Microbiology

(*) e-mail: catia.v.matos@gmail.com

Keywords: Water quality indicators*; Enterococcus;* Antimicrobial resistance; Surface waters; Environment; Public health.

ABSTRACT

The rising of antimicrobial resistance in the environment is a global concern. Surface water systems are exposed to various antimicrobial pollutants (e.g., antibiotics/disinfectants) that variably impact on local microbiota according to diverse weather conditions. We aimed to assess the occurrence and diversity of antimicrobial- resistant (AMR) *Enterococcus* (water-quality-bioindicator) in the water/sediment of rivers used for drinking water production during different seasons.

Samples (n=36-water/n=28-sediments/n=6-rivers) were collected in the Porto region in different seasons (Winter-n=23/Spring/Summer-n=41). *Enterococcus* were isolated by standard water quality methods. Species identification was done by PCR, susceptibility to antibiotics (n=12) by disk diffusion (EUCAST/CLSI,2023) or broth- microdilution (linezolid), and to benzalkonium chloride-BC by broth-microdilution (MIC/MBC; range:0.125-128mg/L; aerobiosis/pH7.3/37ºC/20h). Search of *optrA* and poxtA genes (linezolid resistance) was done by PCR.

A high number of samples (97%; 2-1240 CFU/100ml) presented *Enterococcus*, and its abundance was similar across the seasons analysed (P>0,05). Isolates (n=172) recovered were identified as 9 species, with *E. faecalis* (23%), *E. hirae* (22%), *E. faecium* (16%), *E. lactis* (12%) and *E. durans* (11%) as the most abundant and present in all 6 rivers. Multidrug-resistant (MDR) *Enterococcus* were found in 14% of Winter and 19% of Spring/Summer samples (P>0,05). A high number of samples (>40%) carried isolates resistant to tetracycline and/or erythromycin, independent of the season (P>0,05). *E. faecium*, *E. durans* and *E. gallinarum* (n=1-each species, 3-rivers; different seasons) carried *optrA* and/or *poxtA* genes (linezolid MIC=8 mg/L). All isolates showed BC MIC/MBC=0,5-2mg/L, similar among different seasons (P>0,05).

The year-round presence of diverse MDR-*Enterococcus*, including with linezolid resistance genes, underscores the significant role that surface water bodies eventually play in the dissemination of AMR bacteria, with potential public health implications if treatment barriers are not effective.

Acknowledgements:
This work is funded by national funds from FCT – Foundation for Science and Technology, IP, under the projects 2022.02124.PTDC, UIDP/04378/2020, and UIDB/04378/2020 of the Research Unit in Applied Molecular Biosciences – UCIBIO, and project LA/P/0140/2020 from the Associated Laboratory of the Institute of Health and Bioeconomy – i4HB. Cátia Matos was supported by a scholarship (REQUIMTE 2023-02) associated with project 2022.02124.PTDC, and Andreia Rebelo by a FCT doctoral scholarship (SFRH/BD/137100/2018).

P1.82 - COLISTIN RESISTANCE IN PORTUGUESE WILD BOAR

Paulo Gaspar [1(*)], ***Diana Dias*** [1], ***Sónia Ferreira*** [2], ***Sónia Mendo*** [1], ***Tânia Caetano*** [1]
[1] *CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal.*
[2] *Centro Hospitalar do Baixo Vouga - EPE, Pathology Department, Aveiro, Portugal*

[(*)] *e-mail: paulodegaspar@ua.pt*

Keywords: One Health; wildlife; antibiotic resistance; MALDI-TOF.

ABSTRACT

The One Health concept has significantly raised awareness about the connection between human, animal, and environmental health. It is now known that most emerging infectious diseases have zoonotic origins, particularly wildlife. In the context of antibiotic resistance, wildlife is also recognized as a critical reservoir and disseminator of bacteria and antibiotic-resistance genes (ARG). Thus, environmental monitoring can help find high-risk areas. Colistin, a last-resort antibiotic, treats infections caused by some Gram-negative multidrug-resistant bacteria when other antibiotics fail. However, colistin resistance genes have spread worldwide and can be common in human and livestock fecal samples.

The aim of this study was to investigate the presence of colistin resistance in wild boar in Portugal. To accomplish this, we cultured enrichments of 35 fecal samples collected in distinct areas in Portugal, in Chromagar™ Col-APSE. Colonies were successfully observed on all plates. In an early stage, based on the color phenotype, we isolated 75 colonies that were identified by MALDI-TOF analysis. The results showed that 58% belong to genera known for their natural resistance to colistin, namely, *Hafnia, Moellerella* and *Proteus*. Of the remaining isolates (n=31), nearly 80% were identified as *Escherichia* spp., 10% as *Klebsiella* spp. and 10% as *Enterobacter* spp. As recommended by EUCAST, the MICs for colistin of these strains were performed with the Broth Microdilution Test. Notably, only 18% of the strains tested (n=28) exhibited resistance to colistin (MIC ≥ 4 mg/L), according to clinical breakpoints. Similarly, based on ECOFFs (epidemiological cut-offs), the same percentage was categorized as non-wildtype (NWT). The genetic basis of this resistance is currently under investigation, including sequencing the genome of the NWT strains that include *Escherichia* spp., *Klebsiella* spp. and *Enterobacter* spp. To the best of our knowledge, this is the first study reporting colistin resistance association with wild boar in our country.

Acknowledgements:
Tânia Caetano was funded by national funds (OE) through FCT in the scope of the framework contract (CEECIND/01463/2017). Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), through national funds.

P1.83 - AIR QUALITY IN VETERINARY TEACHING FACILITIES – OCCURRENCE OF MOLDS

Isabel Vaz Carvalho [1,2,3 (*)]***, Kamila Soares*** [1,2,3]***, Justina Oliveira*** [1,2,3]***, Alexandra Esteves*** [1,2,3]***, Patrícia Poeta*** [1,2,3]***, Isabel Pires*** [1,2,3]***, Filipe Silva*** [1,2,3]***, Cristina Saraiva*** [1,2,3 (**)]

[1] *Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*

[2] *Animal and Veterinary Research Centre (CECAV), UTAD, Vila Real, Portugal*

[3] *AL4AnimalS - Animal and Veterinary Research Centre; Portugal*

[(*)] *e-mail: isabelmvazcarvalho@gmail.com;* [(**)] *e-mail: crisarai@utad.pt*

Keywords: Veterinary teaching hospital; Molds; air quality.

ABSTRACT

Veterinary teaching hospitals (VTH) create a unique environment for microorganisms. Assessing air quality allows the evaluation of the exposure to microbiological agents. This study was carried out to assess the air contamination of a VTH and to evaluate the fungal organisms.

Twenty-three samples were collected from the following services: reception hall, companion animals, farm animals and equines, exotic and wild animals, necropsy room, and clinical pathology laboratory.

The quantification of airborne microbiota was carried out by passive air sampling using settle plates based on the 1/1/1 scheme. A 9 cm Petri dish was briefly opened and left on a surface 1 m above the floor, 1 m from the wall for 1 hour. The samples were collected in the morning, before the major rate of movement, and were incubated in Chloramphenicol glucose Agar® (CGA) at 25ºC for 5 days and Plate count Agar® (PCA) at 37ºC for 3 days. Results were expressed in CFU/dm^2/h. The identification of the fungus genera was based on macroscopic and microscopic characteristics.

Mesophilic bacteria count presented a $\bar{x}$=2.89 x10^2 CFU/m^3, and the values varied from 2.33 x10^3 to 5.24 x10^3 CFU/m^3. Regarding to the moulds, a $\bar{x}$= 2.93 x10^2 CFU/m^3 the values ranged from 0 to 8.64x10^2 CFU/m^3. Eighty-three percent of the samples presented a grow of moulds, being the most predominant genera *Penicillium* spp., n=22, in 32.34%, *Mucor* spp., n=18, in 26.47%, *Aspergillus* spp., n=12, in 17.64%, and *Cladosporium* spp., n=5, in 7.35% of the obtained isolates.

The presence of pathogenic organisms and the prolonged human exposure to them can create a health risk for allergic symptoms in staff, that highlight the importance of assessing the air quality as a biosecurity measure.

Acknowledgements:
This work was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Isabel Vaz Carvalho would like also to thank to UTAD FOOD ALLIANZ project, funded by the NORTE-01-0145-FEDER-072687.

P1.84 - ANTIBIOTIC RESISTANCE MONITORING USING AMPLICON SEQUENCING, PCR ARRAY AND SELECTED BIOMARKERS: A COMPARATIVE APPROACH

Inês Leão [1(*)], Jorge T. Antunes [1], A. Margarida Teixeira [1], Inês Baptista [2], Rúben Jorge [2], Ivone Vaz-Moreira [1], Célia M. Manaia [1]

[1] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

[2] WEDOTECH – Companhia de Ideias e de Tecnologias, Lda, Porto, Portugal

[(*)] e-mail: s-mainlesilva@ucp.pt

Keywords: wastewater; antibiotic resistance genes; biomarkers.

ABSTRACT

This study compared quantitative PCR (qPCR), PCR array and class 1 integron amplicon Nanopore sequencing to monitor antibiotic resistance genes (ARGs) in wastewater collected at different treatment stages - raw wastewater, sand filtration+coagulation, ultraviolet (UV) radiation, final effluent and inlet and outlet of storage container, aiming to assess the most suitable and cost-effective procedure.

Monitoring screened different molecular biomarkers (qPCR, 16S rRNA, *intI1, aph(3´´),* crAssphage, *uidA*), profiling of 48 genes (PCR array), and class I integrons amplicon sequencing (MinION, Oxford NANOPORE).

The qPCR analysis showed significant decrease in genes abundance after sand filtration+coagulation compared to raw wastewater (removal 0.9-1.3 log-units), in contrast with UV that did not cause an apparent reduction, although storage led to a decrease (< 1 log-unit) in abundance of the crAssphage, *aph(3")* and *uidA* genes. PCR array showed that raw wastewater had an ARGs profile not distinct of other samples but with significantly higher levels ($p<0.01$) of quinolones, beta-lactams and trimethoprim related ARGs and mobile genetic elements. Class 1 integron amplicon sequencing showed that the 10 most abundant ARGs were present in all samples and suggested that genes such bla_{VIM} were mainly observed in raw wastewater. The relative abundance (annotated reads/total reads) of the genes *aadA2_1* and *dfrB4_1* increased over treatment line, while UV radiation was associated with the increase of relative abundance of some genes, such as *dfrA22_4*, and *dfrB2_1*, and decrease of others - bla_{OXA-10}_1, bla_{OXA-2}_1, ant(3")- Ia_1 and *dfrA16*_2. Storage contributed to decrease the relative abundance of most of the annotated ARGs.

While biomarker monitoring provided a straightforward procedure to assess efficacy of the treatment process, PCR array offered an overview of the genes diversity present throughout the system. As a complement, sequencing of class 1 integrons amplicons may provide important insights about the mechanisms of resistance acquisition or loss over treatment and storage.

P1.85 - MOLECULAR PROFILE, AS OBTAINED BY HIGH-THROUGHPUT FT-MIR SPECTROSCOPY, TO CHARACTERISE AND PREDICT ANTI-BACTERIAL EFFECTS OF BIOACTIVE COMPOUNDS

Igor Leite [1], ***Pedro Sampaio*** [2,3&], ***Cecília R.C. Calado*** [1,4 &(*)]

[1] *ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal*
[2] *COPELABS - Computação e Cognição Centrada nas Pessoas, BioRG—Biomedical Research Group, Faculty of Engineering, Lusófona University, Lisbon, Portugal*
[3] *GREEN-IT BioResources for Sustainability Unit Institute of Chemical and Biological Technology António Xavier, ITQB NOVA, Oeiras, Portugal*
[4] *CIMOSM – Centro de Investigação em Modelação e Optimização de Sistemas Multifuncionais, ISEL, Lisbon, Portugal*

[&] *both authors contributed equally for this work*
[(*)] *e-mail: cecilia.calado@isel.pt*

Keywords: FTIR spectroscopy; High-throughput screening; Bioactive compounds.

ABSTRACT

High-throughput screening associated with Fourier-Transform-Mid-InfraRed (FT-MIR) spectroscopy is a very attractive technique to acquire the molecular fingerprint of biological samples with complex compositions, and to predict its biological activity, such as anti-bacterial effects. In the present work, the technique was used to qualitative predict, by the spectra principal component analysis(PCA), the differences between the molecular fingerprint of extracts obtained from four macroalgae species from Portuguese coastal (*Asparagopsis armata, Fucus vesiculosus, Saccorhiza polyschides, and Stigeoclonium subsecundum*), based on different extraction procedures (e.g., water, methanol, ethanol, acetone, and ether ethyl). The anti-bacterial effect of all these extracts was evaluated against *Escherichia coli*, based either on the conventional agar-dilution method, and by spectroscopy. The new method enabled to evaluate the effect of different algae species and extraction procedures on the extract composition. The spectra of the culture media, enabled to predict the extract effect on the bacteria metabolism, at concentrations much lower than the minimum inhibitory concentration. Furthermore, based on partial least square regression models, most of the extracts reveal a direct proportional effect between the extract concentration and the impact on the bacteria metabolism. It is worthy to note that, the FT-MIR spectra were acquired based on small volume of sample(25 µl), after a simple sample dehydration step, and conducted on a plate with multi-wells, i.e., in a high-throughput mode. The presente method, enables, therefore, to predict the impact of different macroalgae species and extraction process on the extract composition, and even to predict the extract anti-bacterial effect, in a rapid, simple, high-throughput and economic mode. All these characteristics can significantly promote the optimization of extraction processes and the discovery of new compounds with appealing biological effects, such as anti-bacterial.

Acknowledgements:
Project grant DSAIPA/DS/0117/2020 supported by Fundação para a Ciência e a Tecnologia, Portugal.

P1.86 - LEVERAGING THE ANTIMICROBIAL POTENTIAL OF BEE APITOXIN TO DEVELOP PROMISING ANTI-*CANDIDA* AGENTS

Carolina Glória [1,2], Ana Luísa Oliveira [1,2], Dalila Mil-Homens [1,2], Arsénio M. Fialho [1,2], Nélson Moura [3], Ana Azevedo [1,2], Nuno P Mira [1,2(*)]

[1] iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal
[2] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
[3] EcoAPIS, Chaves

[(*)] e-mail: nuno.mira@tecnico.ulisboa.pt

Keywords: anti-Candida treatments; apitoxin; melittin.

ABSTRACT

Candida yeasts are leading causative agents of infections in humans, partly due to a continuous emergence of strains resistant to antifungals used in the clinical setting. This, along with the enhanced difficulties of obtaining molecules that can restrain fungal growth without causing toxicity for the human host, has been boosting research to find alternative anti-*Candida* molecules. In this work we investigated the potential of bee venom (or apitoxin) as an eventual anti-Candida agent. Proteomic characterization, based on LC-MS coupled with MALDI-TOF of several apitoxin samples recovered from hives established in the Northeast region of Portugal demonstrate that melittin and phospholipase A2 are, always, the two more abundant proteins in the complex protein mixtures identified. Apitoxin exerted a strong effect in inhibiting growth of *C. albicans* and, less prominently, of *C. glabrata*, including of strains resistant to azoles more commonly used. This antimicrobial effect of apitoxin could be fully recapitulated by purified melittin recovered from apitoxin samples showing that this peptide underlies the observed anti-*Candida* effect of apitoxin. A strong synergy in inhibiting growth of *Candida* was observed when combining apitoxin/melittin with caspofungin and amphotericin B, indicating potential to be used in combination therapies. Importantly, apitoxin exerted no toxicity against the model wax Galleria mellonella when used in the concentration range that inhibited growth of *Candida*. Treatment with apitoxin/melittin of *G. mellonella* larvae infected with *C. albicans* prominently decreased death of the larvae caused by the yeast showing in vivo efficacy. The results of this work open the design of new approaches for the treatment of infections caused by *Candida* based on the use of melittin/apitoxin, alone or in combination with antifungals already used in the clinical practice.

Acknowledgements:
This study was performed in the framework of the PharmaAPITOX project supported by the European Union through the FEEI program, in the context of COMPETE2020 program. Funds received from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project supporting the Research Unit Institute for Bioengineering and Biosciences (UIDB/04565/2020) and the project supporting Institute for Health and Bioeconomy, i4HB (LA/P/0140/2020).

P1.87 - IS THERE A ROLE FOR TRAMAZEIRA TREE IN OFFERING ALTERNATIVES FOR THE TREATMENT OF INFECTIONS CAUSED BY *CANDIDA* SPECIES?

Joana Valério [1,2], Diogo Santos [1,2(*)], Maria Joana Pinheiro [1,2], Gabriela Vergara [3,4], Natália Seixas [3,4], Dalila Mil- Homens [1,2], Arsénio M. Fialho [1,2], Luís Dias [3,4], Letícia Estevinho [3,4], Nuno P Mira [1,2]

[1] iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal

[2] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

[4] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

[(*)] e-mail: diogo.alexandre.santos@tecnico.ulisboa.pt

Keywords: phytopharmaceuticals; anti-Candida treatments; *Sorbus aucuparia*; Tramazeira.

ABSTRACT

Candida yeasts are prominent Fungal pathogens causative of superficial and systemic infections in humans. In part, the incidence of infections caused by *Candida* is attributable to their capacity to acquire resistance to currently used antifungals, this being particularly worrisome for non-*Candida* albicans Candida species (or NCAS). This, along with the enhanced difficulties of obtaining molecules that can restrain fungal growth without causing toxicity for the human host, has been pressing the search for alternative anti-*Candida* therapies. In this work we will discuss the results obtained within the framework of the TRAMONTE project, a research initiative that aims to analyze what can be the potential of the Portuguese Sorbus aucuparia tree (or Tramazeira) in providing add-value products, including phytopharmaceuticals that can be used against *Candida*. In this context, we will show results of an optimized process to obtain hydro- alcoholic extracts from the berries and the leafs obtained from the tree, as well as their potential to inhibit, in vitro, growth of *Candida*. The effect of combining these extracts with currently used antifungals in the clinical practice (in particular, azoles, echinocandins and polyenes) will also be discussed.

Acknowledgements:
This study was supported by funds received from the TRAMONTE research project in the framework of programa “PROMOVE 2021” (PD21-00013) funded by Fundação LaCaixa, BPI and FCT.

P1.88 - CYANOBACTERIAL DIVERSITY FROM UNDEREXPLORED AREAS AND ENVIRONMENTS ACROSS MOROCCO AND CAPE VERDE

João Morais [1,2(*)], ***Raquel Silva*** [1], ***Flavio Oliveira*** [1,2], ***Pedro Cruz*** [1,2], ***Pedro Leão*** [1] ***and Vitor Vasconcelos*** [1,2]

[1] *CIIMAR – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal*
[2] *FCUP - Faculty of Sciences, University of Porto, Porto, Portugal*

[(*)] *e-mail: joaopmorais@gmail.com*

Keywords: Cyanobacteria; diversity; bioprospection

ABSTRACT

Cyanobacteria are a remarkably diverse group of prokaryotic organisms known for their wide-ranging adaptability and resilience in diverse environmental conditions. Presently, there are over 5,000 documented species, yet it is anticipated that thousands more remain undiscovered.

Exploring underexplored ecosystems and geographical regions has the potential to unveil new cyanobacterial diversity. This could, in turn, be translated into new chemical diversity with potential use in different biotechnological applications that can help to tackle actual societal problems. To expand our understanding of cyanobacterial diversity, investigate their phylogenetic relationships, assess their biosynthetic and toxicological potential, sampling and isolation of these organisms was performed along several locations in São Vicente and São Antão Islands (Cape Verde) in April 2018 and in the Moroccan Atlantic coast from El Jadida to Essaouira in July 2019. A total of more than 250 cyanobacterial isolates were obtained, primarily from marine environments but also from inland locations, including historical monuments. These isolated strains are well distributed within the cyanobacterial tree of life and represent almost all currently recognized orders of cyanobacteria, some of them represents new diversity never described before. Isolated strains are affiliated with or closely related to genera such as *Lyngbya, Okeania, Symploca, Pleurocapsa, Caldora, Xenococcus, Hyella, Phormidium, and Nodularia*, which are known for their production of various families of natural products. Several isolated strains were selected for genome sequencing to facilitate bioprospecting the genomic information for the discovery of biosynthetic gene clusters of natural products but also to help in their taxonomic identification.

P1.89 - UNCOVERING BIOPLASTIC BIOSYNTHESIS POTENTIAL IN MARINE HOSTASSOCIATED BACTERIA USING COMPARATIVE GENOMICS

Ana Maria Fernandes [1,2]***, M. Teresa Cesário*** [1,2]***, Rodrigo Costa*** [1,2 *]

[1] *iBB- Institute for Bioengineering and Biosciences, Bioengineering Department, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*
[2] *Associate Laboratory i4HB, Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*

[(*)] *e-mail: rodrigoscosta@tecnico.ulisboa.pt*

Keywords: Polyhydroxyalkanoates, Marine Bacteria, Functional Annotation

ABSTRACT

Polyhydroxyalkanoates (PHAs) are polyesters of biological origin which, due to their similarity to conventional plastics, biocompatibility, and biodegradability, are of growing biotechnological interest. In bacterial cell metabolism, PHAs are produced under nutrient-limited conditions or stress and function as carbon and energy storage. The marine environment is a vast and so-far non-exhausted reservoir of polymers of microbial origin. Particularly, the diverse and phylogenetically unique microbiomes of sessile marine invertebrates, such as sponges and corals, play pivotal roles in nutrient and carbon cycling and may be promising sources of novel PHA-producing bacteria.

In this study, a panel of 86 bacterial isolates from marine sponges and corals was assessed for potential PHA biosynthesis through genotypic screenings using Protein families- (Pfam) and Clusters of Orthologous Groups of Proteins (COG)-based annotations. From the several genes involved in PHA synthesis, those encoding polyhydroxyalkanoate synthase/polymerase (PhaC) were used as proxy to consider a bacterium a potential PHA producer. The panel of bacterial symbionts analysed in this study is undergoing phenotypic screenings via staining with Nile Red, followed by observation through epifluorescence microscopy. From the 86 bacterial isolates, 55 were positive for the presence of the PhaC gene. Of these, 38 belong to the *Alphaproteobacteria* and 16 to the *Gammaproteobacteria* class in the *Pseudomonadota* phylum, while one isolate belongs to the class Actinomycetes in the *Actinomycetota* phylum. Phylogenetic analysis revealed that PhaC encoding gene relationships did not mirror entirely the corresponding 16S rRNA gene tree topology of the strains under study, suggesting horizontal gene transfer events play a role in the evolution of PHA-encoding genes. Gene duplication and heterogeneity were found in strains of the genera *Kiloniella, Lentilitoribacter, Ruegeria, Sphingorhabdus, and Alcanivorax*, among others. Although confirmation of the PHA production phenotype is still under assessment, this collection has shown promising potential to produce PHA at a larger scale.

Acknowledgements:
This study was supported by the "BLUE BIOECONOMY PACT" (Project Nº. C644915664-00000026), cofunded by Next Generation EU European Fund, under the incentive line "Agendas for Business Innovation" within Component Funding Scheme 5 - Capitalization and Business Innovation of the Portuguese Recovery and Resilience Plan (RRP). Further support was provided by the Portuguese Foundation for Science and Technology (FCT) in the scope of the projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and the project LA/P/0140/2020 of i4HB.

MICROBIOTEC 23

Poster Session

Topic 2

P2.1 - EVALUATION OF PHAGE VIABILITY AT DIFFERENT CONDITIONS FOR PHAGE APPLICATION ON FOOD

Márcia Braz [1]*, Carla Pereira [1], Pedro Costa [1], Carmen S R Freire [2], Adelaide Almeida [1]

[1] Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal
[2] CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

(*) e-mail: marciabraz96@ua.pt

Keywords: *Salmonella enterica*; *Escherichia coli*; Phage; Temperature, pH; Food safety.

ABSTRACT

Salmonella enterica and *Escherichia coli* are among the most prevalent foodborne bacteria. Despite the available food decontamination methods, bacterial contamination of food continues to occur. Therefore, new strategies to overcome this problem are needed. Phages, viruses that only infect bacteria, have been proven to be an effective strategy to inactivate foodborne bacteria. However, one of the main limitations of phage application on food is its vulnerability to various conditions, such as temperature and pH, that must be addressed to ensure the treatment's efficacy. In this study, the viability of three different phages (*E. coli* phage phT4A, *Salmonella* Typhimurium phage phSE-5 and *Salmonella* Enteritidis phage phSE-P1) at different pHs (pH 3, 5, 7 and 8) and temperatures (4, 25, 37 e 45 ºC) was evaluated. The assays were performed in phosphate-buffered saline (PBS) with an initial phage titer of ≈$1x10^7$ plate forming units (PFU)/mL. At pH 3, the phage titer decreased to the detection limit of the method after 10 h, 4 h and 10 days for phT4A, phSE-5 and phSE-P1, respectively. At pH 5, ≈1 and 2 log PFU/mL decrease was observed for phT4A and phSE-5 phages, respectively, while for phSE-P1 phage no significant decrease was observed after 28 days. At pH 7 and 8, no significant decrease was observed for the tested phages. No significant decreases were observed for phSE-P1 phage at the different temperature conditions, and for phT4A and phSE-5 phages at 4 and 25 ºC, after 28 days. At 45 ºC, both phT4A and phSE-5 phages showed a 3 log PFU/mL decrease while at 37 ºC, ≈0.5 and 1 log PFU/mL decrease was observed for phT4A and phSE-5 phages, respectively, after 28 days. Considering the obtained results, with phage titer maintained under most conditions, phages seem to be a promising approach for food decontamination, improving food safety.

Acknowledgements:
The authors acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) and to CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Márcia Braz thanks FCT for the PhD grant (2020.06571.BD). Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Carmen Freire also acknowledges FCT for her research contract (CEECIND/00464/2017). Thanks are also due to the Biology Department and the University of Aveiro, where this research work was performed.

P2.2 - HYPERBARIC STORAGE AS POSSIBLE SIMULTANEOUS MODERATE PRESSURE PASTEURIZATION METHODOLOGY: IMPACT OF PH AND PRESSURE LEVEL ON MICROBIAL INACTIVATION

Vasco Lima [1]*, ***Jorge Saraiva*** [1]

[1] *LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Portugal.*

(*) *e-mail: vasco.lima@ua.pt*

Keywords: hyperbaric storage; moderate pressure pasteurization; watermelon juice; microbial inactivation

ABSTRACT

Hyperbaric storage (HS), a novel food storage under mild pressures, has been proposed as an alternative to conventional refrigeration that can be used at room temperature (RT), allowing for virtually no energy costs and substantially lower greenhouse gas emissions. More recently, moderate-pressure pasteurization (MPP) has been investigated for pasteurization purposes, using hydrostatic pressures up to 200–250 MPa for a few hours.

Watermelon juice has an interesting nutritional composition, associated with health benefits, that includes vitamins, minerals, functionally important amino acids and antioxidants like carotenoids and phenolic compounds. However, it is highly perishable, due to its high pH (5.2–6.7) and high water activity (0.97–0.99), resulting in high microbial growth and enzymatic activity propensity, leading to a short shelf-life.

For the first time, pH's impact on a food (watermelon juice) pasteurized by MPP at RT was studied, focusing on the behaviour of *Escherichia coli, Listeria monocytogenes*, *and Saccharomyces cerevisiae* inoculated in the juice (adjusted to pH 4.0 and 6.5), and stored at 160-250 MPa for up to 12 hours. along with controls stored at atmospheric pressure (AP) under refrigerated or RT conditions.

HS allowed for the inactivation of the three microorganisms, often with reductions over 4 log CFU/g to levels below the detection limit, which was not the case for the AP samples. While increasing the pressure level accelerated the inactivation, the impact of varying the juice's pH depended on the microorganism: for the bacteria tested a lower pH accelerated inactivation, unlike for *S. cerevisiae*.

To conclude, variables like the juice's pH and the storage pressure level should be further studied to better select the MPP conditions. During storage, microbial growth can be controlled and its inactivation can reach levels close to pasteurization, revealing MPP's potential as a nonthermal pasteurization technique at room temperature with *quasi* no energetic costs.

P2.3 - CLEAN LABEL ANTIMICROBIAL STRATEGIES FOR FUNGAL SPOILAGE OF PASTRY FILLINGS

Teresa Bento de Carvalho [1], Miguel Azevedo [2], Beatriz Silva [2], Paula Teixeira [1]*

[1] Universidade Católica Portuguesa, Laboratório Associado, CBQF—Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Porto, Portugal;
[2] Decorgel – Produtos Alimentares S.A., Trofa Portugal

(*) e-mail: pcteixeira@ucp.pt

Keywords: Fungal spoilage; clean label preservatives; pastry fillings

ABSTRACT

Food spoilage is a key concern for/within the food industry, leading to hefty economic losses that impact consumers' views and trust in companies. Consequently, the food waste that follows is also a grave matter not only due to its social or humanitarian aspect but also its environmental impact. The occurrence of fungal spoilage at any stage of the food production chain poses a major issue due to the ability of yeasts and moulds to overcome already implemented control strategies. The aim of this study was to assess the antifungal activity of commercial clean label alternatives to potassium sorbate against fungi isolated from pastry fillings. Five clean label powder antimicrobials, currently available in the market, were tested against six different fungi isolated from a commercial salted caramel pastry filling. Concentrations used for each antimicrobial varied accordingly to each compound's technical specifications. Results show that while fungal growth is not completely inhibited at the concentrations recommended by the manufacturers, the antimicrobial agents are still effective and, therefore, a promising alternative to traditional chemical preservatives for the pastry industry. Fungal spoilage poses a serious threat to the food industry and apart from potassium sorbate there aren't many alternative antifungals. In this context, the clean label movement is trending to offer natural spoilage control strategies while having their organoleptic characteristics and, most importantly, safety in sight.

Acknowledgements:
This work was developed in the scope of the project "Agenda VIIAFOOD project – Platform for Valorization, Industrialization and Commercial Innovation for the Agro-Food Industry, funded by the Portuguese Recovery and Resilience Plan ("PRR") in its component 5 – Capitalization and Business Innovation, under the Regulation of the Incentive System "Agendas for Business Innovation" and Invitation Notice n. 02/C05-i01/2022, and specifically in the sub-project "Development of preservative free preparations to the pastry industry with natural ingredients" Special thank you to Decorgel´s Research & Development Department, Trofa, Portugal, for the collaboration and the opportunity.

P2.4 - PRODUCTION OF EGG WHITE PROTEIN FILMS WITH ANTIMICROBIAL PROPERTIES AS FOOD PACKAGING MATERIALS

Ângelo Luís [1,2*], ***Ana Ramos*** [2,3], ***Fernanda Domingues*** [1,2]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*
[2] *Departamento de Química, Faculdade de Ciências. Universidade da Beira Interior, Covilhã, Portugal*
[3] *FibEnTech – Fiber Materials and Environmental Technologies Research Unit, University of Beira Interior, Covilhã, Portugal*

[(*)] *e-mail: angelo.luis@ubi.pt*

Keywords: egg white; films; essential oil; *Cymbopogon martinii;* food packaging

ABSTRACT

Most of the food packaging is made of plastic materials, which are harmful to the environment since they take a long time to decompose, leading to severe environmental impacts. In this context, natural and sustainable materials are valued because they are more environmentally friendly and can be easily broken down. Several raw materials, such as polysaccharides and proteins are commonly used in films production. Egg white is a natural endogenous protein that has been used in agro-food sector due to its low cost, high nutritional quality, and excellent functional properties, such as foaming, gelling and emulsification.

Cymbopogon martinii are perennial grasses used in the fragrance industry, which are rich in essential oil. *C. martinii* essential oil is extracted by steam distillation of leaves and has been widely used as antibacterial, antiviral, and anti-inflammatory agent.

The main goal of this work was to produce and characterize egg white protein films incorporating *C. martinii* essential oil as new food packaging materials. The optical, mechanical, barrier, and bioactive properties of the films were assessed. The chemical composition of the essential oil was studied by GC-MS, being geraniol its major compound (82.04%). The essential oil demonstrated to possess significant antioxidant activity measured by DPPH assay and β-carotene bleaching method. *C. martinii* essential oil presented antibacterial activity against several strains of foodborne pathogens. The produced films incorporating the essential oil showed better mechanical than those of the control films. The films were transparent (91.81%) and almost colorless. Concerning the water barrier properties, the incorporation of the essential oil in the films reduced the water permeation, probably due to its hydrophobic nature. The antioxidant and antibacterial properties of the essential oil were maintained when it was incorporated in the films, which shown to be antioxidant and active against the growth of the tested foodborne pathogens.

Acknowledgements:
Ângelo Luís acknowledge the contract of Scientific Employment (Microbiology) financed by Fundação para a Ciência e a Tecnologia (FCT) under the scope of DL 57/2016.

P2.5 - CHARACTERIZATION OF FECAL COLIFORM CONTAMINANTS IN SEAWATER AND SEAFOOD SAMPLES COLLECTED FROM NAMIBE IN ANGOLA

Beatriz L Calado [1+]*, Inês C Leal [1+], Eunice Cassoma [2], Isaac Bumba [2], Carmen dos Santos [2], Adelino VM Canário [1], Deborah M Power [1], João CR Cardoso [1]

[1] Comparative Endocrinology and Integrative Biology, Algarve Centre of Marine Sciences (CCMAR-Algarve), Universidade do Algarve, Faro, Portugal
[2] Universidade do Namibe, Campus Farol de Noronha, Moçâmedes, Angola
+ shared first authorship

[(*)] e-mail: ifleal@ualg.pt

Keywords: Coliform bacteria, bivalves, seawater, pathogens

ABSTRACT

Fecal coliform bacteria are microorganisms that are part of the intestinal flora of humans and other warm-blooded animals'. Contamination of aquatic environments by fecal coliform bacteria is a standard indicator of water quality and safety. In less developed countries where sewage treatment is scarce or non-existent and urban water run-offs and waste contaminate coastal waters they represent a major danger to Public Health. The aim of the present study was to conduct a preliminary characterization of fecal coliform bacterial contaminants present in the seawater or in the seafood in the coast of Namibe (Angola) and identify pathogenic bacteria that might pose a risk to human health. Water and bivalve mussel samples were collected from 4 different sites near urban areas and bacteria were isolated by conventional microbiology and by 16S rRNA PCR. The results revealed that fecal indicator bacteria of Proteus spp. and Escherichia coli were abundant in seawater samples, while Klebsiella pneumoniae and *Morganella morganii* were only detected in mussel tissues. E. coli was found in all sampling sites, while the bacteria K. pneumoniae and *M. morgani* were only found at one site (Praia das Conchas) and this was also the samples with the highest diversity of coliform bacteria. This study identifies for the first time the diversity and type of fecal pathogenic bacteria contaminants in coastal areas of Namibe and raises a "red flag" in relation to public health risks particularly of water-borne infections since most of the population depends on marine resources to survive.

Acknowledgements:
This study received Portuguese national funds from FCT - Foundation for Science and Technology through Institutional projects UIDB/04326/2020/2021/2022/2023, and LA/P/0101/2020 and the operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121 and from FCT funded project AGAKHAN/541666287/2019 HealthyBi4Namibe.

P2.6 - ARE GUAIACOL AND HALOPHENOLS THE ONLY ONES TO BLAME FOR THE OFF-FLAVOUR SPOILAGE BY *ALICYCLOBACILLUS SPP.*?

Inês Carvalho Leonardo [1,2], António Ferreira [1], Maria do Rosário Bronze [1,2,3], Maria Teresa Barreto Crespo [1,2],Frédéric Bustos Gaspar [1,2]*

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[3] FFULisboa, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

(*) e-mail: fgaspar@ibet.pt

Keywords: spoilage bacteria, taints and off-flavours, food quality control

ABSTRACT

Industries that produce or use fruit-based products have faced several spoilage events, resulting in economic losses caused by product recalls and loss of consumer confidence. Some of these events correlate to the presence of *Alicyclobacillus* (ACB) in food products since they can produce off-flavours in the final products. Guaiacol (2-methoxyphenol) and halophenols (2,6-dichlorophenol and 2,6-dibromophenol) have been widely explored as the major culprits of off-flavour spoilage by ACB. These compounds are associated with medicinal, disinfectant, or smoky odours.
In this work, the ability of distinct ACB species (*Alicyclobacillus acidoterrestris*, *Alicyclobacillus acidocaldarius*, and *Alicyclobacillus cycloheptanicus*) to produce volatile compounds was evaluated in different conditions (e.g., different time spans, off-flavour precursors added, matrix – growth medium, fruit juice). Relevant metabolites were identified and quantified by GC-MS, while simultaneously investigating their potential as spoilage-related compounds.
For the first time, isobutyric acid (2-methylpropanoic acid) and isovaleric acid (3-methylbutanoic acid) were reported, as soon as two days of incubation, as being produced in different conditions at concentrations which could surpass the described odour threshold. The sweaty, sour, and unpleasant profile of these newly reported compounds is often associated with certain metabolites produced during milk fermentation by lactic acid bacteria in cheese production.Most importantly, isobutyric acid and isovaleric acid were found to be produced by all three ACB species, regardless of their ability to produce guaiacol and/or halophenols. This work clearly shows that even ACB species previously identified as non-spoilage bacteria can also pose a threat to the fruit juice and beverage industries.
Therefore, the risk assessment currently used in the industry for ACB control may need to be revised to accommodate these new findings.

Acknowledgements:
ICL acknowledges Fundação para a Ciência e a Tecnologia (FCT) for the financial support received through PhD scholarship 2020.08210.BD. Funding by iNOVA4Health—UIDB/04462/2020 and UIDP/04462/2020 and LS4FUTURE (LA/P/0087/2020), programs financially supported by FCT and Ministério da Ciência, Tecnologia e Ensino Superior, are also gratefully acknowledged.

P2.7 - MICROBIOLOGICAL HAZARDS IN LOCAL PRE-HARVEST PRODUCTION OF VEGETABLES AND FRUITS AND IRRIGATION WATER

Ariana Macieira [1]* and Paula Teixeira [1]*

[1] CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal;

[(*)] *e-mail: arianamacieira@gmail.com | pcteixeira@ucp.pt*

Keywords: Food Safety, Microbiological hazards, Local Vegetables and Fruits, Irrigation Water

ABSTRACT

Consumers have been demonstrating a high concern regarding agricultural production methods and food safety, leading to an increase in their purchases at local farming markets. Local production and consumption appear to promote more sustainable food consumption. The preference for local products can be attributed to the access to fresher food with a smaller ecological footprint and to the development of closer commercial relationships between producers and customers, shortening the supply chain, minimizing energy costs for distribution and product selling, and provides an easier way to track the product along the food chain. Local products are often used in local recipes which reflect the regional culture and preserve cultural identity.
However, when it comes to local markets, there are some challenges related to food safety that need to be addressed, in order to prevent and control the transmission of foodborne pathogens.

A microbiological characterization was performed on locally produced vegetables and fruits and in irrigation waters in the North of Portugal, in order to estimate the health risks to human exposure to some food pathogens such as *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereus*.

Microbiological hazards in pre-harvested food and irrigation water were detected in this study, namely *Bacillus cereus* and *Listeria monocytogenes*.

This study highlights the need to establish norms and practices that can contribute to prevent pathogens from “entering the fresh production chain, as production has the potential to act as a vehicle for transmission of harmful pathogens in the food-to-fork process”.

Acknowledgements:
This work was developed within the scope of the project HSoil4Food - Healthy soils for healthy foods (NORTE-01-0145-FEDER-000066) co-financed by the European Regional Development Fund (FEDER) through the Northern Regional Operational Program. We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020. Financial support for author Ariana Macieira was provided by a PhD grant DFA/BD/6239/2020 from Fundação para a Ciência e a Tecnologia (FCT), Fundo Social Europeu (FSE), under Programa Operacional Regional do Norte-Norte2020.

P2.8 - PRODUCTION AND QUALITY EVALUATION OF INFANT DIET FORMULATED USING POWDERED PAP AND DIFFERENT NUTS TO ENHANCE FOOD SECURITY

Ayodele Oluwayemisi [1*], Oluwatoyin Modupe [2], Stephen Olanrewaju [1], Gbenga Isaac [1]

[1] Department of Food Technology, The Federal Polytechnic Ado Ekiti, Ekiti state, Nigeria
[2] Department of Biological Sciences, Redeemer's University, Ede, Osun State, Nigeria

(*) e-mail: ogunlade_ao@fedpolyado.edu.ng

Keywords: Powdered pap, Infant diet, Nuts, Food security

ABSTRACT

The aim of this study was to formulate infant diet using powdered pap fortified with different nuts such as almond nuts, ground nuts and cashew nuts in order to improve its nutritive value and enhance food security. To achieve this objective, sorghum was soaked, milled, steeped and oven dried to powdered form while almond, cashew and ground nuts were roasted, oven dried, milled into flour and defatted. Infant diet was then formulated by mixing pap flour with the nuts in ratio 80:20, 70:30, 60:40 and 50:50. Quality evaluation of the sample (proximate, mineral and functional properties) were carried out on the composite flour. Proximate analysis showed that Protein, fat, ash and fibre content of sorghum-almond nuts increased from 14.86-28.03%, 6.86-11.71%, 1.38-1.71%, 3.95-4.02% while, moisture and carbohydrate content reduced from 3.58-3.24% and 69.34-51.30%. Pap fortified with groundnuts had protein, fat and moisture content values increased from 15.74- 22.76%, 11.64- 20.27%, 2.08-2.63%. While the ash, fibre and carbohydrate content reduced from 1.04-0.48%, 4.63-2.64%, 64.87-51.50%. Protein, fat and ash content of pap-cashew nuts increased from 12.39-23.63, 10.02-18.65, 0.77-1.26 while moisture, fibre and carbohydrate content reduced from 4.40-3.34, 5.76- 5.45, 66.83-47.68 respectively. Fortifying the pap with all the nuts increased the mineral contents (Na, Ca, K, P, Mg and Zn) of the samples significantly. The functional properties (WAC, OAC, BD, EA and FC) of sorghum-almond nuts values ranged from 1.8- 2.8g/ml, 1.86-2.55g/ml, 0.32-0.83g/ml 49.00-51.00% and 3.70-11.50%, sorghum-groundnuts values ranged from 1.50-3.00ml/g, 0.78-1.37ml/g, 0.31-1.05g/ml, 54.00-55.00% and 5.60-13.60% and sorghum-cashew nuts ranged from 1.50-1.60g/ml, 0.98-1.37g/ml, 0.67-0.95g/ml, 52.0-55.0% and 7.70-22.60% respectively. Pap fortified with Almond nuts gave the best results in terms of protein increase, carbohydrate reduction, mineral and functional improvement and should be encouraged by infants to meet their nutritional needs.

P2.9 - UNDERSTANDING VIRULENCE VARIABILITY AMONG *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES

Mariana Sousa [1(*)]***, Rui Magalhães*** [1]***, Vânia Ferreira*** [1]***, Paula Teixeira*** [1]
[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, Porto, Portugal*

[(*)] *e-mail: marsousa@ucp.pt*

Keywords: clonal complexes; *in vitro* infection; mutation; risk assessment.

Abstracts

Listeria monocytogenes causes a foodborne disease in humans (listeriosis). Strains of *L. monocytogenes* are grouped into distinct clonal complexes (CCs): hypervirulent CCs, more frequently associated with clinical cases, and hypovirulent CCs, mainly recovered from food and less prevalent in clinical infections. Correlations between the attenuated virulence of hypovirulent CCs and the presence of premature stop codons (PMSC) mutations in the *inlA* gene (that codes for Internalin A protein) have been established. In this study, 16 clinical *L. monocytogenes*, distributed among eight CCs, were selected for analysis, to deepen current knowledge regarding putative virulence-inference based on the *L. monocytogenes* CCs and to enable rapid evaluation of *L. monocytogenes* risk within the food industry. Therefore, both presence of PMSC mutations in the *inlA* gene and differences on the ability to the invade the human colorectal adenocarcinoma cell line (Caco-2 cells) were investigated. The majority of the hypervirulent strains carried a full-length InlA (800 amino acids (aa)), while all hypovirulent isolates harbored *inlA* PMSC mutations. Interestingly, two strains from hypervirulent CCs had an InlA that was 797 aa long, representing a 3 aa deletion at positions 741-743. We are the first to characterize the invasion capacity of *L. monocytogenes* strains that carried these 3 aa deletions and our results showed that it did not impact the invasion into Caco-2 cells. All hypovirulent CCs harboring PMSC mutations showed impaired capability of invading the intestinal epithelial cells (Figure 1). The gathered results support that: (i) hypervirulent CCs showed significantly higher invasion efficiencies; (ii) PMSC mutations in the *inlA* gene are related to a decreased invasion capacity into epithelial cells; and (iii) the achievement of a zero *L. monocytogenes* infection risk is challenging since strains from hypovirulent CCs can also cause human listeriosis.

Acknowledgements:
This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GenoPhenoTraits4Persitence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021).

P2.10 - RHAMNOLIPIDS-BASED NANOEMULSIONS LOADED WITH NATURAL PIGMENTS: EFFECT OF PIGMENT CONCENTRATION AND PARTICLE SIZE ON COLOR AND NANOEMULSIONS STABILITY

Mariana B N. Alves [1(*)]***, Miguel A. Cerqueira*** [2]***, Jorge F. B. Pereira*** [1]

[1] *University of Coimbra, CIEPQPF, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, Coimbra, Portugal.*

[2] *International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, Braga, Portugal*

[(*)] *e-mail: mbna@uc.pt*

Keywords: Rhamnolipids; Nanoemulsions; Curcumin; Lycopene; Beta-Carotene; Chlorophyll.

ABSTRACT

Color plays a fundamental role in shaping consumers' sensory perceptions and preferences towards food products. The integration of vibrant colors into consumables is a common strategy to enhance consumers' expectations. Recent legislative instruments, the continued restriction of synthetic colorants, and the growing consumer demand for natural and wholesome products have increased the quest for naturally derived colorants. Despite their numerous advantages compared to their synthetic counterparts, natural pigments face some limitations, including poor water solubility, limited bioavailability, and reduced stability. Consequently, it is imperative to address these limitations, with nanoemulsions emerging as an effective potential solution. Herein, we explored the potential of rhamnolipids to produce nanoemulsions loaded with different natural pigments, such as lycopene, β-carotene, curcumin, and chlorophyll. We studied the influence of pigment concentration on the physical properties and stability of these emulsions over a 7-day period. Subsequently, we identified the conditions that exhibited the most promising outcomes and subjected them to a 30-day stability evaluation. These assessments implicated the measurement of critical parameters, including droplet sizes, polydispersity index, zeta potential, turbidity, and color. Emulsions showcased remarkable stability throughout the experimental period. By changing the sonication periods, the influence of different particle sizes on emulsion color and stability was determined. For lower particle sizes, emulsions demonstrated higher stability against droplet aggregation and gravitational separation. Furthermore, our findings indicated that the nanoemulsions with the smallest droplet sizes exhibited the minimal color variation. This study underscores the efficacy of rhamnolipids as promising alternatives for the creation of stable nanoemulsions loaded with natural pigments. Such innovations hold immense potential for their application in creating naturally colored food products, aligning with the growing consumer preference for clean -label and natural ingredients.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e Tecnologia (FCT). Mariana Alves and Jorge F. B. Pereira also acknowledge the financial support from FCT through the project DRI/India/0044/2020.

P2.11 - EVALUATE THE EFFECTIVENESS OF ULTRAVIOLET LIGHT-EMITTING DIODES FOR FOOD MICROBIOLOGICAL SAFETY

Ana Paula Marques [1,2(*)], Katia Luz [1,3], Maria Teresa Barreto Crespo [1,2], Vanessa Jorge Pereira [1,2]

[1] *Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal;*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, Oeiras, Portugal.*

[3] *Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal;*

[(*)] *e-mail: ana.marques@ibet.pt*

Keywords: Ultraviolet C light emitting diodes; food disinfection; pathogenic bacteria; lettuce.

ABSTRACT

In the last decade, the market of fresh produce had a high consumption increase[1,2]. However, uncooked products may be a common source of microorganisms, including pathogenic bacteria. Foodborne outbreaks associated with the consumption of fresh produce have increased[3]. Every year foodborne diseases are responsible for the loss of 33 million healthy life years[4]. On the other hand, data indicates that about 1.3 billion tons of food suitable for human consumption are wasted every year, with 44% attributed to fruits and vegetables[5]. Disinfection has been described as the most important processing step to guarantee the quality, safety, and shelf-life of fresh produce ready-to-eat. Chlorine has been widely applied due to its disinfection efficacy. However, it is known to produce hazardous by-products[6]. Light emitting diodes (LEDs) coud be an alternative disinfection solution[7]. Three single small LEDs that emit light at 260 and 280 nm were used for the inactivation of *Salmonella enterica* Typhimurium and *Listeria monocytogenes*, that have been associated with foodborne outbreaks, spiked in phosphate-buffered saline and lettuce leaves. Washing water collected at a food industry company was also tested unspiked and spiked with a cocktail of *S. enterica, L. monocytogenes* and *Escherichia coli* before and after exposure to LEDs. The effect of UV-C LEDs on cyclobutane pyrimidine dimers (CPDs) formation was analysed. The results show that a 4-log and 6-log reduction of the target bacteria was achieved applying a UV fluence of 2 mJ/cm^2 and 4 mJ/cm^2, respectively. Unspiked lettuce leaves were also exposed to the LED system and a 2-log reduction of colony count at 30 °C was reach after exposed to a UV fluence of 7 mJ/cm^2 on each side of the leaves. The concentration of CPDs formed were similar for both bacteria and for the same UV fluence of approximately of 6 mJ/cm^2. This disinfection method proved to be promising to inactivate bacteria associated to foodborne diseases.

References:

[1] Esua, O., Chin, N., Yusof, Y., & Sukor, R. (2020). A review on individual and combination technologies of UV-C radiation and ultrasound in postharvest handling of fruits and vegetables. Processes, 8(11), 1433.

[2] Betts, R. (2014). Microbial update fruits & Salad. International Food Hygiene, 23, 9-12.

[3] Callejón, R. M., Rodríguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M. C., & Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes. Foodborne Pathogens and Disease, 12(1), 32-38.

[4] World Health Organization (WHO), Estimating the burden of foodborne diseases. (Access: September 15, 2023): https://www.who.int/activities/estimating-the-burden-of-foodborne-diseases.

[5] FAO. 2018. Food loss and waste and the right to adequate food: making the connection. Rome. 48 pp. Licence: CC BY-NC-SA 3.0 IGO. https://www.fao.org/food-loss-and-food-waste/flw-data

[6] Gadelha, J., Allende, A., López-Gálvez, F., Fernández, P., Gil, M., & Egea, J. (2019). Chemical risks associated

with ready-to-Eat Vegetables: Quantitative Analysis to estimate formation and/or accumulation of disinfection byproducts during washing. EFSA Journal, 17.
[7] Meireles, A., Giaouris, E., & Simões, M. (2016). Alternative disinfection methods to chlorine for use in the fresh-cut industry. Food Research International, 82, 71-85.

Acknowledgements:
This research was funded by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through the Exploratory Research Project SafeFood (Reference 2022.01340.PTDC), National funds to iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). We also thank Professor Paula Teixeira from Escola Superior de Biotecnologia, Universidade Católica Portuguesa for providing the bacterial strains used on this study.

P2.12 - MICROBIAL HYDROLYSATES OF WHEY PROTEIN: GENERATING PROMISING ANTICOAGULANT PEPTIDES

Tiago Paiva [1], Duarte Toubarro [1(*)], Nelson Simões [1]

[1] *CBA - Açores Biotechnology Center (CBA), Faculty of Science and Technology, University of Açores, Ponta Delgada, Portugal.*

[(*)] *e-mail: duarte.nt.tiago@uac.pt*

Keywords: Whey proteins; anticoagulant peptides; functional foods.

ABSTRACT

Whey proteins, which results from the waste of manufacture of cheese, contain about 20% of the proteins found in milk. These proteins are a source of bioactive peptides capable of modulating physiological responses in the body. The best way to obtain bioactive peptides is through enzymatic hydrolysis. Most of the work carried out on the hydrolysis of whey proteins used commercial enzymes with perfectly known cutting sites. In this work we used proteases encoded by a Bacillus mycoides (isolate s102A), from the bank of the CBA, to hydrolyze whey proteins to produce peptides with antithrombotic activity. Enzymatic extract of B. mycoides was used to hydrolyze whey proteins sourced from a local cheese factory. The hydrolysate was fractionated through molecular exclusion and ion exchange FPLC chromatography, and the fractions tested for inhibitory activity in an euglobulin assay. Two active fractions were selected, which exhibited 85% and 96.5% inhibition on euglobulin coagulation. The active fractions were further analyzed by HPLC and mass spectrometry. MS/MS analysis allowed the identification of low molecular weight peptides derived from k-casein, beta-casein, beta-lactoglobulin, and alpha-lactalbumin in active fractions. Among the six peptides synthesized, two of them (βLac1 and Kcase2) exhibited inhibitory effects on euglobulin coagulation. Furthermore, enzymatic and bioinformatic assays unveiled that both βLac1 and Kcase2 peptides share structural features and work synergistically by targeting thrombin's exosite-I and exosite-II. The present work showed that the use of unknown enzymes can provide new bioactive peptides generated from food proteins with great potential as functional foods and nutraceuticals.

Acknowledgements:
This work was supported by the projects UIDP/05292/2020 e UIDB/05292/2020 Financed by FCT. T. Paiva received a PhD fellowship from FCT.

P2.13 - MOLECULAR PROFILING OF HIDDEN INSECT INFESTATION IN STORED RICE GRAINS: A COMPREHENSIVE RT-PCR APPROACH FOR SPECIES DISCRIMINATION AND LIMIT OF DETECTION ASSESSMENT

Ana Campos [1(*)], Inês Gonçalves de Sousa [1,2], Carina Almeida [1,3], Carla Brites [1,4]

[1] *National Institute for Agricultural and Veterinary Research (INIAV), Oeiras, Portugal*

[2] *LEAF (Linking Landscape, Environment, Agriculture and Food) Research Center, Universidade de Lisboa, Tapada da Ajuda, Portugal*

[3] *LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, AliCE – Associate Laboratory in Chemical Engineering, Porto, Portugal*

[4] *GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal*

[(*)] *e-mail: anamaria.campos@iniav.pt*

Keywords: Rice hidden infestation; insect infestation; *Sitophilus oryzae*; *Sitophilus zeamais*; real-time PCR; DNA barcoding; COI.

ABSTRACT

Hidden insect infestation in stored rice grains, inflicted by *Sitophilus oryzae* and *Sitophilus zeamais*, continues to be a critical challenge to both producers and consumers across the globe and has far-reaching implications for food security, economic stability, and public health. These two genetically close insect species, commonly known as rice weevils, have long plagued rice storage facilities and are particularly adapted to attack rice grains, spending a considerable part of their life cycle inside them. Visual inspection cannot successfully detect these insects, and traditional detection methods often fall short in reliably identifying them, making it necessary to develop more robust and sensitive techniques, for precise identification of these insidious insect infestations. To address this pressing issue, this work focused on developing a specific and fast multiplex real- time polymerase chain reaction (qPCR) methodology. Firstly, adopting a DNA barcoding approach, where a standardized region of insect genomes - the cytochrome oxidase I (COI) gene - was used for species-specific primer and probe design and then their evaluation for efficiency. Secondly, an assay to determine the limit of detection (LOD) of the qPCR was done. The designed primers showed 98% and 92% efficiency, for *S. oryzae* and *S. zeamais,* respectively. Regarding the LOD of the qPCR, this methodology can detect until 0,00005 ng/µL of *S. zeamais* DNA and until 0,0025 ng/µL of *S. oryzae* DNA, showing that this technique could detect very low concentrations of these insect species. By doing so, this work offers a suitable tool for stakeholders seeking more effective means to detect, monitor, and combat hidden insect infestation in rice grains.

Acknowledgements:
Funding for this research has been received from TRACE-RICE with Grant nº 1934, (call 2019, section 1 Agrofood) that is part of the PRIMA Programme supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation.
C. Almeida also acknowledges the financial support by LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC).
This study was also funded by the Foundation for Science and Technology (Portugal), through a PhD grant number 2023.02433.BDANA to Inês Sousa.

P2.14 - CHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF TWO QUINOA (CHENOPODIUM QUINOA) FLOUR VARIETIES FROM PERU

Silvia Melissa García-Torres [1,3(*)], José A. Teixeira [2], Christian René Encina-Zelada [3], Cristina Luisa Miranda Silva [1], Ana Maria Gomes [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia - Porto, Portugal*

[2] *Centre of Biological Engineering, School of Engineering, University of Minho - Braga, Portugal*

[3] *Departamento de Tecnología de Alimentos, Facultad de Industrias Alimentarias, Universidad Nacional Agraria La Molina - Lima, Perú*

[(*)] *e-mail: s-smgtorres@ucp.pt*

Keywords: quinoa; nutritional; functional.

ABSTRACT

Food insecurity, malnutrition, and environmental problems are critical challenges for which a solution has been sought for decades. However, no great progress has been made. Although organizations such as FAO, UNICEF, WHO, and WFP make efforts to eradicate hunger and malnutrition by 2030, the goal is far from being achieved, making the situation even worse with the pandemic and the Ukrainian war. One of the strategies proposed to solve this problem is the transformation of food systems, considering sustainability and protection of biodiversity, in addition to increasing access to affordable and healthy diets. In this context, researching and disseminating information about ancestral grains such as quinoa is important, considering that this crop originating from the Andes is world-renowned for its nutritional value, functional properties, and agronomic versatility. Two varieties of quinoa flour from Junín – Peru, were evaluated: Rosada de Huancayo (RH) and Pasankalla (PK). RH was characterized by a good grain size and white color, while the PK showed red color, indicating potential antioxidant properties. In the proximal evaluation, the protein, insoluble dietary fiber, and soluble dietary fiber contents showed significant differences ($p<0.05$). RH variety presented the highest protein content (19.41% ± 0.67, dry basis) compared to the PK (17.35% ± 0.54, DB), while for insoluble and soluble dietary fiber, this was higher in the PK variety (14.60% ± 0.46; 0.96 ± 0.13%, DB respectively). In the case of minerals, zinc, manganese, and copper contents were higher in PK, while a higher phosphorus and potassium content was found in RH. Regarding polyphenols and antioxidant capacity, the PK variety had the highest values and showed significant differences ($p<0.05$) with respect to RH. Both varieties of quinoa presented good nutritional and functional quality, showing values equal to or higher than those of the most consumed cereals such as wheat and corn.

P2.15 - IMPACT OF *CHLORELLA VULGARIS* IN THE SOURDOUGH MICROBIOTA AND CHARACTERISTICS OF SOURDOUGH BREAD

Carla Pestana [1], Nancy Mahmoud [1], Cristiana Nunes [1], Anabela Raymundo [1], Catarina Prista [1(*)]

[1] LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Lisbon, Portugal.

(*) e-mail: cprista@isa.ulisboa.pt

Keywords: Microalgae biomass; wheat bread; sourdough; microbiota; bioactivity.

ABSTRACT

The recent surge in consumer demand for healthier and more delicious wheat bread has prompted the widespread adoption of sourdough in bread from artisanal bakeries employing natural fermentation methods. Microalgae represent a promising and sustainable food ingredient, offering health benefits, but there is limited knowledge regarding the effects of this addition on the sourdough microbiome and on the technological performance of the bread products [1]. In the present work, a sourdough starter containing 4% of the microalga *Chlorella vulgaris* was prepared, monitoring its pH until stabilization over time. Then the microbial diversity of this microalga-enriched sourdough was analyzed and compared to the control sourdough (without microalgae). As expected, the predominant species identified belonged to the *Saccharomycetaceae*, *Lactobacillaceae,* and *Acetobactereacea* families. Interestingly, both growth and diversity of bacteria and yeasts were notably higher in the sourdough with microalgae. Subsequently, a bread dough using either 20% sourdough or 20% sourdough with 0.5% commercial yeast, achieving 1% of microalgae incorporation in relation to the flour weight, was prepared. The quality of bread focused on attributes such as nutritional composition, antioxidant properties, texture and volume, was evaluated. Breads prepared with the microalga-enriched sourdough starter exhibited higher firmness and lower volumes than those fermented by sourdough starter in mixture with baker yeast, but there were no significant differences when microalgae was added at this level for both bread formulations. Moreover, it was observed that the incorporation of *C. vulgaris* biomass into the sourdough starter led to an increase in total phenolic compounds and enhanced antioxidant capacity, measured by DPPH and FRAP assays in the final breads. The results obtained suggest that traditional breadmaking methods involving sourdough and microalgal biomass have a positive impact on the techno-functional performance of these wheat bread doughs. This innovation enhances the potential of this traditional food product.

References:

[1] Perez-Alva et al. (2022). Fermentation of algae to enhance their bioactive activity: A review. Algal Research, 64: 102684.

Acknowledgements:

This work was financially supported by: (i) Project PT-INNOVATION-0075, YUMAlgae - enzYmes for improved sensory qUality of MicroALGAE ingredients in foods (EEA Grants) and (ii) Portuguese Foundation for Science and Technology (FCT), UIDB/04129/2020 of LEAF Research Center.

P2.16 - DISINFECTION OF IRRIGATION WATER USING ULTRAVIOLET LIGHT EMITTING DIODES

Nicole Ferreira [1(*)], Ana Paula Marques [1,2], Maria Teresa Crespo [1,2], Vanessa Pereira [1,2]

[1] *Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, Oeiras, Portugal.*

(*) *e-mail:* nicole.ferreira@ibet.pt

Keywords: Ultraviolet light emitting diodes; irrigation water; disinfection; microorganisms.

ABSTRACT

The development of effective disinfection treatment processes will be crucial to help food producers cope with the inevitable challenges resulting from the increase in human population and climate change. The main goal of this study is to evaluate the implementation of UV-C LEDs at pilot scale using a flow through system that emits light at 280 nm. Prior to the pilot scale experiments, real water samples were collected and characterized in terms of: chemical parameters (chemical oxygen demand, ammonia, chlorine, total nitrogen, nitrate and phosphate), microbiological parameters (total microorganisms that grow at 22°C and 36°C, fungi and yeasts, total coliforms, *Escherichia coli* and *enterococci*) as well as parameters that influence photolysis such as the percent transmittance of the irrigation water (that varied between 40 to 55%). Since the level of microorganisms present in the irrigation water was very low (none of the target microorganisms were detected in the samples after 10min of exposure to three small LEDs), the inactivation efficiency of UV-C LEDs that emits light at 280 nm was also tested in real irrigation water samples spiked with *Escherichia coli* (DSM18039). The pseudo-first order inactivation rate constant obtained was 0.031±0.001s^{-1}. A low UV fluence of 15mJ/cm^2 led to a 6-log reduction of *E. coli*. The obtained results prove that this treatment is extremely effective to achieve inactivation of irrigation water and will be evaluated at pilot scale in an irrigation line, under real conditions.

Acknowledgements:
This work was supported by iBET through the iBETXplore FoodLED project. It was also funded by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through national funds to iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). The authors thank AquiSense Technologies for the PearlAqua Deca™ system that will be used in the pilot scale inactivation assays.

P2.17 - PATHOGENICITY AND VIRULENCE OF *LISTERIA MONOCYOTOGENES* ISOLATES FROM DAIRY FARMS

Ana Rita Barata [1,2], Bárbara Nunes [1,3], Teresa Nogueira [1,4], Maria José Saavedra [2,3,5], Gonçalo Almeida [1,6(*)]

[1] *National Institute of Agricultural and Veterinary Research, I.P (INIAV), Vila do Conde, Portugal*

[2] *Escola de Ciências da Vida e do Ambiente, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[3] *Departamento das Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[4] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & Global Change and Sustainability Institute (CHANGE), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal*

[5] *Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB) & Institute for Innovation, Capacity building and Sustainability of Agri-Food production (Inov4Agro), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[6] *Center for Animal Science Studies - Institute of Agricultural and Agro-Food Sciences and Technologies (CECA-ICETA), University of Porto, Porto, Portugal*

(*) *e-mail:* goncalo.almeida@iniav.pt

Keywords: *Listeria monocytogenes*; Microbiology; Dairy farms; WGS; Food safety.

ABSTRACT

Listeria monocytogenes serves as foodborne pathogen capable of inducing listeriosis in human and animal hosts. It has evolved a remarkable capacity to adapt to diverse stress conditions encountered in various environments has contributed to its pervasive distribution. Given that certain food preservation methodologies and disinfection protocols employed in food-processing environments exhibit limited efficacy in preventing contamination, *L. monocytogenes* represents a substantial threat to human health and a challenge to food safety. In the present study detection of *L. monocytogenes* was performed using the real-time PCR method. Colonies were confirmed using microbiological methods described in ISO 11290. A collection of 9 *L. monocytogenes* isolates were sequenced. Our results demonstrated that 44% of the isolates were identified as IVb, a serogroup related to a high number of outbreaks of human listeriosis, and 22% to IVbv1, referred to as an emerging risk. The present study also reveals that CCs already found in foods and clinical cases are present in farm environment. The virulence gene cluster essential for intracellular parasitism was detected in all isolates. Genes such as *sigB* and *SSI-1* that allow survival when bacterial cells are exposed to lethal conditions, have been detected. The diversity of virulence genes was confirmed in the present study. This study has evaluated the presence of *L. monocytogenes* on dairy farms and discover the main clonal complexes present. The prevalence in the agricultural environment highlights the importance of understanding the ecology and transmission of *L. monocytogenes* and the need to increase measures that can reduce its presence through food chain, increasing food safety.

Acknowledgements:
The authors thank CECA-ICETA UIDB/00211/2020; CITAB UIDB/AGR/04033/2020 and to FCT project GenoPheno4trait Ref. PTDC/BAA-AGR/4194/2021 for their support.

P2.18 - ENZYMATIC SYNTHESIS OF MONOSACCHARIDE FATTY ACID ESTERS

Bruna Basto [1,2(*)], Mara Guadalupe Freire [3], José António Teixeira [1,2], Sara Cruz Silvério [1,2]

[1] *CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[3] *CICECO-Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal*

(*) *e-mail:* bb_basto@hotmail.com

Keywords: Butyric Acid; Esterification; Lipase, Monosaccharides; Sugar fatty acid esters.

ABSTRACT

The non-ionic and biodegradable surfactant family known as sugar fatty acid esters (SFAEs) includes a wide range of products broadly used in several industrial applications. They are considered as important compounds in the food due to their remarkable technological properties. SFAEs are composed of lipophilic fatty acid moieties and hydrophilic sugar head groups which result in particular physicochemical characteristics that are the basis for their broad range of applications. A great variety of coupling possibilities between hydrophilic sugar head groups and hydrophobic alkyl chains can be explored, leading to the production of different SFAEs with promising industrial features. SFAEs can be obtained by chemical esterification. However, the alternative synthesis by enzymatic route using lipases as biocatalysts has gained increased attention in the last decades. When enzymes are used as catalysts, reactions are carried out in mild conditions, avoiding product degradation. Moreover, enzymes are biodegradable and present higher specificity, thus minimizing the formation of undesirable side-products. In this work, the enzymatic synthesis of SFAEs in *t*-butanol was followed and qualitatively evaluated by thin layer chromatography. The esterification between different mono-, di- or trisaccharides and butyric acid was performed by commercial immobilized Lipase B from *Candida antarctica*, at 60°C. The results showed that SFAEs were successfully biosynthesized under these conditions using monosaccharides.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020. BB acknowledges her PhD grant from FCT (2020.05612.BD).

P2.19 - ASSESSING THE PREBIOTIC POTENTIAL OF XYLOOLIGOSACCHARIDES PRODUCED BY ONE-STEP FERMENTATION USING AGRO-RESIDUES

Ana Cordeiro [1,2,3(*)]***, Andreia Fernandes*** [3]***, Joana Sousa*** [3]***, Beatriz Cardoso*** [3]***, Joana Alves*** [3,4]***, Sara Silvério*** [3,4]***, António Peres*** [1,2]***, Lígia Rodrigues*** [3,4]***, Cláudia Amorim*** [3,4]

[1] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança;*

[2] *Laboratório Associado para a Sustentabilidade e Tecnologia em Região de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal;*

[3] *CEB – Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga, Portugal;*

[4] *LABBELS – Associate Laboratory, Guimarães, Braga, Portugal;*

(*) *e-mail:* anacordeiro.ac03@gmail.com

Keywords: Coffee silver skin; Human faecal inocula; Olive Stones; Prebiotic Effect; Xylooligosaccharides.

ABSTRACT

The prebiotic effect is a fundamental concept in the fields of nutrition and gut health, referring to the beneficial effects of specific non-digestible dietary components on the gut microbiota, including xylooligosaccharides (XOS). These compounds function as food sources for beneficial gut bacteria, fostering their growth and activity. In this work, *in vitro* studies were performed to evaluate the prebiotic potential of XOS produced from olive stones (OS) and coffee silver skin (CSS) via a one-step fermentation using a recombinant *Bacillus subtilis* 3610 harbouring the xylanase gene *xyn2* from *Trichoderma reesei*. This potential was compared with a commercially available prebiotic oligofructose (Orafti®, BENEO, Germany). A mixture of human faeces from four healthy donors aged between 24 and 28 years old was used as inoculum. The pH variation and the production of short-chain fatty acids (SCFAs), gases, and ammonia were analysed during the 48 hours fermentations. The prebiotic supplementation resulted in a reduction of the pH value over time, with oligofructose presenting the most significant pH drop at 48 hours (ΔpH=3.65). The addition of prebiotics also significantly increased the production of beneficial SCFAs, with oligofrutose exhibiting a notable increase in the production of lactic and acetic acid production after 48 hours (28.0±0.1 and 28±1 mM, respectively), while OS-XOS and CSS-XOS demonstrated a more prominent rise towards the production of acetic acid (14.8±0.4 and 20.4±0.1 mM, respectively), butyric acid (2.5±0.3 and 3.29±0.04 mM, respectively), and valeric acid (75±1 and 110±14 mM, respectively) at 48 hours. Remarkably, the gas analysis revealed that the addition of OS/CSS-XOS fully suppressed the production of CH_4 and increased the CO_2 generation after 48 hours (2.6±0.7 and 5.20±0.05 $mmol.L^{-1}$medium, respectively). These findings strongly suggest that the XOS produced from OS and CSS holds potential prebiotic properties for human health.

Acknowledgements:
CA, AC and JS acknowledge the junior researcher contract (CEECIND/00293/2020) and the PhD scholarships (UI/BD/153689/2022 and 2022.11695.BD), respectively, from the Portuguese Foundation of Science and Technology (FCT). This study was supported by FCT under the scope

of the strategic funding of CIMO (UIDB/00690/2020 and UIDP/00690/2020), CEB (UIDB/04469/2020) and Associate Laboratory SusTEC (LA/P/0007/2020).

P2.20 - HIGH-THROUGHPUT MICRO-PLATE FLUORESCENCE QUENCHING FOR SCREENING THE BINDING OF MYCOTOXINS TO ALBUMINS

Heidar Adalsteinsson [1], Lavínia Pinto [1], Diana G. Rodrigues [1], Lucília Domingues[1,2], António A. Vicente [1,2], Tatiana Q. Aguiar [1,2], Luís Abrunhosa [1,2] (*)

[1] CEB – Centre of Biological Engineering. University of Minho, Braga, Portugal
[2] LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

(*) e-mail: luisjap@deb.uminho.pt

Keywords: Food safety; Mycotoxin; Analytical control; Patulin; Ochratoxin A; Zearalenone.

ABSTRACT

Filamentous fungi, which commonly contaminate food and animal feed, produce toxic metabolites called mycotoxins. Legal limits on their maximum levels in food require food producers to carry out highly specific analytical tests for mycotoxins, such as Ochratoxin A (OTA), patulin (PAT) and zearalenone (ZEN). Serum albumins are known to bind some mycotoxins, presenting an opportunity for the development of novel capture and clean-up strategies. The aim of this study was to validate a fluorescence quenching micro-plate methodology for high-throughput screening of the binding constants of OTA, PAT and ZEN to albumins.

Herein, we present measurements of equilibrium binding constants of these mycotoxins to several albumins using fluorescence quenching, measured in 96-well micro-plates. In addition, commercial native human serum albumin (HSA) was compared to recombinant HSA produced by *Pichia pastoris* KM71H transformed with the pPICZ9ssHSAH6 vector. Both OTA and ZEN showed slightly higher affinity for recombinant *versus* native HSA, whereas the difference was negligible for PAT. The order of affinity for native HSA is OTA>ZEN>PAT, with binding constants, Ksv (L/mol), of 6.22 x 10^5, 2.97 x 10^4 and 9.50 x 10^3, respectively. The use of different buffers (pH 7.0-7.4) was also tested, where the affinity of ZEN for HSA decreased slightly in PBS compared to 10 mM Tris-HCl and 20 mM PB, whereas PAT binds more strongly to HSA in PB. The affinity of mycotoxins to bovine- and rat-serum albumins (BSA, RSA) was also tested and compared to HSA. For ZEN the order of affinity was found to be RSA>HSA>BSA, and HSA> BSA>RSA for OTA and PAT. The results will aid in the development of novel protein-based extraction columns for analytical control.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and project MycoProAffinity-2022.03438.PTDC (https://doi.org/10.54499/2022.03438.PTDC).

P2.21 - EFFECT OF *MELISSA OFFICINALIS* ESSENTIAL OIL ON BIOFILM FORMED BY *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM MEAT INDUSTRIES

Alexandra Coimbra [1(*)], ***Susana Ferreira*** [1], ***Felice Panebianco*** [2]
[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[2] *Department of Veterinary Sciences, University of Turin, Turin, Italy*

[(*)] *e-mail:* alexandra.coimbra@hotmail.com

Keywords: *Listeria monocytogenes*; *Melissa officinalis*; antimicrobial activity; anti-biofilm.

ABSTRACT

Among the pathogenic microorganisms that may be found in the food industries, *Listeria monocytogenes* is a major causative agent of foodborne illness that carries high mortality rates. The ability to persist in different environments due to its biofilm forming capacities allows this bacterium to survive on surfaces for extended periods of time with little access to water and nutrients. The essential oils, due to their potential bioactivities, have been considered as potential alternatives to conventional biocides and could be employed to counteract microbial biofilm in food processing environments. *Melissa officinalis* is a medicinal plant that is known for its biological properties, including antimicrobial effects. Thus, this work aimed to evaluate the activity of *M. officinalis* essential oil against biofilm formed by different *L. monocytogenes* strains.

The antibacterial activity of *M. officinalis* essential oil against *L. monocytogenes* (six wild-type strains isolated from meat processing industries and two reference strains) and the attenuation of the bacterium's virulence were evaluated. The antibacterial activity of *M. officinalis* essential oil was observed with the determination of minimum inhibitory concentration (MIC) and the minimum lethal concentration (MLC), obtaining values between 0.125 and 2 µL/mL. Further, the essential oil showed effect on *L. monocytogenes* virulence related factors like biofilm formation and even the elimination of previously formed biofilms, which was evaluated using the Crystal Violet Staining method for determination of inhibition percentage and the MTT assay for evaluating the metabolic activity. The concentration equal to twice the MLC value inhibited biofilm formation and the preformed biofilm by more than 50% in almost all strains under study.

These results indicate that *M. officinalis* essential oil has a good antibacterial activity against *L. monocytogenes*, and it has the potential to be used as sanitizer to ensure the microbiological safety of surfaces that promote biofilm development.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES, and within the scope of the project "Assessment of innovative eco- friendly technologies to contrast Listeria monocytogenes biofilm in the food industry"-PANF_GFI_22_01_F- Grant for Internationalization 2022 of the University of Turin.

P2.22 - ANTIFUNGAL CAPACITY OF A COMMERCIAL FLAVOURING AGENT AGAINST SPOILAGE YEASTS

Beatriz Nunes Silva [1,2], Teresa Bento de Carvalho [1], Miguel Azevedo [3], Beatriz Silva [3], Paula Teixeira [1(*)]

[1] Universidade Católica Portuguesa, Laboratório Associado, CBQF—Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Porto, Portugal
[2] CEB—Centre of Biological Engineering, University of Minho, Braga, Portugal
[3] Decorgel – Produtos Alimentares S.A., Trofa Portugal

[(*)] e-mail: pcteixeira@ucp.pt

Keywords: clean label preservatives; fungal spoilage; antimicrobial; Zygosaccharomyces.

ABSTRACT

Yeasts are involved in the spoilage of foods and beverages, causing undesirable changes in the physicochemical and sensory properties of the products that are, often, very evident. Prevention of food spoilage requires good manufacturing and hygienic practices, but additives are also frequently used to hinder yeast growth. Nowadays, consumers and companies are moving from chemical additives towards other options that can be perceived as more natural and less harmful to human health.

With this in consideration, the goal of this study was to assess the antifungal capacity of a commercial flavouring against four *Zygosaccharomyces* species isolated from pastry fillings, *Z. bailii*, *Z. parabailii*, *Z. bisporus* and *Z. rouxii*, and two yeasts isolated from thermo-sealed packaging used by a pastry company. The flavouring concentrations tested were 0.15%, 0.3%, 0.9%, 1.5% and 3% (w/v), in accordance with the manufacturer's dosage recommendations.

After 48 hours at 25 °C, the commercial flavouring did not inhibit any of the four *Zygosaccharomyces* yeasts, even at 3%. The two yeasts isolated from thermo-sealed packaging were unaffected by flavouring concentrations of 1.5% and below; however, at 3%, the growth of one of the yeasts was reduced, whereas the other was completely inhibited, which indicated a reduction of around 5 log CFU/mL.

While this commercial flavouring did not exhibit antifungal activity against all the tested yeasts, the results are still encouraging. The flavouring tested offers a natural alternative to the chemical preservatives currently used in the pastry industry, such as potassium sorbate. This natural alternative can help to hamper the growth of yeasts that may be detected during the manufacturing process. The presence of yeasts poses a serious threat in terms of food spoilage, product loss and consumer perception.

Acknowledgements:
This work was developed in the scope of the project “Agenda VIIAFOOD project – Platform for Valorization, Industrialization and Commercial Innovation for the Agro-Food Industry, funded by the Portuguese Recovery and Resilience Plan (“PRR”) in its component 5 – Capitalization and Business Innovation, under the Regulation of the Incentive System “Agendas for Business Innovation” and Invitation Notice n. 02/C05-i01/2022, and specifically in the sub-project “Development of preservative free preparations to the pastry industry with natural ingredients”. Special thank you to Decorgel´s Research & Development Department, Trofa, Portugal, for the collaboration and the opportunity.

P2.23 - PURIFICATION AND CHARACTERIZATION OF A BACTERIOCIN PRODUCED BY *ENTEROCOCCUS FAECALIS* L3A21K6

Susana C. Ribeiro [1], ***Célia C.G. Silva*** [1,2(*)]

[1] *Institute of Agricultural and Environmental Research and Technology (IITAA), University of the Azores, Angra do Heroísmo, Azores, Portugal*
[2] *Faculdade de Ciências Agrárias e do Ambiente (FCAA). Universidade dos Açores, Angra do Heroísmo, Portugal*

[(*)] *e-mail: celia.cg.silva@uac.pt*

Keywords: *E. faecalis* L3A21K6; Bacteriocin; purification; characterization.

ABSTRACT

Food safety and food security are a global concern and remain a major challenge for the food industry. In the search for different biopreservatives, as an alternative to the use of chemical preservatives, bacteriocins have attracted attention as novel food preservatives. Bacteriocins are ribosomally synthesized peptides that in most cases exhibit antibacterial activity against bacteria closely related to the producing bacteria. Bacteriocins produced by lactic acid bacteria (LAB) have been widely explored as a new type of biological preservative due to their safety, high efficiency, and non-toxic properties. In this study, a bacteriocin produced by *Enterococcus faecalis* L3A21K6 isolated from an Azorean artisanal cheese was purified and characterized. This strain exhibited antimicrobial activity against *Listeria monocytogenes* and *Clostridium perfringens*. The common bacteriocin structural genes were not detected by PCR amplification with specific primers. Purification of bacteriocin was performed in a three-step procedure using ammonium sulphate precipitation, a Sep-Pak cartridge microcolumn and RP-HPLC. Peptides were precipitated from the CFS with 80% ammonium sulphate. The pellet was dissolved with phosphate buffer (5mM, pH 6.5) and passed through a Sep-Pak cartridge microcolumn. Further purification was carried out by RP-HPLC. The chromatogram showed a single pick that exhibited anti-listeria activity at a retention time of 32 min. This purified peptide showed an anionic charge that is unusual for bacteriocins described in the literature. The molecular weight of the bacteriocin was approximately 3.0 to 3.5 kDa, as revealed by tricine sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis. Characterisation studies showed that the antimicrobial activity of bacteriocin was not affected by pH (2 to 12), heating (100°C) and surfactants. It showed bacteriostatic mode of action against *L. monocytogenes* and maximum production was observed during stationary phase. These results suggest that this bacteriocin has great potential for use as a biopreservative in the food industry.

Acknowledgements:
This work was supported by Fundação para a Ciência e Tecnologia (FCT), project UIDP/00153/2020.

P2.24 - MICROBIOLOGICAL CONTAMINATION OF FRESH CHICKEN MEATS AND OFFALS IN THE RETAIL MARKETS

Sónia Saraiva [1,2,3(*)], ***Cristina Saraiva*** [1,2,3 (**)], ***Kamila Soares*** [1,2,3], ***Ana Leite*** [1], ***Thomai Lazou*** [4]

[1] *Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*

[2] *Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes e Alto (UTAD), Vila Real, Portugal*

[3] *AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences; Portugal*

[4] *Department of Hygiene and Technology of Food of Animal Origin, School of Veterinary Medicine, Aristotle University of Thessaloniki | AUTH*

[(*)] *e-mail: soniasarai@gmail.com;* [(**)] *e-mail: crisarai@utad.pt*

Keywords: *Escherichia coli; Salmonella* sp.; *Pseudomona*s spp.; contamination of chicken meat; offals contamination; spoilage.

ABSTRACT

Microbial contamination of chicken meats and offals can occur during poultry production and slaughter processes. *Enterobacteriaceae, Escherichia coli* and *Salmonella* sp. can be used to monitor enteric contamination and *Pseudomonas* spp. as an indicator of spoilage.

The aim of th*is* study was to assess the bacterial contamination of chicken meat products and offals, from local supermarkets and butcheries. A total of 72 samples, namely neck (n=12), liver (n=14), heart (n=16), gizzard (n=14) and feet (n=16), were analysed for *E. coli*, *Enterobacteriaceae*, *Salmonella* sp. and *Pseudomonas* spp., total mesophilic and psychrotrophic quantification, according to ISO methods. *E. coli* was isolated in 63 (87.5%) samples and *Salmonella* spp. in 9 (12.5%). A significant effect of the type of establishment was observed for *E. coli* with samples from local butcheries showing higher counts (2.60 cfu/g vs. 2.18 cfu/g; *P*<0.001). Neck is the product with highest average levels (3.69±0.77 cfu/g). Gizzard (1.24±1.08 cfu/g) showed the best results. Higher counts of *Enterobacteriaceae*, total mesophilic and psychrotrophic were observed in samples from local butcheries and, for *Pseudomonas* spp. in supermarkets.

Significant differences were observed in relation to the type of product, with feet showing the highest average levels for *Pseudomonas* spp. (6.41 ±1.09), mesophilic (6.93±0.77) and psychrotrophic (6.41±1.09), and liver showing the lowest counts for *Pseudomonas* spp. (4.69± 0.72) and psychrotrophic (5.59± 0.86) and also gizzard for mesophilic (5.55 ±0.66).

Monitoring the microbiological quality and exploring the sources of contamination are crucial to maintain high quality of lower price products and the safety of the consumers.

Acknowledgements:

The authors would like to thank CECAV and the support of the projects UIDB/CVT/00772/2020 and LA/P/0059/2020, funded by the Portuguese Foundation for Science and Technology (FCT).

P2.25 - EXPLORE LIGHT EMITTING DIODES AND PHOTOCATALYTIC SURFACES TO PREVENT BIOFILM FORMATION IN FOOD INDUSTRY

Carolina Santos [1(*)], ***Ana Paula Marques*** [1,2], ***João Sério*** [1,2], ***Maria Teresa Barreto Crespo*** [1,2], ***Vanessa Jorge Pereira*** [1,2]

[1] *iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

[(*)] *e-mail: carolina.santos@ibet.pt*

Keywords: Surface disinfection; UV-C LEDs; Photocatalytic surfaces; *Listeria monocytogenes: Escherichia coli.*

ABSTRACT

Pathogenic microorganisms can contaminate food and food contact surfaces and form biofilms (aggregates of microorganisms from one or more species that are enclosed in a matrix of extracellular polymeric substances) that can be the source of foodborne diseases and contribute to the deterioration of food contact surfaces. The removal of biofilms is a very challenging task.

This work aimed to explore the effectiveness of the inactivation of *Listeria monocytogenes* biofilms with ultraviolet light using the emergent technology of light emitting diodes (LEDs) as a potential effective alternative to the use of ultraviolet (UV) mercury lamps. *Listeria monocytogenes* biofilms were produced at room temperature and at 4 ˚C in stainless-steel discs. Biofilms were analysed before and after short exposures (2.5 min and 5 min) to three small LEDs that emit UV light at 265 nm. 1 to 2-log reductions were obtained in all the inactivation assays. This disinfection method was also tested to disinfect stainless-steel discs that were inoculated with a cell suspension of *Listeria monocytogenes* as well as a multidrug resistant environmental *Escherichia coli* isolate. A 2-log reduction was achieved after 5 min of exposure. Scanning electron microscope images were obtained before and after the exposure to UV-C LEDs and some changes on the cell morphology were observed.

Photocatalytic surfaces were also tested for biofilm removal. Stainless steel discs modified with a titanium dioxide solution were also used to produce *Listeria monocytogenes* biofilms that were then exposed to LEDs. A 0.8-log reduction was achieved in this experiment.

The use of UV-C LEDs proved to be promising approach to disinfect surfaces and could be used by food, medical and pharmaceutical industries.

Acknowledgements:
This research was funded by iBET through the LightUp project, iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020)., a program financially supported by Fundação para a Ciência e Tecnologia/ Ministério da Ciência, Tecnologia e Ensino Superior, through national funds, is gratefully acknowledged. We also thanks to Professor Paula Teixeira from Escola Superior de Biotecnologia, Universidade Católica Portuguesa for providing the Listeria monocytogenes strain that was used in this study.

P2.26 - MODELING LACTIC ACID PRODUCTION IN *LACHANCEA THERMOTOLERANS*

Marcos Esteves [1,2], ***Véronique Gomes*** [1], ***Rogério Tenreiro*** [2], ***Marco Reis*** [3], ***Nuno P. Mira*** [4], ***Ana Mendes-Ferreira*** [1,2(*)]

[1] *WM&B - Wine Microbiology & Biotechnology Laboratory, Universidade de Trás-os-Montes e Alto Douro*

[2] *BioISI - Biosystems and Integrative Sciences Institute*

[3] *Department of Chemical Engineering, University of Coimbra, CIEPQPF*

[4] *Department of Bioengineering - Instituto Superior Técnico; iBB - Institute of Bioengineering and Biosciences*

[(*)] *e-mail: anamf@utad.pt*

Keywords: *Lachancea thermotolerans*; lactic acid; fractional factorial experimental design.

ABSTRACT

After a long period in which non-*Saccharomyces* genera were seen exclusively as spoilage yeasts in the wine industry, presently various species are being increasingly investigated because they can provide solutions to emerging problems through their metabolic systems. Among them, *Lachancea thermotolerans* has the unique trait of fermenting grape-juice sugars into both ethanol and lactic acid, positively affecting wine acidity. This trait is appealing for wine industry since in several regions of the globe, due to climate change, wines are being more prone to contain excessive ethanol and lack acidity, as a direct result of grapes overripening. Previous reports have shown that *L. thermotolerans* strains exhibit great variations in lactic acid production, with the mechanisms underlying this production still not well known to allow the understanding of its variability.

This study aimed to evaluate how four fermentation parameters, sugar and nitrogen levels, pH, and temperature, affect the growth and lactic acid production of *L. thermotolerans*. To achieve this, a 2-level fractional factorial (IV resolution) experimental design was defined, resulting in a total of eleven fermentations that were conducted in synthetic grape-juices. Through Response Surface Methodology (RSM), the impact of the parameters on lactic acid production was assessed and the optimal conditions that enable its maximum production were identified. Our promising results provide further knowledge towards the understanding of the physiology of this yeast species and its rational application in wine industry as an acidity modulator.

Acknowledgements:
This work was financially supported by SMARTWINE 2.0 project with the reference 2022.06777.PTDC, financed by FCT - Portuguese Foundation for Science and Technology. Marcos Esteves is a recipient of a PhD grant with the reference PD/BD/150587/2020 from the FCT Doctoral Program in Applied and Environmental Microbiology (DP_AEM).

P2.27 - FRUIT PEELS AS SOLID-STATE FERMENTATION SUBSTRATES FOR THE SUSTAINABLE PRODUCTION OF MICROBIAL PROTEIN

Helena Vilela [1], Liliana Araújo [1(*)], Isabel Belo [1,2], Rita Pinheiro [3], Marlene Lopes [1,2]

[1] CEB–Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal
[2] LABBELS–Associate Laboratory, Braga/Guimarães, Portugal
[3] Center for Research and Development in Agrifood Systems and Sustainability, Polytechnic Institute of Viana do Castelo, Viana do Castelo, Portugal

[(*)] e-mail: lparaujo@ceb.uminho.pt

Keywords: mycoprotein; *Aspergillus ibericus*; *Rhizopus oryzae*; orange peels; banana peels.

ABSTRACT

Protein consumption is continuously increasing owing to the rise of the global population, which is expected to reach 9.7 billion by 2050, resulting in an unsustainable increase in demand for animal and plant foods. Microbial protein, an alternative protein source produced by microorganisms, has the potential to be incorporated into food and feed diets.

This study evaluated the potential of fruit by-products (orange and banana peels) as substrates for solid-state fermentation (SSF) to produce microbial protein with filamentous fungi. The main effects of several factors were studied in a Plackett-Burman experimental design at 2 levels: moisture content (60% and 75%), incubation time (7 days and 14 days), inoculum size (1×10^5 spores/g and 5×10^5 spores/g), nitrogen supplementation (0 g/g and 0.01 g/g), and fungal species (*Aspergillus ibericus* and *Rhizopus oryzae*).Total protein of fermented orange and banana peels increased by 179% and 46%, respectively, relative to non-fermented peels. The antioxidant activity of fermented peels increased 2.7- and 5-fold for orange and banana peels, respectively. Thereafter, the effect of moisture (50%, 60%, 70%), ammonium sulphate (0 g/g, 0.005 g/g, 0.01 g/g), and corn steep liquor (0 g/g, 0.005 g/g, 0.01 g/g) were studied in a Box-Behnken experimental design to optimize protein production by *A. ibericus*. The SSF process improved the nutritional value of fruit peels, increasing the total protein content by 239 % and 121 % for orange and banana peels, respectively. The results demonstrate that orange and banana peels are potential substrates for biotechnological production of microbial protein by *A. ibericus.* This process represents a step forward for food and industrial biotechnology owing to its potential to obtain a promising protein source with nutritional value and its positive impact on the environment through the valorization of discarded food-grade by-products.

Acknowledgements:
Portuguese Foundation for Science and Technology (FCT): UIDB/04469/2020, 2022.01705.PTDC; LABBELS–Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

P2.28 - HIGH-PRESSURE HOMOGENIZATION TREATMENT TO OBTAIN CREAMS USING TOMATO WASTE

Helena Ribeiro [1,2(*)], ***Boelo Schuur*** [2], ***Jorge F. B. Pereira*** [1]

[1] *University of Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal*
[2] *University of Twente, Faculty of Science and Technology, Green Energy Initiative, Sustainable Process Technology Group, The Netherlands*

[(*)] *e-mail:* helenaribeiro270@gmail.com

Keywords: Creams; High-pressure homogenization; Lycopene; Tomato waste.

ABSTRACT

Food waste valorization, through the recovery and production of valuable products in a biorefinery concept, improves the sustainability and economic competitiveness of agro-food industries. Tomato waste is one representative example, in which 20-40% of its fresh weight is discarded (including peels, pomace and seeds), still containing highly valuable biological active compounds, such as carotenoids, lipids, crude fibers, carbohydrates and crude proteins [1, 2]. Lycopene is the major carotenoid present, mainly found in the skin fraction (72–92%) of tomatoes [3, 4]. This tomato fraction is of upmost interest since peels are one of the main residues of tomato-processing industries. However, the selection of the extraction method to recover the added-value compounds is still the most challenging step, due to the complexity and presence of several physicochemical obstacles in these food materials [5]. Thermal and mechanical treatments have been applied to reduce the mass transfer issues, and/or to reduce the use of organic solvents [6]. Among these, high-pressure homogenization (HPH) has been pointed as a fast and effective method to micronize plant tissue in suspension and to release the bioactive compounds entrapped in cells, enhancing high extraction yields [7].

In this work, we evaluated the extraction of bioactive compounds from the tomato waste using HPH. Solutions containing 10 wt% of tomato waste and water and water + sunflower oil were prepared and subjected to a HPP treatment for up to 10 passes. Samples were taken after different passes for further analysis.

The development of efficient, simple, and economical cell disruption operations with bio-based solvents will not only allow the development an effective rupture of the cell wall, but also the solubilization and maintenance of the biological activities of the intracellular solutes, which still represents a major challenge in the recovery of bioactive compounds from food waste.

References:

[1] D. D. Adi, I. Oduro, and B. K. Simpson, “Biological and Microbial Technologies for the Transformation of Fruits and Vegetable Wastes,” in Byproducts from Agriculture and Fisheries: Adding Value for Food, Feed, Pharma, and Fuels, 2020, pp. 403–420.
[2] R. K. Saini, S. H. Moon, and Y. S. Keum, “An updated review on use of tomato pomace and crustacean processing waste to recover commercially vital carotenoids,” Food Res. Int., vol. 108, pp. 516–529, 2018.
[3] C. Fritsch et al., “Processing, valorization and application of bio-waste derived compounds from potato, tomato, olive and cereals: A review,” Sustain., vol. 9, no. 8, pp. 1–46, 2017, doi: 10.3390/su9081492.
[4] Y. P. A. Silva, T. A. P. C. Ferreira, G. B. Celli, and M. S. Brooks, “Optimization of Lycopene Extraction from Tomato Processing Waste Using an Eco-Friendly Ethyl Lactate–Ethyl Acetate Solvent: A Green Valorization Approach,” Waste and Biomass Valorization, vol. 10, no. 10, pp. 2851–2861, 2019, doi: 10.1007/s12649-018-0317-7.

[5] B. J. Allison and C. W. Simmons, "Valorization of tomato pomace by sequential lycopene extraction and anaerobic digestion," Biomass and Bioenergy, vol. 105, pp. 331–341, 2017, doi: 10.1016/j.biombioe.2017.07.019.
[6] S. Jurić, M. Jurić, G. Ferrari, A. Režek Jambrak, and F. Donsì, "Lycopene-rich cream obtained via high-pressure homogenisation of tomato processing residues in a water–oil mixture," Int. J. Food Sci. Technol., vol. 56, no. 10, pp. 4907–4914, 2021, doi: 10.1111/ijfs.15243.
[7] W. Mustafa, G. Pataro, G. Ferrari, and F. Donsì, "Novel approaches to oil structuring via the addition of high-pressure homogenized agri-food residues and water forming capillary bridges," J. Food Eng., vol. 236, pp. 9–18, 2018, doi: 10.1016/j.jfoodeng.2018.05.003.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e Tecnologia (FCT) through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. Helena Ribeiro acknowledges to FCT for PhD Grant (UI/BD/150909/2021).

P2.29 - CHARACTERIZATION OF *CAMPYLOBACTER* SPP. ISOLATES FROM POULTRY SLAUGHTERED FOR HUMAN CONSUMPTION IN PORTUGAL

Sónia Ramos [1], Maria-Leonor Lemos [2,3], Ludovica Di Bella [1,4], Selma Marques [2], Margarida Penteado [1], Margarida Alves [1], Adriana Belas [1], Isabel Santos [1], Ana Lima [1], Laurentina Pedroso [1], João Paulo Gomes [1,5], Alexandra Nunes [1,5(*)], Mónica Oleastro [2]

[1] Veterinary and Animal Research Centre (CECAV), Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal
[2] National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
[3] ICBAS-Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal.
[4] Faculty of Veterinary Medicine, University of Sassari, Sassari, Italy
[5] Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

(*) e-mail: alexandra.nunes@insa.min-saude.pt

Keywords: One Health; Zoonosis; Public Health; *Campylobacter*; Antimicrobial resistance; MDR; *Helicobacter pullorum.*

ABSTRACT

Campylobacteriosis is the most reported zoonosis in the European Union (EU), and antimicrobial resistance can be of concern, with Portugal reporting one of the highest rates of resistance to critical antibiotics in the EU. Although food-products of animal origin are one of the main sources of campylobacteriosis for humans, data on *Campylobacter* resistance in Portuguese animals is not abundant. Focusing on the food-producing poultry reservoir, this study aims to understand the epidemiology and population structure of multi-resistant *Campylobacter* isolates in Portugal, in order to clarify the sources and transmission dynamics.

A total of 215 samples of poultry feces (90 chickens, 125 turkeys) were collected in 13 different Portuguese slaughterhouses. After isolation according to ISO 10272-1:2017 (E), suspected *Campylobacter* spp. isolates were confirmed and identified by MALDI-TOF. Antimicrobial susceptibility to a panel of clinically relevant antibiotics was tested. All multidrug-resistant isolates were also genetically characterized by WGS.

Although no *Campylobacter* spp. was found in turkeys, eight isolates of *Campylobacter coli* were identified on chicken samples. Seven *C. coli* isolates showed a multidrug resistance (MDR) profile to ciprofloxacin, tetracycline, erythromycin and ampicillin, with four of them even showing decreased susceptibility to the combination of amoxicillin with clavulanic acid. Among the targets responsible for these resistances, the presence of the *tet*O and *bla*OXA-61 genes stands out, as well as known mutations in the *gyr*A and 23SrRNA genes. Comparative phylogenomic analysis showed a segregation of these isolates according to ST-MLST.

Interestingly, for 83 samples (24 chickens and 59 turkeys), oxidase-positive colonies suggestive of "*Campylobacter*" were also detected, which were identified by WGS as *Helicobacter pullorum*.

Overall, the detection of MDR *C. coli* isolates and the emerging pathogen *H. pullorum* in chickens slaughtered for human consumption, raises a public health problem that must be considered when establishing strategies control of foodborne illnesses.

Acknowledgements:
This work was developed under the project "ResisCampyOH - Multidrug resistant *Campylobacter jejuni* and *Campylobacter coli* in Portugal: a One Health study to understand the sources, transmission and persistence", 2022-2023, funded by the Faculty of Veterinary Medicine from Lusófona University (FMV-ULHT). Maria-Leonor Lemos is the recipient of a Ph.D. grant (2022.10133.BD) from FCT.

P2.30 - GENOMIC DIVERSITY OF *KLEBSIELLA PNEUMONIAE* IN CHICKEN PRODUCTION CHAIN: ANTIBIOTIC RESISTANCE AND METAL TOLERANCE INTERPLAY

Joana Mourão [1,2], Marisa Ribeiro-Almeida [1,3], Mafalda Magalhães [1,4], Andreia Rebelo [1,3,5], Carla Novais [1], Luísa Peixe [1], Ângela Novais [1], Patrícia Antunes [1,4(*)]

[1] UCIBIO/Associate Laboratory i4HB - Institute for Health and Bioeconomy. Laboratory of Microbiology, Faculty of Pharmacy, Porto University, Porto, Portugal
[2] Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
[3] School of Medicine and Biomedical Sciences (ICBAS-UP), Porto University, Porto, Portugal
[4] Faculty of Nutrition and Food Sciences, Porto University, Porto, Portugal
[5] School of Health, Polytechnic Institute of Porto, Porto, Portugal

(*) e-mail: patriciaantunes@fcna.up.pt

Keywords: antimicrobial resistance; WGS; poultry; one-health.

ABSTRACT

Farm-to-fork antimicrobial strategies influence the spread of antibiotic resistance-ABR across the food-chain. Although non-clinical reservoirs have been linked to multidrug- resistant *Klebsiella pneumoniae*-Kp transmission to humans, Kp genomic diversity in poultry production remains understudied. We conducted a comprehensive genomic characterization of Kp recovered in chicken intensive production chain after colistin ban and in the context of copper-supplemented feeds.

Sixty-eight Kp (flocks+environment+meat samples/different farms/KL-types/2019-2022) were characterized by whole-genome-sequencing-WGS (Illumina). Assembled contigs underwent multi-locus sequence typing (MLST), core-genome MLST (cgMLST), and plasmid replicon analysis (Pathogenwatch). Kleborate predicted ABR and virulence and in-house database metal tolerance-MeT genes. MOB-recon allowed ABR/MeT location and plasmid reconstruction.

We identified 31 STs/37 kp lineages, some globally distributed (ST11-KL106/KL111, ST15-KL19, ST147-KL-64, ST280-KL23 and ST307-KL102). In some cases, we found strains (ST11-KL111/KL27, ST15-KL19/KL146, ST280-KL23, ST392-KL27, ST1537-

KL64 and ST1997-KL28) with genetic similarities (<10 allele/≤21 SNPs) with strains causing human infections. Highly related strains (<10-alleles/0-207-SNPs difference) were found in different samples from the same farm throughout time (e.g., ST11-KL106 in chicken/water, or ST11-KL111, ST6405-KL109, ST6406-CG147-KL111 in chicken/ derived meat). We found 38 ABR genes (7 classes; 75% ≥3 classes), including clinically- relevant ones (*bla*CTX-M-15/*qnrB*/*qnrS*). Chromosomal mutations linked to fluoroquinolones (*gyrA-parC*, 59%) or colistin (32%) resistance were frequent. ST1228 isolates carried yersiniabactin locus ybt15-YbST378/ICEKp11. MeT clusters *sil+pco*-copper (68%) were frequently associated with *ars*-arsenic (77%), *mer*-mercury (49%) and/or *ter*-tellurite (51%) operons mainly in IncFIBK-IncFIIK plasmids (51%/~80-350 Kb) alongside with variable ABR genes.

Chicken farms and their derived meat are important reservoirs of diverse Kp clones enriched in ABR/MeT genes, some with genetic similarities with human clinical strains. Further investigations are needed to elucidate the factors driving Kp persistence and dissemination within poultry

production for improvement of food safety risk management. This study emphasizes the importance of understanding the interplay between antimicrobial control strategies and non-clinical sources to effectively tackle ABR spread.

Acknowledgements:
This work was supported by the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia (UIDP/04378/2020 and UIDB/04378/2020), by the Associate Laboratory Institute for Health and Bioeconomy-i4HB (LA/P/0140/2020), by the AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (Programa Operacional Regional do Norte 2014/2020) and by the University of Porto (Grant Nº IJUP-Empresas-2021-14). Joana Mourão and Ângela Novais were supported by national funds through FCT/MCTES in the context of the Scientific Employment Stimulus (2021.03416.CEECIND and 2021.02252.CEECIND, respectively). Marisa Ribeiro-Almeida and Andreia Rebelo were supported by PhD fellowships from FCT (SFRH/BD/146405/2019 and SFRH/BD/137100/2018, respectively).

P2.31 - LONG-TERM PERSISTENCE OF MCR-1 CARRYING *ESCHERICHIA COLI* IN INTENSIVE RABBIT FARMS AFTER COLISTIN BAN

Marisa Ribeiro-Almeida [1,2], André Pinto de Carvalho [2,3], Joana Mourão [1,4], Paulo Martins da Costa [2,5], Luísa Peixe [1], Patrícia Antunes [1,6(*)]

[1] UCIBIO/REQUIMTE and Associate Laboratory i4HB - Institute for Health and Bioeconomy. Laboratory of Microbiology, Faculty of Pharmacy, Porto University, Porto, Portugal
[2] School of Medicine and Biomedical Sciences, Porto University, Porto, Portugal
[3] NANTA, Marco de Canaveses, Portugal
[4] Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal
[5] CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, Porto University, Portugal
[6] Faculty of Nutrition and Food Sciences, Porto University, Porto, Portugal

(*) e-mail: patriciaantunes@fcna.up.pt

Keywords: mcr; colistin; rabbit farms; one-health.

ABSTRACT

The global escalation of colistin resistance and *mcr* genes spread in *Enterobacteriaceae* from livestock emphasizes the need of limiting its veterinary use. Although Portuguese rabbit farms decreased/banned colistin use, their long-term effects remain unknown. We assessed the occurrence and molecular characteristics of *mcr*-carrying *E. coli*-Ec in intensive rabbit farms post-colistin withdrawal for three consecutive years.

Fecal samples were collected from 18 groups of reproductive females-M and their offspring at two stages (30-39 days weaning rabbits-R1 and 58-80 days pre-slaughter rabbits-R2) across eight rabbit farms (three farms: two-year colistin ban; five farms: one- year colistin ban) in 2020/2021. Additionally, environmental samples (feed-n=14, nest- n=8; water-n=24) were analyzed. Samples with/without enrichment were plated on TBX+colistin plates. Ec identified by MALDI-TOF-MS/PCR were screened for *mcr* genes presence (*mcr*1-9), antibiotic susceptibility (14 antibiotics-disk-diffusion/microdilution- colistin) and clonality (PFGE/MLST). Selected isolates underwent WGS (Illumina- HiSeq). Farms with *mcr*-carrying-Ec were resampled over two consecutive years, including samples from new reproductive females-GP.

We found *mcr*-1-carrying Ec (n=28 isolates; 16 PFGE-types; colistin MIC=4mg/L) in all rabbit groups (n=21-R1+R2; n=4-M) and feed (n=3) samples from one single farm (one- year colistin ban). Subsequent visits revealed polyclonal (21 PFGE-types) *mcr-1*- carrying Ec across various rabbit samples (2022: n=20-R1+R2, n=11-M; 2023: n=10-R2, n=1-GP). All isolates exhibited multidrug-resistance (MDR), sharing similar phenotypes (ampicillin, ciprofloxacin, gentamicin, tetracycline, chloramphenicol, sulfonamide, trimethoprim), with *mcr*-1 gene detected in IncHI2 plasmids. WGS revealed diverse clones, some globally distributed (B1-ST40/EPEC, B1-ST1196/ExPEC and B1-ST1589/ExPEC), including highly related strains (<20-allelic differences) in different samples and years (e.g., ST40:R2/feed-2020/21; ST1196:R1/feed-2020/21 and GP- 2023; ST1589:R1-2020/21 and R2-2023).

Despite colistin ban, one rabbit farm had polyclonal MDR Ec carrying *mcr-1* in IncHI2 plasmids for

over three-years. Identifying rabbit strains genetically similar to those from external farm samples (feed and GP) highlights the importance of controlling the farm environment to effectively contain the global spread of colistin resistance.

Acknowledgements:
This work was supported by the Applied Molecular Biosciences – UCIBIO which is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia (UIDP/04378/2020 and UIDB/04378/2020), by the Associate Laboratory Institute for Health and Bioeconomy-i4HB (project LA/P/0140/2020), by the AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by the European Regional Development Fund (ERDF), through NORTE 2020 (Programa Operacional Regional do Norte 2014/2020) and by MicroLab – ICBAS/CIIMAR. Marisa Almeida was supported by a FCT PhD fellowship (SFRH/BD/146405/2019). Joana Mourão was supported by national funds through FCT/MCTES in the context of the Scientific Employment Stimulus (2021.03416.CEECIND).

P2.32 - PHAGE THERAPY IN THE CONTROL OF VIBRIO ALGINOLYTICUS IN THE AQUACULTURE LIVE FOOD ARTEMIA FRANCISCANA NAUPLII

Amanda Tadeu [1], João Duarte [1*], Davi Trindade [1], Carla Pereira [1], Adelaide Almeida [1]

[1] CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal

[(*)] *email: Joao.md.w@gmail.com*

Keywords: Bacteriophages; Phage Therapy; *Vibrio alginolyticus*; Live feeds; *Artemia franciscana*

ABSTRACT

Vibrio species are a major cause of diseases in marine fish and invertebrate hatcheries, resulting in significant economic losses. Moreover, these bacteria can be transmitted to humans through the consumption of undercooked or raw seafood, posing a risk to public health. Artemia nauplii, commonly used as live food in aquaculture, has been considered a possible source of Vibrio species contamination, including *V. alginolyticus*. It serves as a vehicle for these bacteria into culture tanks and affecting fish health and consumers. Antibiotics are still often used to address this issue, but they contribute to aggravate the antimicrobial resistance. Therefore, eco-friendly biological strategies, like phage therapy, should be adopted to control bacterial infections. The use of phages in live feeds can reduce bacterial levels and enhance the microbiological safety of aquaculture food products. In this study two new phages (TDD and SRI) were isolated, characterized and their efficacy was evaluated separately and in cocktail (TDD/SRI) to control *V. alginolyticus* in vitro and in vivo. In TSB, both phages were effective against *V. alginolyticus*, but phage TDD (reduction of 5.7 log CFU/mL after 10 h of treatment) was more effective than phage SRI (maximum reduction of 4.6 log CFU/mL after 10 h of treatment). In artificial marine water, phage TDD and SRI led to a maximum reduction of *V. alginolyticus* of about 4.2 and 2.5 log CFU/mL, respectively. When artificially contaminated, *Artemia franciscana* was treated with phage TDD and SRI, and 3.0 log CFU/mL of reduction was reached after 12 h. The use of the cocktail (TDD/SRI) was not significantly more effective than the use of single phages. The results of this study suggest that the phages TDD and SRI can be an effective alternative to control *V. alginolyticus* in *A. franciscana*, validating their potential for application on live feed.

Acknowledgements:
The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020). João Duarte was supported by a PhD grant 2021.05519.BD. Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Thanks are also due to the Department of Biology and University of Aveiro, where this research work was carried out.

P2.33 - BACTERIOPHAGES TO CONTROL VIBRIO PARAHAEMOLYTICUS: A TECHNOLOGY FOR BIVALVE DEPURATION

João Duarte [1], Carla Pereira [1], Ricardo Calado [2], Adelaide Almeida [1].*

[1] *CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal*
[2] *2ECOMARE, CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal*

[(*)] *email: Joao.md.w@gmail.com*

Keywords: Bacteriophages; Phage therapy; *Vibrio parahaemolyticus*; Bivalve depuration

ABSTRACT

Vibrio parahaemolyticus is a pathogenic bacterium commonly found in marine or estuarine environments. This bacterium can cause wound infections and sepsis but it is mostly associated with gastroenteritis caused by the consumption of raw or undercooked seafood. Bivalves are filter feeding animals that are considered the main vector for human contamination. Despite mandatory depuration, bacteria from the genus Vibrio seem to be somewhat resistant to this process due to their ability to form strong biofilms. When bacterial loads are not efficiently reduced, the consumption of undercooked or raw bivalves can pose a serious threat to public health. Bacteriophages are viruses that infect bacteria and therefore, have innumerous potential applications in many different fields. Since these viruses are target specific, applying phages in depuration units may allow a targeted decontamination of certain bacteria and an improvement in the overall safety of seafood. To test this hypothesis, four different bacteriophages have been isolated using as host the environmentally isolated strain *V. parahaemolyticus* O22C. The phages (S1; VPP; S/H and H) were isolated by their plaque morphology using water samples collected in the Ria de Aveiro estuarine system. When the bacterial host was challenged with phages individually in tryptic soy broth, all four phages efficiently controlled the growth of the strain. At a multiplicity of infection (MOI) of 1, phages S1, VPP, S/H and H presented a maximum inactivation of 4.5, 4.0, 4.6 and 4.4 Log (CFU/mL) after 6 to 10 hours, respectively. With higher phage dose (MOI10) all phages presented a very similar maximum inactivation (varying between 4.4 to 4.7 Log CFU/mL). Interestingly, when a higher dose was used, all phages reached their maximum inactivation potential at the same moment (6 hours of incubation). These results suggest that phages S1, VPP, S/H and H can be successfully used to control *V. parahaemolyticus*.

Acknowledgements:
The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) for the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020). João Duarte was supported by a PhD grant 2021.05519.BD. Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Thanks are also due to the Department of Biology and University of Aveiro, where this research work was carried out.

P2.34 - EFFECTS OF THE NON-SUGAR SWEETENERS XYLITOL AND SUCRALOSE ON *SACCHAROMYCES CEREVISIAE* CHRONOLOGICAL LIFESPAN

Conceição Gonçalves [1,2], Sara Dourado [1,2], Beatriz Ferreira [1,2], Maxime Gomez [1,2], Belém Sampaio- Marques [1,2], Joris Winderickx [1,2,3], Paula Ludovico [1,2]

[1] Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal
[2] ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal
[3] Department of biology, KULeuven, Belgium

Keywords: aging; sweeteners; yeast.

Abstract

The worldwide obesity epidemic has the consumption of sugar-rich foods as a key factor. Non-sugar sweeteners (NSS), sweetening agents with no or low calories, have emerged as an option in aiding the decrease of sugar intake. Sweeteners are approved and regulated by international food agencies, but some doubts remain about their impact on body weight, incidence of diabetes, cardiovascular disease, and mortality, as well as toxicity and carcinogenicity.

Saccharomyces cerevisiae cells display well conserved hallmarks of cellular aging; hence, this microorganism is widely used as a model for cellular and organismal ageing. Ras2 is part of a cellular pathway of nutrient sensing. This work was developed with the objective of furthering our limited understanding on the effects of NSS (xylitol, sucralose) on cellular aging.

The yeast chronological lifespan (CLS) model was used to evaluate the effects of sweeteners. BY4741 (WT) and *RAS2* deleted yeast cells non-supplemented were used as negative control and glucose supplemented cells as positive control. Growth and CLS were assessed by optical density and colony forming units count, respectively. Cellular mechanisms of aging were also accessed: an Atg8-GFP plasmid was used to assess autophagy, and flow cytometry to evaluate reactive oxygen species (ROS) superoxide anion and hydrogen peroxide accumulation and cell cycle. Osmolarity of each sweetener was measured in the growth medium.

Sucralose did not have impact on chronological aging while xylitol extended lifespan. ROS formation was found to be altered by xylitol but not by sucralose. Neither sweetener exhibited effects on cell cycle. While sucralose was demonstrated to induce a lower osmolarity on medium and impact autophagy, xylitol did not.

Overall, these results showed opposite effects of xylitol and sucralose on chronological aging due to their impact on intracellular signaling pathways. Further research is needed to ascertain the effects of sweeteners that although not metabolized could interfere with relevant intracellular pathways.

Acknowledgements

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020.

P2.35 - THE ACTION OF THE SWEETENERS CYCLAMATE AND SACCHARIN ON THE CHRONOLOGICAL AGING OF *SACCHAROMYCES CEREVISIAE*

Sara Dourado[1,2], Conceição Gonçalves[1,2], Beatriz Ferreira[1,2], Maxime Gomez[1,2], Belém Sampaio- Marques[1,2], Joris Winderickx[1,2,3], Paula Ludovico[1,2]

[1]Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal
[2]ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal
[3]Department of biology, KULeuven, Belgium

Keywords: aging; sweeteners; yeast.

Abstract

With the increasing prevalence of obesity, Diabetes mellitus and other metabolic diseases, sweeteners have become an alternative to replace glucose in food, as well as in oral hygiene and pharmaceutical products, since they provide sweetness without supplying calories or glycemic effects. Sweeteners are not only a palatable substitute for sugar but are also used for therapeutic and clinical purposes being classified as natural or synthetic/artificial. Although many sweeteners have been approved for use by International Health Agencies, there is still a lack of studies relating them to aging, which is a complex and multifactorial biological process determined by the combination of genetic and environmental factors. The main objective of this work was to investigate the effects that common sweeteners as cyclamate and saccharin might have on aging by exploring the chronological yeast Saccharomyces cerevisiae lifespan, as a cellular model of aging.

To achieve the proposed objectives, the chronological life span (CLS) of S. cerevisiae BY4741 cells was evaluated with glucose, cyclamate or saccharin supplementation and compared to control cells without any additional sugar. Cellular mechanisms associated with aging were assessed (autophagy by immunobloting; cell cycle and accumulation of the reactive oxygen species - ROS

- superoxide anion and hydrogen peroxide by flow cytometry).

The results showed that cyclamate extends CLS, accompanied by increased autophagy and reduced ROS accumulation. In contrast, saccharin did not have a major impact in CLS or ROS formation, although cells exhibited a decreased autophagy flux. Sweeteners did not impact cell cycle profile.

Overall, these results highlight that sweeteners, so commonly used as food supplements in the human diet, might impact on intracellular signaling pathways and that more research is needed to understand the long-term effects of sweeteners.

Acknowledgements

This work has been funded by National funds, through the Foundation for Science and Technology (FCT) - project UIDB/50026/2020 and UIDP/50026/2020.

P2.36 - SEA BASS EMBRYONIC CELL CULTURE USING MICROCARRIERS FOR CULTIVATED SEAFOOD PRODUCTION

Laura Alves [1,2], Diana M. C. Marques [1,2], Diogo E.S. Nogueira [1,2], Catarina Toste [1,2], William, O.S. Salvador [1,2], Paola Sanjuan-Alberte [1,2], Joaquim M.S. Cabral [1,2], Frederico C. Ferreira [1,2], Alexandrea Duscher [3], Carlos A.V. Rodrigues [1,2]

[1] Department of Bioengineering and iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

[2] Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

[3] Affiliate Faculty, Virginia Polytechnic Institute and State University (VT), Blacksburg, USA

(*) e-mail: carlos.rodrigues@tecnico.ulisboa.pt

ABSTRACT

Cellular agriculture is the production of animal-sourced products from cell cultures rather than farming. Cellular Agriculture brings new challenges for scalable fish cell cultivation, such as cell characterization and availability of suitable food grade/edible microcarriers. This work contributes to address these two challenges providing experimental results on fish cell characterization and a route to develop novel microcarrier processes.

In this context, culture conditions for the Sea bass embryonic cell line DLEC were optimized. Different seeding densities were tested and different basal culture media. A screening of adequate antibodies for pluripotency markers and preliminary evaluation of differentiation potential were conducted.

Cell growth kinetics were investigated and a 8±1- and a 18±4- fold increase in cell number was observed after 4 and 8 days, respectively. Initial efforts to establish animal product- free culture conditions were performed but the present results were obtained with FBS- containing media. Preliminary experiments of DLEC culture on microcarriers were also performed using commercially available microcarriers.

This study is a preliminary proof of concept for scalable expansion of Sea bass cells for cultivated seafood products. The results provide improved understanding of fish stem cell biology and essential data for future bioprocess development.

Acknowledgements:
The authors acknowledge funding from The Good Food Institute (VitaminSea project) and Fundação para a Ciência e Tecnologia (FCT), Portugal through iBB – Institute for Bioengineering and Biosciences (UIDB/04565/2020 and UIDP/04565/2020), PhD scholarship (2022.12900.BD) and CleanFish project (2022.07677.PTDC).

MICRO BIOTEC 23

Poster Session

Topic 3

P3.1 - CROSS EFFECT OF A POLYCLONAL ANTIBODY AGAINST CYSTIC FIBROSIS BACTERIAL PATHOGENS AS A NEW ALTERNATIVE THERAPY

António M.M. Seixas [1,2], Sílvia A. Sousa [1,2], Carolina Silva [1,2], Sara C. Gomes [1,2], Mirela R. Ferreira [1,2], Leonilde M. Moreira [1,2], Jorge H. Leitão [1,2]*

[1] Department of Bioengineering, IBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
[2] Associate Laboratory, i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

(*) e-mail: jorgeleitao@tecnico.ulisboa.pt;

Keywords: *Pseudomonas aeruginosa, Burkholderia cenocepacia,* cystic fibrosis, immunotherapy, *Galleria mellonella*

ABSTRACT

Bacterial chronic respiratory infections remain a life threat to cystic fibrosis (CF) patients. Two of the most difficult infections to treat in these patients are those caused by *Pseudomonas aeruginosa* and bacteria of the *Burkholderia cepacia* complex, due to a variety of factors including biofilm formation and antimicrobial resistance. The absence of an effective antimicrobial eradication strategy led to the search for immunotherapies able to viably control and reduce the damages caused by these infections and protect CF patients. Previous studies performed by our research group have shown that a polyclonal goat IgG antibody against the *Burkholderia cenocepacia* J2315 outer membrane protein BCAL2645 was able to interfere with the infection process of this bacteria. In this work, we demonstrate that the genomes of strains of *P. aeruginosa* and *B. multivorans* encode a protein with a significant identity degree to BCAL2645. In this work, we show that the antibody also recognized the protein expressed by *P. aeruginosa* F69A isolate IST27, and *B. multivorans* BM1 strains. As such, a cross-effect of neutralization of the infection process *of P. aeruginosa* and polysaccharide-producing *B. multivorans* by our antibody was tested. The antibody was found to strongly decrease the adhesion and invasion of these strains to the human bronchial epithelial cell line CFBE41o-, significantly impacting *P. aeruginosa* biofilm formation in both the attachment to surfaces and to other cells, and also reducing the virulence of these strains in the animal model *Galleria mellonella*. Altogether our results show that an antibody targeting BCAL2645 protein has a high potential for use in the development of new immunotherapies against some of the most problematic strains infecting CF patients.

Acknowledgements:
The authors acknowledge funding from FCT (Project 2022.07091.PTDC) and iBB— Institute for Bioengineering and Biosciences through project UIDB/04565/2020. The authors also acknowledge FCT for Ph.D. fellowships to AMMS (PhD Program in Applied and Environmental Microbiology, PD/BD/150420/2019), to S.C.G. (SFRH/BD/144198/2019) and M.R.F. (BIOTECnico PhD program, PD/BD/150337/2019).

P3.2 – IMPROVING THE MANUFACTURING OF MRNA NANOMEDICINES USING THERMOREVERSIBLE AQUEOUS BIPHASIC SYSTEMS AND IONIC LIQUIDS

Maria I. Sousa [1]*, Luís C. V. Silva [1], Andreia Correia [1], Mara G. Freire [1], Francisca A. e Silva [1], Augusto Q. Pedro [1]

[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal

(*) e-mail: minessousa@ua.pt

Keywords: mRNA nanomedicines; ionic liquids; thermoreversible aqueous biphasic systems; in vitro transcription.

ABSTRACT

It is undeniable the potential of messenger RNA (mRNA) vaccines as effective tools to manage infectious disease outbreaks. However, mRNA nanomedicines production is complex and expensive, requiring improved technologies to produce stable and widely accessible products, whilst meeting required production times and fulfilling market demands.

Ionic liquids (ILs) are composed of organic cations and organic or inorganic anions. Given their remarkable structural diversity, ILs can be properly designed to improve RNA stability. Moreover, as phase-forming agents of aqueous biphasic systems (ABS), ILs contribute to develop highly selective purification processes. This work aims to integrate production-clarification steps of mRNA vaccines, using thermoreversible IL-based ABS, simplifying subsequent purification steps. Several quality control methods were designed to evaluate integrity and purity of mRNA manufactured resorting to *in vitro* transcription. ABS formed by dextran from Leuconostoc spp. with an average molecular weight of 450.000-650.000 g/mol (Dex 500) and polyethylene glycol (PEG) 3350 g/mol, containing ILs as adjuvants were characterized regarding their thermoreversible nature.

Preliminary mRNA partition experiments using IL-based ABS indicate that mRNA is preferentially partitioned toward the IL-PEG-Rich phase. Promising systems for mRNA purification have been identified. Ongoing work focuses on selecting the most promising IL-based ABS for initial mRNA clarification. In the future, mRNA production-clarification will be integrated into the best performing system to overcome current challenges in mRNA nanomedicine manufacturing, such as sustainability and production speed, whilst improving mRNA stability and yield.

Acknowledgements:
This work was developed within the scope of: CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through FCT/MCTES (PIDDAC); and mVACCIL (EXPL/BII-BTI/0731/2021), financially supported by national funds (OE), through FCT/MCTES. Augusto Q. Pedro and Francisca A. e Silva acknowledge FCT respectively, for the research contracts CEEC-IND/02599/2020 and CEEC-IND/03076/2018 under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.

P3.3 - CREATION OF A FUNGAL LIBRARY AND SCREENING OF ANTIMICROBIAL AND ANTICANCER ACTIVITY

Diogo Ferreira [1,2,5]*, Lara Areal Hermida [1], Ana Catarina Rocha [3,4,5], Pilar Baylina [2,4,5], Carmen Sieiro [1], Rúben Fernandes [4,5]

[1]Departamento de Bioloxía Funcional e Ciencias da Saúde. Área de Microbioloxía. Universidade de Vigo,Vigo, España.
[2]TBIO, Center for Translational Health and Medical Biotechnology Research, Escola Superior De Saúde do Porto, Porto, Portugal
[3]FMUP, Faculdade de Medicina da Universidade do Porto, Porto, Portugal
[4]I3S, Instituto de Investigação e Inovação em Saúde, Porto, Portugal
[5]HE-FP, Hospital Fernando Pessoa, CECLIN - Center of Clinical Studies, Gondomar, Portugal

(*) e-mail: diogo90f@gmail.com

Keywords: *Fungi,* secondary metabolites, antimicrobial, anticancer.

ABSTRACT

According to the World Health Organization, cancer and infectious diseases are two of the most problematic diseases nowadays. Cancer kills 10 million people every year and the emergence of resistance to antitumoral drugs is an important medical challenge. At the same time, antimicrobial resistance (AMR) is also a serious threat to human and environmental health. Besides mortality, AMR burdens healthcare services and dampens medical procedures such as surgeries, cancer treatments and other invasive procedures. The development of new drug therapies to fight drug resistance is essential to contest the rising of resistant bacteria and reduction of the effectiveness of antitumoral drugs.

Microorganisms have been a major source for natural compounds throughout the years. Fungi, renowned for their ability to produce an array of broad and diverse secondary metabolites, offer a rich resource for drug discovery.

We built a collection of fungal species, isolated from chestnuts, sunflower seeds, and chestnut flour, and explored their extracts for potential antimicrobial and anticancer activity. Fungi cultures for secondary metabolite biosynthesis were done in submerged fermentation in Malt Extract broth for 15 days at 26 °C. Liquid-liquid extraction techniques, with ethyl acetate as a solvent, were applied to obtain crude secondary metabolite extracts.

Clinical resistant bacteria, yeasts, and prostate cell lines (human prostate epithelial cells – HpepiC; human caucasian prostate adenocarcinoma cells - PC3) were exposed to fungal extracts at a single concentration of 100 µg/mL.

Our results so far show several extracts with antimicrobial and/or anticancer activity without decreasing cell viability of non-tumoral cells, showing their potential as therapeutic drugs without possible secondary effects. Although, more studies should be done, and pending fungal identification will allow us to select which extracts will be further investigated to find if the displayed bioactivity could be happening due to unknown natural compounds.

Acknowledgements:
This work was funded by a research grant PORTIC/LABMI/BI72021/02 through project BIOTECH4HEALTH from Instituto Politécnico do Porto and it was done during the mobility Program IACOBUS.

P3.4 - MOLECULAR BEACONS FOR PRE-MIRNA SECONDARY STRUCTURE CHARACTERIZATION AND MIRNA QUANTIFICATION

Micaela Riscado [1]*, Leonor Mariano [1], Carla Cruz [1], Chantal Pichon [2], Fani Sousa [1]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.
[2] CNRS - Center for Molecular Biophysics, University of Orléans, Orléans, France

(*) e-mail: micaelariscado@gmail.com

Keywords: pre-miRNA, Molecular Beacons, FRET, Hairpin Structure

ABSTRACT

MicroRNAs (miRNAs) are involved in post-transcriptional gene expression regulation and are promising as innovative therapeutic agents. They are processed from a stem-loop precursor, the pre-miRNA, recognized and cleaved into the active form. Selecting the pre-miRNA as the biopharmaceutical, instead of the mature miRNA, is expected to guarantee sample stability and improve its recognition and processing in the cell, upon administration. Thus, after the recombinant pre-miRNA production, verifying if it presents the natural secondary structure is necessary so that the cell machinery can properly recognize and process it to generate the miRNA. This work aims to optimize a molecular beacon (MB) assay that simultaneously confirms the pre-miRNA hairpin structure and quantifies the mature form. The MBs, also in hairpin shape, are nucleic acid probes labeled with a reporter fluorophore at the 5' end and a quencher at the 3' end. In this work, the MB loops were designed to be complementary to the mature form of the respective pre-miRNA. Since this sequence is present in the pre-miRNA stem region, it is not available for MB hybridization when the hairpin is established. In this study, two MBs were designed, one complementary to the miR-29-1-3p and the other complementary to the miRNA-9-1-5p. Both MBs specifically recognized the respective miRNA, even in the presence of mixtures, and the fluorescence was shown to be directly proportional to the miRNA concentration. Noteworthy is that no significant signal was achieved in the presence of pre-miRNAs, allowing the distinction between both forms. These results prove that this method can, not only be used to determine if the pre-miRNAs present hairpin structure but also to quantify the corresponding mature form.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT), through the projects UIDB/00709/2020 and UIDP/00709/2020. Micaela Riscado acknowledges FCT for the Ph.D. fellowship 2021.07658.BD.

P3.5 - IONIC LIQUIDS: AN INNOVATIVE CELL DISRUPTION METHOD FOR THE EXTRACTION OF NUCLEIC ACIDS-BASED BIOPHARMACEUTICALS FROM YEAST

Ana S. C. Marques [1]*, Augusto Q. Pedro [1], Cristine Gonçalves [2], Ana P. M. Tavares [1], Chantal Pichon [2], Mara G. Freire [1]

[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal.
[2] Centre de Biophysique Moléculaire, CNRS, Orléans, France.

[(*)] e-mail: anascm10@ua.pt

Keywords: Nucleic acid biopharmaceutical, Bioprocess, Extraction, Integrated process, Ionic liquids, Stability.

ABSTRACT

Messenger RNA (mRNA) based-vaccines have revolutionized the COVID-19 fight, however their production through *in vitro* transcription (IVT) remains time-consuming and costly. Thus, there is a demand for a more efficient and cost-effective process to help meet future market demands for mRNA. Yeasts are currently being investigated as an alternative for this purpose, but a disruption step is required to recover the intracellular biopharmaceutical. Considering the tunable nature of ionic liquids (ILs) and ability to stabilize nucleic acids, this work aims to investigate their use in the cell disruption step for the recovery of nucleic acid-based biopharmaceuticals. For this purpose, new ILs were identified, synthesized and characterized. The target biopharmaceutical was biosynthesized resorting to a modified yeast strain. Yeast cells were incubated with specific concentrations of ILs, after which the biopharmaceutical was recovered and its integrity evaluated using agarose gel electrophoresis, the concentration determined using UV spectroscopy and the identity evaluated by *in vitro* translation. The effect of ILs on biopharmaceutical stability and integrity was additionally evaluated. Results revealed that some ILs were able to promote yeast cell disruption and extraction of biopharmaceutical of interest and simultaneously maintain its stability. Ongoing work focus the optimization of IL-mediated cell disruption envisaging to increase the yields of extracted biopharmaceutical. Overall, this study highlights the potential of ILs as an effective method for cell disruption and biopharmaceutical extraction, requiring however further optimization.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). This work was additionally funded by the EIC-Pathfinder YSCRIPT project with reference 101047214, supported by the budgets of the Horizon Europe Program. Ana Marques, Augusto Pedro and Ana Tavares acknowledge FCT, respectively, for the PhD grant 2023/04854/BD and the research contracts CEECIND/2020/02599 and CEECIND/2020/01867.

P3.6 - OREGANO ESSENTIAL OIL: AN EFFECTIVE AND NON-TOXIC APPROACH FOR PREVENT OR TREAT RESISTANT CANDIDA SPECIES

Liliana Fernandes [1]*, Inês Silva [1], Elena Blázquez [2], Ainara Tejada [2,3], Artur Ribeiro [1,4], Sónia Silva [1,4], Nuno Mira [5], Lorena Cussó [3,6], Sofia Costa-de-Oliveira [7], Maria Elisa Rodrigues [1,4], Mariana Henriques [1,4]

[1] Centre of Biological Engineering, University of Minho, Braga, Portugal;
[2] Laboratorio de Imagen Médica. Unidad de Medicina y Cirugía Experimental. Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain;
[3] Departamento de Bioingeniería. Universidad Carlos III de Madrid, Madrid, Spain
[4] LABBELS – Associate Laboratory, Braga/ Guimarães, Portugal;
[5] iBB, Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal;
[6] Unidad de Imagen Avanzada. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain;
[7] Division of Microbiology, Department of Pathology, Faculty of Medicine, University of Porto, Portugal; Center for Health Technology and Services Research – CINTESIS@RISE, Faculty of Medicine, University of Porto, Portugal

(*) e-mail: lilianafernandes@ceb.uminho.pt

Keywords: *Vulvovaginal candidiasis, Lactobacillus spp;* Phytotherapeutic applications; vapor-phase of essential oil; Keratin nanocapsules; Alternative treatment

ABSTRACT

Vulvovaginal candidiasis (VVC) is one of the most prevalent vaginal infectious diseases, and the emergence of drug-resistant *Candida* strains has presented a growing challenge in its treatment. This highlights the urgent need to develop effective and non-toxic alternative treatments. In this context, essential oils (EOs) have emerged as a promising alternative considering low toxicity and high antimicrobial activity.

This work is divided into two parts, the first consists of evaluating the effect of the vapor phase of oregano EO (VP-OEO) on biofilms of antifungal-resistant *Candida* species (*Candida albicans* and *Candida glabrata*) quantified by colony forming units' enumeration and determine their mode of action by flow cytometry. Interestingly, the VP-EOs has shown to be more effective against *Candida* growth than their liquid form. Indeed, the results revealed high antifungal activity of VP-OEO against these drug-resistant strains, significantly reducing biofilm formation and mature biofilms, with impact on membrane integrity and metabolic activity of the fungal cells. The second part consists of the design and evaluation of nanoencapsulated OEO (KNP-OEO) as another alternative application of OEO for VVC treatment. These nanoparticles provided stability to OEO and controlled release of the EO. The results demonstrated complete inhibition of *C. albicans* growth. Moreover, in *in vivo* assay with BALB/C female mice, a single intravaginal application of KNP-OEO reduced *C. albicans* growth and preserved a healthy vaginal microbiota, including *Lactobacillus* species.

In conclusion, these studies highlight the promising efficacy of OEO as an alternative for VVC treatment. Both approaches, VP-OEO and OEO-KNP, showed effective antifungal activity against drug-resistant strains while preserving vaginal health. These therapeutic options not only combat antifungal resistance, but also potentially propose a safer option for women's health due to their

natural characteristics. However, further research is needed to confirm these promising results and advance the development of these alternative VVC therapies.

Acknowledgements:
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and grant ref 2020.05720.BD for Liliana Fernandes. Also, this study was supported by LABBELS—Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020 and Maria Elisa Rodrigues thanks FCT for funding through program DL 57/2016—Norma transitória. Sofia Costa de Oliveira acknowledges national funds through FCT, I.P., within the scope of the project "RISE - LA/P/0053/2020. Nuno Pereira Mira acknowledges support from FCT through its funding of research focused on Candida-lactobacilii interactions through LactoCan project (contract number: PTDC/BIA-MIC/31515/2017), iBB (contract: UIDB/04565/2020) and i4HB funding (contract: LA/P/0140/2020). Lorena Cussó acknowledges support by Agencia Estatal de Investigación (PRE2020-095268 MCIN / AEI /10.13039/501100011033 and by "ESF Investing in your future"), by Instituto de Salud Carlos III (PT20/00044) and co-funded by European Union (ERDF, "A way to make Europe").

P3.7 - NOVEL PLATFORMS FOR HUMAN SERUM PRETREATMENT AND BREAST CANCER BIOMARKERS EXTRACTION USING IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS

Maria S. M. Mendes [1]*, Maria C. Souza [1], João P. Conde [2,3], Mara G. Freire [1], Francisca A. e Silva [1]

[1]CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal.
[2]Instituto de Engenharia de Sistemas e Computadores-Microsistemas e Nanotecnologias (INESC MN), Portugal
[3]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

(*) e-mail: msilvinamm@ua.pt

Keywords: breast cancer; biomarkers; pretreatment; aqueous biphasic systems; ionic liquids.

ABSTRACT

Breast cancer ranks as the most common cancer among women. Improved disease management can be accomplished through the analysis of biomarkers present in human serum, namely human epidermal growth factor receptor 2 (HER2). Nevertheless, achieving accurate quantification of HER2 has critical challenges due to the complex composition of serum. Consequently, it becomes imperative to incorporate a sample pretreatment step into the analytical process to mitigate the influence of serum complexity during analysis. In this context, aqueous biphasic systems (ABS) have emerged as effective pretreatment techniques for the extraction of biomarkers. However, polymer-based ABS exhibit limited selectivity and efficiency. By selecting the right combination of cations and anions, ionic liquids (ILs) can serve as attractive alternatives for traditional phase-forming agents in ABS. This work proposes the use of IL-based ABS for the simultaneous removal of HSA and IgG while extracting HER2, and further compares their performance against conventional ABS. The most effective IL-based ABS system achieved complete removal of IgG and 69% removal of HSA at the interphase, allowing the extraction of 93% of HER2 in the top phase. Furthermore, IL-based ABS surpass the efficiency of conventional ABS as sample pretreatment and biomarker extraction tools. These outcomes highlight the practicality of IL-based ABS in improving HER2 extraction, offering alternative tools for breast cancer biomarkers analysis

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). This work was developed within the project PTDC/EMD-TLM/3253/2020 (ILSurvive), funded by national funds (OE), through FCT/MCTES. M.S.M.M. acknowledges FCT for the doctoral grant 2022.11229.BD. F.A.eS. acknowledges FCT for the researcher contract CEECIND/03076/2018 under the Scientific Employment Stimulus – Individual Call 2018.

P3.8 - DEVELOPMENT OF A POSTBIOTIC-BASED ORODISPERSIBLE FILM TO PREVENT DYSBIOSIS OF *STREPTOCOCCUS MUTANS* IN THE ORAL CAVITY

Mariana Rebelo [1]***, Cláudia Oliveira*** [1]***, Freni Tavaria*** [1]*

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal.*

(*) *e-mail: ftavaria@ucp.pt*

Keywords: Oral dysbiosis; Postbiotics; Oral health; *Lactobacillus spp.; Streptococcus mutans; Orodispersible films*

ABSTRACT

Oral diseases affect over three billion people worldwide. Recent studies show that to reduce the risk of caries or periodontitis, the control of the ecology of the oralome, instead of the complete removal of both harmful and beneficial microorganisms, is more effective. This is based on the knowledge that oral diseases are not caused by a pathogen but rather by a shift in homeostasis, called dysbiosis. Implementing strategies to prevent and control oral dysbiosis to avoid complications, is of utmost importance. Conventional treatments are based on the use of broad-spectrum antibiotics, which disrupt homeostasis. Postbiotics, due to their ability to modulate the oralome and decrease dysbiosis, arise as an interesting alternative. However, their mechanisms of action need to be addressed to clarify their possible applications as preventive strategies.

Lactobacillus plantarum and *Lactobacillus paracasei* were grown in MRS broth, centrifuged and filtered after 48h. The postbiotics were diluted to different concentrations and co-incubated with *Streptococcus mutans*. The antimicrobial activity was assessed by plate counting. Additionally, the minimal inhibitory concentration and the time needed for *S. mutans* inhibition were evaluated. Antibiofilm capacity was determined by the crystal violet method. Finally, an orodispersible film based on polymers and plasticizers was developed to serve as an administration vehicle.
Postbiotics demonstrated antimicrobial activity against *S. mutans* after 24h in co-incubation. The formulation of a postbiotic-based orodispersible film based on polymers was optimized.
This study offered an overview of the potential of postbiotics to prevent oral dysbiosis, focusing on their antimicrobial and anti-biofilm activity. Antimicrobial action against *S. mutans* in preliminary studies was observed. Given the obtained results, orodispersible films impregnated with postbiotics should be considered as a potential alternative to target oral dysbiosis.

Acknowledgements:
This work was supported by National Funds from Fundação para a Ciência e a Tecnologia (FCT) through the project [UIDB/50016/2020] and by the Be@t – Textile Bioeconomy Project (Investment TC-C12-i01 – Sustainable Bioeconomy [02/C12-i01/202]).
The authors would also like to thank the staff of the CBQF for their assistance in this project.

P3.9 - NOVEL COMPOUNDS RESCUE P53 AND INDUCE APOPTOSIS IN HPV- POSITIVE CELLS: PROSPECTS FOR ANTI-HPV DRUG DEVELOPMENT

Diana Gomes [1,2,3*], ***Carolina Ribeiro*** [1], ***Vanessa Brito*** [1], ***Samuel Silvestre*** [1,4,5,6], ***Luís Passarinha*** [1,2,3,4], ***Ângela Sousa*** [1]

[1]*CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*
[2]*UCIBIO–Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal.*
[3]*Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA, Caparica, Portugal.*
[4]*Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal.*
[5]*CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.*
[6]*C4–Cloud Computing Competence Centre, UBIMedical, University of Beira Interior, Covilhã, Portugal.*

[(*)] *e-mail: dianarouca@gmail.com*

Keywords: Apoptosis; Cervical cancer; E6 protein inhibitors; Human Papillomavirus; p53.

ABSTRACT

Human papillomavirus (HPV) accounts for 5% of the global incidence of human malignancies, with 99% of cervical cancer cases linked to HPV infection. The oncogenic potential of high-risk HPVs is mainly due to the E6 and E7 oncoproteins, which function by inactivating p53 and pRb tumor suppressor proteins, respectively. Given the pivotal role of E6 protein in driving malignant tumor formation, it stands as a well-recognized therapeutic target for the identification of innovative treatments for cervical cancer [1]. In this work, computational studies with E6 protein led to the identification of seven potential inhibitors capable of impeding the molecular interaction between E6 and p53. Subsequently, a thermofluor assay employing recombinant E6 protein [2] validated the computational findings, considering that all identified compounds exhibited stable binding interactions with E6. The efficacy of seven small molecules was assessed in both HPV-positive (Caski and HeLa) and HPV-negative (C33A and NHDF) cell lines. According to MTT assays, all compounds reduced cell viability, specifically in HPV-positive cells, without affecting HPV-negative cells. Furthermore, the new molecules demonstrated the capacity to prevent the long-term proliferation and migration of HPV-positive cells. Notably, four of these compounds, including two barbiturates and two steroid derivatives, exhibited the ability to inhibit E6-mediated p53 degradation, thereby restoring p53 levels and inducing apoptosis in HPV-positive cells. In a near future, further studies will be undertaken to corroborate the activity of p53 protein and conduct cell cycle analyses. Altogether, our data can be relevant for the discovery of prospective candidates in the pursuit of specific anti-HPV drug development.

References:

[1] Almeida AM, et al. Cervical cancer and HPV infection: ongoing therapeutic research to counteract the action of E6 and E7 oncoproteins. *Drug Discov Today*. 24(2019):2044.
[2] Gomes D, et al. Integrated approaches for the separation and purification of recombinant HPV16 E6 protein from Escherichia coli crude extracts. *Sep. Purif. Technol.* 315(2023):123647.

Acknowledgements:
Foundation for Science and Technology supports this work through European Regional Development Fund Portugal 2020 Program with projects UIDB/00709/2020, UIDB/04378/2020, the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020), the research contract from the call CEECINST/00016/2021 under the CEEC Institutional 2021, and PhD fellowship 2020.06792.BD.

P3.10 - A POTENTIAL EFFECT OF CIRCADIAN RHYTHM IN THE DELIVERY/THERAPEUTIC PERFORMANCE OF PACLITAXEL-DENDRIMER NANOSYSTEMS

Tânia Albuquerque [1], Ana Raquel Neves [1], Milan Paul [2], Swati Biswas [2], Elena Vuelta [3,4,5], Ignacio García-Tuñón [4], Manuel Sánchez-Martin [3,5,6], Telma Quintela [1,7], Diana Costa [1(*)]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *Department of Pharmacy, Nanomedicine Research Laboratory, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Medchal, Hyderabad 500078, Telangana, India.*

[3] *Servicio de Transgénesis, Nucleus, Universidad de Salamanca, 37008 Salamanca, Spain.*

[4] *IBSAL, Instituto de Investigación Biomédica de Salamanca, 37007 Salamanca, Spain.*

[5] *Departamento de Medicina, Universidad de Salamanca, 37008 Salamanca, Spain.*

[6] *Unidad de Diagnóstico Molecular y Celular del Cáncer, Instituto Biología Molecular y Celular del Cáncer (USAL/CSIC), 37007 Salamanca, Spain.*

[7] *UDI-IPG-Unidade de Investigação para o Desenvolvimento do Interior, Instituto Politécnico da Guarda, Guarda, Portugal.*

(*) *e-mail:* dcosta@fcsaude.ubi.pt

Keywords: Bmal1 silencing; PAMAM; apoptosis; cancer therapy; caspases; circadian rhythm; nano-delivery systems.

ABSTRACT

The circadian clock is a remarkable timing system responsible for the control of several metabolic, physiological and behavioral processes. Nowadays, the connection between the circadian clock and cancer development/progression is consensual. Additionally, circadian rhythms can also affect mechanisms involved in the efficacy of anticancer drugs. Chronotherapy, a treatment scheduling approach, might consequently improve treatment efficacy of many types of cancer, increase patient survival and diminishing associated costs for cancer therapy. In parallel, to overcome the major obstacles of conventional therapies, a variety of nanosized drug delivery systems have been considered for the targeted drug release to cancer cells. Joining chronobiology and nanotechnology emerges as an innovative attempt to provide new knowledge and methodologies for novel/creative outcomes in cancer treatment. In the present work, we synthesized nanosystems based on an octa-arginine (R8)-modified poly(amidoamine) dendrimer conjugated with the anticancer drug paclitaxel (PTX), G4-PTX-R8, and its physicochemical properties were revealed to be appropriate for *in vitro* delivery. The influence of the circadian rhythm on its cellular internalization efficiency and potential therapeutic effect on human cervical cancer cells (HeLa) was studied. Cell-internalized PTX and caspase activity, as a measure of induced apoptosis, were monitored for six time points. Higher levels of PTX and caspase-3/9 were detected at T8, suggesting that the internalization of G4-PTX-R8 into HeLa cells and apoptosis are time-specific/-regulated phenomena. For a deeper understanding of the impact of the circadian clock on cellular uptake and apoptosis, the clock protein Bmal1—the main regulator of rhythmic activity, was silenced by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. Bmal1 silencing was revealed to have an impact on both PTX release and caspase activity, evidencing a potential role for circadian

rhythm on drug delivery/therapeutic effect mediated by G4-PTX-R8.

Acknowledgements:
D. Costa acknowledges the Fundação para a Ciência e a Tecnologia (FCT) her 2021.03946.CEECIND Researcher Contract. T. Albuquerque (SFRH/BD/148406/2019) and A. Neves (2020.08310.BD) acknowledge their FCT PhD grants, co-supported by the European Social Fund via Programa Operacional Regional Centro. Swati Biswas acknowledges the Indian Council of Medical Research (ICMR) for providing funding through a research grant (F-20218945). Milan Paul acknowledges ICMR for providing the senior research fellowship (45/11/2022/NAN/BMS). The presented research was developed within the scope of CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese FCT/MCTES.

P3.11 - SYNTHESIS AND CHARACTERIZATION OF GALLIC ACID-TRIETHYLENE GLYCOL APTADENDRIMERS

André Miranda [1], Roi Lopez-Blanco [2], Jéssica Lopes-Nunes [1], Ana M. Melo [3,4], Maria P. C. Campello [5,6], António Paulo [5,6], Maria Cristina Oliveira [5,6], Jean-Louis Mergny [6], Paula A. Oliveira [7], Eduardo Fernandez-Megia [2(*)], Carla Cruz [1(*)]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain*

[3] *iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa, Portugal;*
d Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[4] *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Bobadela LRS, Portugal.*

[5] *Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Bobadela LRS, Portugal.*

[6] *Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS, INSERM, Institut Polytechnique de Paris, 91128 Palaiseau, France*

[7] *Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, Vila Real, Portugal;*

(*) *e-mail:* ef.megia@usc.es | carlacruz@fcsaude.ubi.pt

Keywords: *Gallic acid-triethylene glycol dendrimers; G-quadruplex aptamers; aptadendrimer; biophysical studies.*

ABSTRACT

Cancer is a major public health problem that, unfortunately, is growing up worldwide. Chemotherapy, radiotherapy, and surgery are the preferred therapies; however, off-target activity/reactivity and toxicity are major shortcomings. One of the best ways to address these weaknesses is using drug delivery systems that enhance the tumor targeting, uptake, and internalization of the drug by the tumor cells. Several nanostructures, such as dendrimers, have been used to deliver drugs [1]. Dendrimers are synthetic tree-like macromolecules composed of repetitive layers of branching units that are prepared in a controlled iterative process, through generations with discrete properties [2]. Aptamers have been widely used as promising targeting moieties due to hallmark properties, such as low immunogenicity, easy synthesis, and high specific binding affinity [3]. They can be used to modify drug-loaded nanocarriers for targeted drug delivery. Herein, we described the synthesis of an aptadendrimer by covalent bioconjugation of a gallic acid-triethylene glycol (GATG) dendrimer with the G-quadruplex (G4) AT11 aptamer (a modified version of AS1411) at the surface. We evaluated the loading and interaction of an acridine orange ligand, termed C8, that acts as an anticancer drug and binder/stabilizer of the G4 structure of AT11. Both steady-state and time-resolved fluorescence anisotropy evidenced the interaction between the

aptadendrimer and C8. Release experiments show a delivery of C8 after 4 h. Also, the cell viability, uptake and delivery were evaluated into prostate cancer and healthy cell lines. Aptadendrimers tend to localize in the cytoplasm of various cell lines studied as demonstrated by confocal microscopy. The internalization of the aptadendrimers is not nucleolin-mediated or by passive diffusion, but via endocytosis. MTT studies with prostate cancer cells and non-malignant cells evidenced high cytotoxicity mainly due to the C8 ligand. The rapid internalization of the aptadendrimers and their fluorescence properties make them attractive for the development of potential nanocarriers.

References:

1. Sherje, A.P.; Jadhav, M.; Dravyakar, B.R.; Kadam, D. Dendrimers: A Versatile Nanocarrier for Drug Delivery and Targeting. Int J Pharm 2018, 548, 707–720, doi:10.1016/j.ijpharm.2018.07.030.
2. Caminade, A.-Marie. Dendrimers : Towards Catalytic, Material and Biomedical Uses. 2011, 538.
3. Zhou, J.; Rossi, J. Aptamers as Targeted Therapeutics: Current Potential and Challenges. Nature Reviews Drug Discovery 2016 16:3 2016, 16, 181–202, doi:10.1038/nrd.2016.199.

Acknowledgements:

André Miranda acknowledges the research fellowship from “Rede Nacional de Ressonância Magnética Nuclear” (ref. PINFRA/22161/2016-B4) and the doctoral fellowship Grant from FCT—Foundation for Science and Technology (ref. 2021.04785.BD). J. Lopes-Nunes acknowledges the doctoral fellowship grant from the FCT—Foundation for Science and Technology ref. 2020.05329.BD. A.M.M. acknowledges the Junior Researcher position under the FCT CEECindividual call (CEECIND/00884/2017). The authors thank Alexander Fedorov for assistance with time-resolved fluorescence measurements. C.C. acknowledges the grant from FCT ref. UIDP/00709/2020, project PAPILOMA ref. CENTRO-01-0145-FEDER-181235, “Bolsa de Investigação em Oncologia Dr. Rocha Alves do Núcleo Regional do Centro da Liga Portuguesa Contra o Cancro” and project INOVC+ Ecossistema de Inovação Inteligente, funded by CENTRO 2020, through Fundo Europeu de Desenvolvimento Regional ref. CENTRO-01-0246-FEDER-000044.

P3.12 - UREA DETECTION BASED ON A PROTOTYPE OF A MINIATURED NEAR-INFRA-RED SPECTROMETER TO MONITOR IN REAL TIME AND IN SITU KIDNEY FUNCTION

Paulo Zoio [1,2], Diogo Barradas [2], Luís P. Fonseca [1(*)], Bernardo B. Carvalho [3(*)], Cecília R.C. Calado [2,3(*)]

[1] Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico (IST), Universidade de Lisboa (UL), Lisboa, Portugal

[2] ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro, Lisbon, Portugal.

[3] Instituto de Plasmas e Fusão Nuclear, Instituto Superior Técnico (IST), Universidade de Lisboa (UL), Lisboa, Portugal.

[4] CIMOSM – Centro de Investigação em Modelação e Optimização de Sistemas Multifuncionais, ISEL, Rua Conselheiro Emídio Navarro, Lisbon, Portugal.

(*) e-mail: cecilia.calado@isel.pt

Keywords: Kidney function; *In situ* monitoring; Portable devices; NIR spectroscopy.

ABSTRACT

Acute kidney injury (AKI) affects 13.3million people worldwide per year and causes up to 1.7million deaths annually. Depending on, if the renal function recovers, AKI survivors are at high risk of transitioning to chronic kidney disease (CKD) and, in some cases, to end-stage kidney disease. A new method, enabling to monitor kidney function, based on a drop of blood, and on a portable equipment can strongly promote on time and adequate therapies to attenuate the AKI-CKD progression. The present work evaluates the application of a prototype of a portable miniaturized near-infrared (miniNIR) spectrometer to detect urea, which may provide crucial in real time and on-site information to clinical staff concerning the kidney function. To enable the device miniaturization and cost optimization, the mini-NIR spectrometer, is based on powered (5 V) and controlled via USB port of a computer and operates in the spectral range 1300–2100 nm (7692–4762cm^{-1}) with a matrix of 6 independent LEDs. Spectra of diverse samples of urea in bovine serum solutions (between 1 and 50mg/dl) were considered to mimic kidney malfunction, since levels above a value of 43 mg/dl are considered indicative of AKI. The spectra principal component analysis highlighted that it was possible to reproducible detect the evaluated urea concentrations, since scores for a defined concentration were clustered together and generally apart from other concentrations. Indeed, partial least regression models, based on a low number of latent variables(n=5), of leave-one-out-cross-validation data, resulted in a reasonable regression coefficient (r^2 > 0.80) and root mean square errors (5.9 mg/l). This presents a high potential to monitor kidney function based on portable and economic equipment's, enabling an *in situ* and real time monitoring.

Acknowledgements:
Project grant DSAIPA/DS/0117/2020 from Fundação para a Ciência e a Tecnologia, Portugal.

P3.13 - IONIC LIQUIDS AS ADDITIVES FOR STABILIZATION AND FORMULATION OF PROTEIN- BASED PRODUCTS

Nathalia V. Veríssimo [1], Qi Han [2], Valéria C. Santos-Ebinuma [1], Tamar L. Greaves [2], Jorge F.B. Pereira [3(*)]

[1] *Department of Bioprocesses Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil*

[2] *STEM College, RMIT University, Melbourne, Australia*

[3] *University of Coimbra, CIEPQPF, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, Coimbra, Portugal*

(*) e-mail: jfbpereira@eq.uc.pt

Keywords: Ionic liquids; Proteins; stabilization; formulation; additives.

ABSTRACT

The low stability and high production costs of proteins is the main bottleneck limiting access to life-saving protein-based bioproducts in countries and communities of low income. To improve the instability of proteins, ionic liquids (ILs) have been employed as stabilizers of proteins. However, several variables can impact the effect of ILs on proteins, including the nature, biocompatibility, and concentration of ILs, environmental conditions such as temperature and pH, and the intrinsic properties of proteins. Hence, this work compiled and analyzed the effect of ILs on proteins considering the protein properties, ILs classes, type of protein stability, and IL solutions concentrations to find trends that indicate the impact of each variable in protein stability. Considering the top four major IL families in this field, imidazolium and ammonium-based ILs are the predominant classes for protein stabilization studies. However, the most compatible classes with proteins are ammonium and cholinium ILs, followed by imidazolium and pyridinium/pyrrolidinium ILs. Moreover, ILs have a great aptitude to prevent protein aggregation (more than half of samples decreased aggregation) and activity (more than 40%), including some IL families that are also adequate for the preservation of structural and thermal stability of proteins (one-third of samples). Finally, we also experimentally evaluated the effect of different concentrations and IL classes on the short and long-term stability of the Green Fluorescent Protein (GFP). For the GFP, imidazolium- and cholinium-based ILs increased GFP short and long-term stabilization at room temperature by decreasing its aggregation. This work provides a clear overview about which ILs families can be used as protein stabilizers, with the potential to help expand the applications of unstable proteins and increase access to biological products.

Acknowledgements: CIEPQPF is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UIDB/ EQU/00102/2020 and UIDP/EQU/00102/2020. J.F.B. Pereira acknowledge FCT for funding the project DRI/India/0044/2020. N. V. Veríssimo thanks the support from PROPG/PROPe call 05/2022. V. C. Santos-Ebinuma thanks the financial support of FAPESP (2021/06686-8).

P3.14 - PREVENTING *CLOSTRIDIODES DIFFICILE* INFECTION BY USING AN IMMOBILIZED BILE SALT HYDROLASE TO INTERFERE WITH SPORE GERMINATION

Daniela Silva [1]***, Mónica Serrano*** [1]***, Adriano O. Henriques*** [1]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, NOVA University, Lisbon, Portugal.*

(*) *e-mail: daniela.silva@itqb.unl.pt*

Keywords: *Bacillus subtilis*; spore display; CotA; *Lactobacillus salivarius;* bile salt hydrolase.

ABSTRACT

The anaerobe *Clostridioides difficile* is a leading cause of healthcare-associated infections. *C. difficile* infection occurs after faecal-oral transmission of spores that germinate in the gut in response to certain bile salts. Commensal microbiota can prevent spore germination by modulating the bile acid pool. Therefore, spore germination, toxin production and disease only occurs under dysbiosis, normally caused by antibiotic therapy. A bile salt hydrolase produced by a commensal bacterium catalyzes the deconjugation of taurocholate in cholate and taurine without any known cofactors and inhibits C. difficile spore germination and colonization in vivo.

Fusing the bile salt hydrolase to a *Bacillus subtilis* spore surface protein allows the use of the resulting spores as a source of the enzyme during antibiotic treatment. *B. subtilis* has a GRAS (generally regarded as safe) status and spore display has been successfully used in several biotechnological applications. The display of the bile acid hydrolase relies on the use of an abundant spore surface protein as the carrier. Our strategy includes: structure-based design of the fusion protein using *Alphafold2* and the known and potential interactions of the carrier to avoid occlusion of the carrier regions needed for recruitment and association to the spore surface; transcriptional and translational optimization of the carrier gene, using site-directed mutagenesis, transcriptional lacZ fusions and analysis of the spore coat composition; assays of spore resistance and germination, to verify maintenance of a normal structure and functional properties of the recombinant spores; scoring the number of molecules displayed, on average, per spore; activity assays of the purified and spore-displayed bile acid hydrolase, as well as *C. difficile* germination assays *in vitro* in the presence/absence of the recombinant spores.

The implementation of this strategy and its preliminary results will be presented.

P3.15 - APTAMERS FOR BLOCKING ENTEROTOXIGENIC *ESCHERICHIA COLI*

Maria Margarida Barros [1,2(*)], ***Ricardo Oliveira*** [1,3,4], ***Joana Castro*** [1,5], ***Daniela Araújo*** [1], ***Sónia Silva*** [1,5,6], ***Ana Campos*** [1], ***Divanildo Outor-Monteiro*** [2], ***Carina Almeida*** [1,3,4]

[1] *I.P—National Institute for Agrarian and Veterinariay Research (INIAV), Vila do Conde, Portugal*

[2] *Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[3] *LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal*

[4] *ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal*

[5] *Centre of Biological Engineering, University of Minho, Braga, Portugal*

[6] *LABBELS –Associate Laboratory, Braga/Guimarães*

(*) *e-mail: margarida.barros@iniav.pt*

Keywords: Aptamer; *Galleria mellonella*; Cell-SELEX, ETEC, F4-fimbria.

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is the major cause of enteric infections in swine, resulting in significant costs for the swine industry. Among other virulence factors, fimbriae are essential for the initial adhesion of ETEC to the intestinal epithelial cells. In particular, the F4-type (K88) fimbriae are commonly associated with neonatal infections and most post-weaning diarrhoeal infections. These diseases are traditionally prevented or treated with antibiotics, but the use of antibiotics is being highly restricted due to the growing phenomenon of antimicrobial resistance. Therefore, novel strategies such as aptamers, which are small single-stranded oligonucleotides capable of binding to target molecules, seem to be a promising alternative to block the initial adhesion of F4-ETEC. The present study focuses on two parallel studies, the first in which two pre-selected aptamers (31/37) were tested in an *in vivo* model, *Galleria mellonella,* to evaluate their toxicity at three inoculated concentrations (1µM, 10µM, 20µM) and the performance as treatment (C_{apt}=500 nM) with infection (10^8 CFU/mL) of five strains (F4-ETEC, F18-ETEC, *Escherichia coli* K12, *Klebsiella pneumonia* ATCC 43216, *Staphylococcus aureus* ATCC 25929). Secondly, new specific DNA aptamers were selected through an innovative cell-SELEX approach against F4-ETEC bacteria, which involved four main steps: library incubation, partitioning, elution, and amplification. Both pre-selected aptamers showed no toxicity in *Galleria mellonella* after 96 h, regardless of the inoculated concentration. Furthermore, inoculation of 'aptamer 31 + F4-ETEC' in *Galleria mellonella* increased the larval survival rate and health index when compared to inoculation with F4-ETEC alone. Regarding the new selection of DNA aptamers, 12 rounds of SELEX were successfully carried out and a final pool of potential aptamers against F4-ETEC was obtained, which will now be evaluated for their specificity and affinity. This work demonstrates the potential of aptamers in the treatment of ETEC infections as an alternative to antibiotics.

Acknowledgements:

This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). The authors are also grateful to FCT for the PhD Grant SFRH/BD/2023.04664.BDANA and for the CEEC Individual (2022.06886.CEECIND).

P3.16 - OPTIMIZATION OF THE PRODUCTION AND PURIFICATION OF NON-VIRAL VECTORS FOR GENE THERAPY APPLICATIONS

Ana I. Valente[1(*)], Leonor S. Castro [1], Inês D. Rios [1], Augusto Q. Pedro [1], Ana P. M. Tavares[1], Fani Sousa [2], Mara G. Freire [1]

[1] *CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Portugal*

[2] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[(*)] *e-mail:* ***anaivalente@ua.pt***

Keywords: *Escherichia coli*; fermentation; induction; mcDNA biopharmaceuticals; non-viral vectors.

ABSTRACT

Cancer ranks the second leading cause of death globally and has significant societal and economic costs. To address this issue, the production of biopharmaceuticals is standing out. Nucleic acids are gaining popularity in preventing infections and as therapeutic agents in gene therapy, aiming to fix the target dysfunction by transfecting eukaryotic cells with gene-based products. Non-viral vectors, including plasmid DNA and minicircle DNA (mcDNA), attracted increased importance among these. Despite their clinical relevance, current manufacturing strategies are still complex and involve multi-step purification processes, ultimately increasing their cost. To overcome this obstacle, this work investigates applying ionic-liquid-based aqueous biphasic systems (IL-ABS) as a primary capture strategy of p53-mcDNA biopharmaceuticals. Considering the medium complexity in which the p53-mcDNA is produced, a clarification and concentration step with IL-ABS is critical before moving to high-resolution chromatographic purification. p53-mcDNA was produced resorting to recombinant *Escherichia coli* cells under optimized conditions to promote cell growth and parental plasmid (PP) bioproduction. Afterward, recombination was induced using L-arabinose, yielding the p53-mcDNA. The fraction containing the PP and p53-mcDNA was subsequently isolated using a commercial kit, and their partitioning behavior in ABS comprising bromide-based ILs and citrate potassium salt was investigated. Ongoing studies are focused on optimizing the separation of PP and p53-mcDNA using the designed IL-ABS, after which a sample of increased complexity will be applied.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC) and the CICS-UBI project UIDB/00709/2020. This work was additionally developed within the project 2022.03394.PTDC (PureDNA), financially supported by national funds (OE), through FCT/MCTES. Ana Valente, Leonor Castro, Augusto Pedro, and Ana Tavares acknowledge FCT, respectively, for the PhD grants 2020/05090/BD and SFRH/BD/08352/2021 and research contracts CEECIND/02599/2020 and CEECIND/2020/01867 under the Scientific Stimulus – Individual Call.

P3.17 - ON THE USE OF ISOTHERMAL AMPLIFICATION WITH A NUCLEIC ACID LATERAL FLOW ASSAY READ-OUT FOR THE DIAGNOSTIC OF RNA VIRUSES

***João M. Vindeirinho*[1,2,3 (*)], *Eva Pinho*[1,2,3], *Raquel Guiomar*[4], *Nuno F. Azevedo*[2,3], *Ahmed El Abd Wahed*[5], *Carina Almeida*[1,2,3]**

[1] *INIAV, I.P - National Institute for Agrarian and Veterinarian Research, Rua dos latidos, Lugar da Madalena, Vairao, Portugal*

[2] *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering , University of Porto, Rua Dr. Roberto Frias, Porto, Portugal*

[3] *ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal*

[4] *INSA, I.P - National Institute of Health Dr.Ricardo Jorge, Avenida Padre Cruz, Lisboa, Portugal*

[5] *Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, An den Tierkliniken, Leipzig, Germany.*

[(*)] *e-mail: jo.vindeirinho@gmail.com*

Keywords: Nucleic Acid Amplification; Isothermal Amplification; RT-RAA; RNA viruses.

ABSTRACT

SARS-CoV-2 stressed the need for the adoption of affordable, simple-to-operate and effective diagnostic methods, aimed at the detection of nucleic acids; the vast majority of the methods devoted to such end, demand a nucleic acid amplification step. RT-RAA (Reverse Transcription Recombinase-Aided Amplification) consists of an isothermal amplification method, which can be performed in only 15-20 minutes. Nucleic Acid Lateral Flow Assays (NALFAs) are a group of methods aimed at the detection of amplicons after an amplification step. These can use reporter- and capture- oligonucleotide probes to accurately detect target nucleic acid sequences by naked eye, following an isothermal nucleic acid amplification step. In this context, there is a promising NALFA approach that relies in the tailing of DNA amplicons during the step of amplification, with single strand DNA sequences (ssDNA), allowing their interaction with the NALFA´s capture and reporter oligonucleotide probes. This NALFA approach generates results in 10-15 minutes. In this work, RT-RAA was chosen to produce tailed amplicons and then combined with an in- house developed NALFA, using SARS-CoV-2 as a model. The limit of detection (LOD) of this assay was found to be 10-10^2 copies of RNA/µL, which is suitable for use in point-of-care detection of SARS-CoV-2 and other viruses. There was no cross-reactivity when testing a panel of related and non-related viruses. Moreover, 30 clinical samples were tested, with 13/14 positives and 13/16 negatives being correctly detected. The RT-RAA together with NALFA colorimetric device comprehends a simple and fast diagnostic approach, which can be integrated in a device for the detection of SARS-CoV-2 at the point-of-care, exhibiting a suitable cross-reactivity and LOD. In addition, this assay is promising for simple, end-point detection of other RNA viruses.

Acknowledgements:
This work was financed by LA/P/0045/2020 (ALiCE), UIBD/00511/2020 e UIDP/00511/2020 (LEPABE), using national funds through FCT/MCTES (PIDDAC). The authors also thank FCT for the PhD fellowship 2020.10243.BD.

P3.18 - INTERACTION OF NUCLEOLIN AND RNA G-QUADRUPLEX MOTIF AND PRELIMINARY CLINICAL EVALUATION IN LUNG CANCER

Joana Figueiredo[1(*)], André Miranda[1], Jéssica Lopes-Nunes[1], Daniela Alexandre[1], Salete Valente[2], Jean-Louis Mergny[3] Carla Cruz[1,4(*)]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[2] *Pneumology Department, Centro Hospitalar e Universitário Cova da Beira, Covilhã, Portugal*

[3] *Laboratoire d'Optique et Biosciences, Institut Polytechnique de Paris, CNRS, INSERM, Université Paris-Saclay, Palaiseau cedex, France*

[4] *Departamento de Química. Facultade de Ciências. Universidade da Beira Interior, Covilhã, Portugal.*

(*) e-mail: figueiredo_joana@hotmail.com | carlacruz@fcsaude.ubi.pt

Keywords: G-quadruplex; pre-MIR150; Nucleolin; Molecular Interaction; Lung Cancer.

ABSTRACT

Nucleolin (NCL) is a versatile protein involved in several biological processes and it is commonly overexpressed in cancer cells and not in healthy tissues. Elevated NCL expression levels are frequently associated with a poor prognosis of patients with lung cancer (LC), suggesting that NCL might serve as a potential biomarker. NCL has been observed to display a strong binding to G-quadruplexes (G4). Here, we investigated NCL interaction/recognition by the pre-MIR150 G4-forming sequence (designated as "rG4"). Circular dichroism (CD) spectra of the rG4 sequence showed a parallel quadruplex structure formation in KCl conditions or when complexed with the ligand PhenDC3. The thermal stability of rG4 is very high (ΔT_m= 54 ºC), and further increases in the presence of PhenDC3 (ΔT_m= 17ºC). The binding affinities of rG4 to PhenDC3 and NCL RBD1,2 are in the nanomolar range. PAGE results suggest the formation of a ternary rG4-ligand-protein, indicating that PhenDC3 does not prevent the binding of rG4 to NCL RBD1,2. Finally, labeled rG4 can recognize NCL-positive cancer cells and can be used as a probe for this protein. ELISA experiments indicate altered NCL expression patterns in liquid biopsies of LC patients in a non-invasive manner, potentially helping the clinical treatment.

Acknowledgements:
Joana Figueiredo, André Miranda Daniela Alexandre and Jessica Lopes Nunes acknowledges doctoral fellowship grants from FCT references SFRH/BD/145106/2019, 2021.04785.BD, 2021.07695.BD and 2020.05329.BD, respectively. CC acknowledges INSTRUCT ERIC project APPID 2473.

P3.19 - LEPT-IFA: AN INDIRECT IMMUNOFLUORESCENCE TEST FOR THE DIAGNOSIS OF LEPTOSPIROSIS

Ana Armada[1(*)], Wilmer Fernandes[2], Maria Luísa Vieira[1]

[1] Microbiology Unit, Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal

[2] Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal.

(*) e-mail: aarmada@ihmt.unl.pt

Keywords: *Leptospirosis;* Laboratory diagnosis; *Leptospira interrogans*; MAT; Immunofluorescence.

ABSTRACT

Leptospirosis is a reemerging zoonosis with worldwide distribution, with more than 1,000,000 severe cases notified annually in the human population. It is caused by pathogenic spirochetes of the genus *Leptospira*. The definitive diagnosis of leptospirosis, along with compatible clinical and epidemiological findings, is based on the Microscopic Agglutination Test (MAT), a serological reference test recommended by the World Health Organization (WHO). However, MAT is sometimes not feasible due to the high complexity of its execution, since it is a serovar-specific test, requiring a battery of 20 to 26 different serovars. Thus, this study aims to optimize an indirect immunofluorescence assay (Lept- IFA) for the diagnosis of leptospirosis, using three pathogenic serovars, recognized for their high and ubiquitous prevalence in Portugal. *L interrogans* s.l.. and *L. biflexa* s.l. (saprophytic species), were maintained in Ellinghaussen, McCullough, Johnson and Harris (EMJH) medium. To preserve surface leptospiral proteins, spirochetes were harvested by low-speed centrifugation and loaded to each spot of the IFA slide coated with lysine and then fixed with formalin. Fifty human serum samples, selected from the BioBank of the IHMT-NOVA/Leptospirosis laboratory, with a clinical indication for leptospirosis and analyzed by MAT, were tested by Lept-IFA. A representative serum from a healthy population and sera tested for two other spirochetes (*Borrelia burgdorferi* s.l. and *Treponema* sp), were used as negative controls. Lept-IFA confirmed all positive samples tested by MAT (19+; 38%), 12% (n=6) of the samples showed inconclusive results, and the remaining 50% were negative (n=25). In the inconclusive sera group, both Lyme borreliosis sera (controls) cross-reacted with Patoc serovar.

Acknowledgements:
This study pointed out that Lept-IFA is a useful tool as a first-line screening test for the serological diagnosis of leptospirosis in laboratories that do not have the reference test recommended by the WHO.

P3.20 - SCREEN-PRINTED ELECTROCHEMICAL IMMUNOSENSORS FOR IMPROVING HEART FAILURE PATIENT MANAGEMENT

***Bernardo Servolo*[1,2(*)], *Miguel Castelo-Branco*[1], *Julia Arcos-Martinez*[3], *Ana Cristina Dias-Cabral*[1,2]**

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[2] *Department of Chemistry, Faculty of Sciences, University of Beira Interior, Covilhã, Portugal*

[3] *Department of Chemistry, Faculty of Sciences, University of Burgos, Burgos, Spain.*

[(*)] *e-mail:* bernardo.miguel.viegas.servolo@ubi.pt

Keywords: Cardiac biomarkers; proBNP; Electrochemical immunosensor; SPCEs; Direct monitoring.

ABSTRACT

Heart failure (HF) is a prevalent disease worldwide, with a significant impact in Portugal. Patients may have a stable and controlled disease, or they may have symptoms that worsen regularly, requiring repeated visits to the emergency department and often prolonged hospital stays. It has been proven that significant decompensations shorten survival time, reduce quality of life, and cause the clinical state to deteriorate more quickly. It is therefore essential to detect decompensations at an early stage. The effectiveness of home monitoring is well recognized, but the accuracy of early detection of decompensations needs to be increased. To this end, we intend to develop a multiplexed biosensor to identify HF biomarkers in saliva that can complement clinical data and improve the ability to recognize decompensations in these patients before they become severe. Screen-printed electrodes (SPCEs) will be used as transducers, due to their low cost and ease of manufacture, while antibodies will be used as recognition elements due to their high affinity. As a first step, an analytical technique based on direct monitoring is examined. Direct observation reduces the complexity of building the biosensor. Specific antibodies to BNP (brain natriuretic peptide) will be functionalized on modified carbon electrodes. Square wave voltammetry measurements will be carried out using a redox mediator to monitor the decline in the peak reduction current as a function of analyte binding. The initial results of this research will be presented and discussed.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

P3.21 - RESCUE OF *MYCOBACTERIUM BOVIS* DNA OBTAINED FROM CULTURED STRAINS DURING OFFICIAL SURVEILLANCE OF ANIMAL TUBERCULOSIS

Daniela Pinto [1,2], Gonçalo Themudo [1], André C. Pereira [1,2], Ana Botelho [3], Mónica V. Cunha [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*

[3] *National Institute for Agrarian and Veterinary Research (INIAV IP), Av. da República, Quinta do Marquês, Oeiras, Portugal.*

[(*)] *e-mail: mscunha@ciencias.ulisboa.pt*

Keywords: animal tuberculosis; *Mycobacterium bovis*; whole genome sequencing; whole genome amplification; computational biology; mixed infection.

ABSTRACT

Epidemiological surveillance of animal tuberculosis (TB) has greatly advanced with whole genome sequencing (WGS) of *Mycobacterium bovis*, the main etiologic agent. WGS offers high-resolution data for infection source identification, pathogen population characterization, and contact tracing. However, in this model system, the workflow from bacterial isolation to sequence data analysis faces technical challenges that can hinder understanding of epidemiological scenarios and outbreak response. When implementing genomic surveillance of animal TB in Portugal and using archived DNA from strains isolated from animal samples during official surveillance, we encountered three major challenges: limited concentration of *M. bovis* DNA, contamination with DNA from other organisms, and co-occurrence of multiple *M. bovis* strains in a single sample (mixed infection). Losing an isolate's genome impairs the accurate reconstruction of transmission chains, impacting biological and epidemiological interpretations, hence the importance of recovering as many genomes as possible. To address these challenges, we developed an integrated solution involving whole genome amplification and a specialized computational pipeline. This approach minimized challenges inherent to previously established routine procedures and allowed us to recover 62 out of 100 samples that would have otherwise been lost. Based on these results, we discuss best practice adjustments for official and research laboratories to streamline sequential implementation of bacteriological culture, PCR, genomics, and computational methods. All these steps supporting timely, data-driven intervention. While we report the key rescue strategies for the animal TB model system, these recovery approaches have a broader application in a clinical and One Health context.

Acknowledgements:
Fundação para a Ciência e a Tecnologia, IP [PTDC/CVT- CVT/29783/2017, UIDB/00329/2020 UIDB/04046/2020, LA/P/0121/2020], PORLisboa [LISBOA-01-0145-FEDER-029783], COMPETE [POCI-01-0145- FEDER-029783].

P3.22 - PORTABLE COLORIMETRIC SENSORS BASED ON SUPPORTED IONIC LIQUID MATERIALS FOR L-ASPARAGINE DETECTION

João Nunes, Márcia Neves (*), Mara Freire, Ana Paula Tavares

CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

(*) *e-mail: mcneves@ua.pt*

Keywords: L-asparaginase; supported ionic liquid materials; portable colorimetric sensors; L-asparagine detection.

ABSTRACT

L-asparaginase (ASNase) is an amidohydrolase enzyme applied in the pharmaceutical industry as an anticancer agent in the treatment of lymphoproliferative disorders[1]. ASNase is also used in biosensors for the detection of L-asparagine in blood serum samples of acute lymphoblastic leukemia (ALL) patients[2]. However, the high cost of non-portable and sensitive L-asparagine detection techniques, being mainly carried out by chromatography, reinforces the need of developing portable and low-cost colorimetric sensors for L-asparagine detection. Supported ionic liquid (SIL) materials comprise ionic liquids (ILs) covalently attached to the support, enabling distinct interactions to be established among the target compounds and the support. SIL materials with quaternary ammonium cations and Cl^- as the counterion have been successfully applied in the immobilization of ASNase[1]. In this work, SIL materials based on silica fabric functionalized with quaternary ammonium cations and the Cl^- anion, viz. silica fabric functionalized with dimethylbutylpropylammonium chloride ([SF][N3114]Cl) and silica fabric functionalized with triethylpropylammonium chloride ([SF][N3222]Cl), were synthesized, characterized through several distinct techniques. These novel materials were also investigated as portable colorimetric sensors for L-asparagine detection in aqueous samples, i.e., aqueous solution of L-asparagine or commercial blood serum spiked with different L- asparagine concentration levels (10^{-1} M – 10^{-5} M). The developed low-cost and portable silica fabric SILs allows the fast and accurate detection of L-asparagine at different concentration levels.

References:

[1] Nunes JCF, Almeida MR, Bento RMF, Pereira MM, Santos-Ebinuma VC, Neves MC, Freire MG Tavares APM, Molecules, 2022, 27, 929.

[2] Westmacott KL, Crew AP, Doran O, Hart JP, Biosens. Bioelectron., 2020, 150, 111837.

Acknowledgements:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP. APM Tavares and MC Neves acknowledge FCT for the research contract CEECIND/2020/01867 and CEECIND/00383/2017, respectively. JCF Nunes acknowledges SPQ and FCT for the PhD fellowship (SFRH/BD/150671/2020).

P3.23 - COMPUTER-AIDED DESIGN OF AFFINITY LIGANDS FOR RNA PURIFICATION USING MOLECULAR DOCKING

***Rita Carapito*[1(*)], *Paulo Almeida*[1], *Matheus M. Pereira*[2], *Mara G. Freire*[3], *Fani Sousa*[1]**

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[2] *CIEPQPF - Chemical Engineering Processes and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Portugal*

[3] *CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal.*

[(*)] *e-mail: ritacarapito@fcsaude.ubi.pt*

Keywords: Oligonucleotides; pre-miRNA-29b; Affinity ligands; Molecular Docki ng.

ABSTRACT

Nucleic acids have been intensively used as biopharmaceuticals for diagnosis and treatment of several diseases. RNA has gained considerable attention due to its widespread use in vaccines, however, non-coding RNAs are also promising therapeutic tools. In patients with Alzheimer's disease (AD), the levels of pre-miRNA-29b are decreased and consequently, human β-secretase (hBACE1) is overexpressed. With this, pre-miR-29b can be a potential therapeutic agent, as restoring its levels in AD could reduce hBACE1 expression and consequently the accumulation of Amyloid-β peptides in neuronal cells. Nevertheless, its application highly depends on purity, stability, and biological activity. In this work, affinity chromatography, which is known for its high performance and selectivity, is being explored to purify pre-miR-29b. For this, Molecular Docking (MD) was used to screen a variety of customized oligonucleotides which were designed to be complementary to pre-miRNA-29b structure. First, the oligonucleotides structures were created and applied to Chem3D-MM2 protocol to minimize their energy. MD analysis was conducted using an FFT-based search algorithm, with the oligonucleotides acting as ligands and pre-miRNA-29b as the receptor. Through MD, we evaluated 26 oligonucleotides and identified four as most promising for pre-miRNA-29b purification, which were chosen for further immobilization. The docking score, binding site, and ligand-receptor interactions were the key factors to select these ligands. The most common interactions observed in MD results were hydrogen bonds, which increase the stability of the complex, and may have a positive impact on pre-miR-29b purification.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT), through the projects UIDB/00709/2020, UIDP/00709/2020, and within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). CIEPQPF is supported by the FCT through the projects UIDB/EQU/00102/2020 and UIDP/EQU/ 00102/2020. R. Carapito acknowledges FCT for the PhD fellowship (2020.07980.BD).

P3.24 - NIPHARMINS: NOVEL LOW RESISTANCE-INDUCING ANTIMICROBIALS WITH ANTIBIOFILM ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*

João Pedro Carrilho [1,2(*)], **Nuno Candeias** [3], **Alexandra Nunes** [1,2,4]

[1] *Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal*

[2] *School of Psychology and Life Sciences, Lusófona University, Lisbon, Portugal*

[3] *LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal*

[4] *Veterinary and Animal Research Centre (CECAV), Faculty of Veterinary Medicine, Lusófona University, Lisbon, Portugal.*

(*) *e-mail: jmcarrilho1462000@gmail.com*

Keywords: *Staphylococcus aureus*; Antimicrobial resistance; NiPharmins; Biofilms; *In vitro* Selective Pressure; Whole Genome Sequencing.

ABSTRACT

The rapid increase of *Staphylococcus aureus* antimicrobial resistance (AMR) together with their impact in healthcare highlights the urgent need of alternative therapeutic compounds. We have recently demonstrated that novel alkylaminophenols (NiPharmins) are promising effective antibacterial agents against *S. aureus*, evidencing a very low potential of inducing AMR. Herein, we intend to get a step further in NiPharmins' validation as a promising prophylactic option against *S. aureus* infections by evaluating its antibiofilm activity. *S. aureus* (SA-1) isogenic clones were continuously propagated *in vitro* without and under sub-MIC of one of the most potent NiPharmins (Nph1). Phenotypic tests coupled with WGS were used to assess the putative AMR acquisition by expressing staphylococcal biofilm. The impact of Nhp1 prolonged exposure at sub-MIC conditions on bacterial susceptibility against a panel of 16 antibiotics was also evaluated. Moreover, the capacity of NiPharmins for inhibiting biofilms from the staphylococcal populations evolved under selective pressure was studied. Overall, the biofilm expression of SA-1 lead to AMR, with all staphylococcal populations growing to at least 4xMIC. Furthermore, for each antibiotic tested, no significant differences in antimicrobial susceptibility were seen among all bacterial populations (wild-type, positive control, and Nph1-evolved populations), suggesting that prolonged exposure to Nph1 sub-MIC does not adversely affect the bacterial original AMR profile to traditional antibiotics. This compound also demonstrated great efficiency in inhibiting the formation of biofilms, at MIC concentration, of all SA-1 populations. Genomic analyses revealed nonsynonymous mutations on important transcriptional regulators, namely AraC and AcrR, both related to biofilm formation. Mutations in Pur operon repressor (PurR), also related with the expression of biofilms, was likewise observed. This work reinforces the potential of these novel antibacterial compounds as promising prophylactic alternatives for the prevention of *S. aureus* infections.

P3.25 - COPING WITH ANTIMICROBIAL RESISTANCE SCENARIO RESORTING TO AVIAN IMMUNOGLOBULIN Y (IGY) ANTIBODIES

Catarina Almeida[1,2(*)], Débora Sousa[1], Márcia Neves[1,2], Mara Freire[1,2]

[1] *CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal*

[2] *RYA Purification Technologies, Ílhavo, Portugal.*

[(*)] *e-mail: ac.almeida@ua.pt*

Keywords: Antimicrobial Resistance; Methicillin-resistant *Staphylococcus aureus*; Biopharmaceuticals; Avian Immunoglobulin Y; Stability; Antibody-antigen recognition; Toxicity.

ABSTRACT

Antimicrobial Resistance (AMR) is increasing owning to antibiotic's misuse, being Methicillin-resistant *Staphylococcus aureus* (MRSA) one of the most hazardous pathogenic bacteria [1]. Therefore, efficient alternative therapeutics, namely biopharmaceuticals, are needed to tackle this scenario. Avian Immunoglobulin Y (IgY), present in hen's egg yolk, is a promising solution since by being a polyclonal antibody can detect multiple epitopes on an antigen, that provides benefits in the treatment of these microbial diseases [1]. Nonetheless, their recovery at high purity and yields, and preservation is difficult to achieved due to their matrix complexity, limiting their use as biopharmaceuticals.

In this work, the potential of IgY to fight infectious diseases caused by MRSA was assessed. To this end, specific IgY were purified from the egg yolk of hyperimmune eggs with the recombinant Penicillin-Binding Protein 2a (PBP2a) of MRSA. Subsequently, formulations comprising anti-PBP2a IgY were developed and stored at 4 or -20 ºC, up to 3 months, being their stability and percentage of aggregates determined through Circular Dichroism (CD) Spectroscopy and Size Exclusion-High Performance Liquid Chromatography (SE-HPLC), respectively. Moreover, the anti-PBP2a IgY recognition for antigen, as well as their *in vitro* and *in vivo* toxicity were assessed. Accordingly, promising stabilizers for anti-PBP2a IgY were identified, being this antibody able to recognize the antigen, while exhibit nontoxicity to cells and rodents. Altogether, uphold IgY potential as a safe, stable, and effective biopharmaceutical to tackle MRSA-related diseases, while allowing to reduce the burdens linked to AMR.

References:

[1] S. A. El Kafrawy et al., Front. Immunol. 14 (2023)

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). CA acknowledge FCT for the doctoral grant 2022.11570.BD. MCN acknowledge FCT for the research contract CEECIND/00383/2017 under the Scientific Stimulus—Individual Call.

P3.26 - PH-RESPONSIVE NANOPARTICLES FOR EFFICIENT RNA DELIVERY IN CANCER CELLS

Bruno Baptista [1(*)]***, Andreia S. R. Oliveira*** [2]***, Patrícia V. Mendonça*** [2]***, Arménio C. Serra*** [2]***, Jorge F. J. Coelho*** [2]***, Fani Sousa*** [1]

[1] *CICS-UBI – Health Sciences Research Centre, Universidade de Beira Interior, Av. Infante D. Henrique, Covilhã, Portugal*

[2] *University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Chemical Engineering, Rua Sílvio Lima-Polo II, Coimbra, Portugal.*

[(*)] *e-mail:* bruno.m.baptista@hotmail.com

Keywords: pH-responsive nanoparticles; small RNAs; POEOMA-b-PDPA; Cancer.

ABSTRACT

The growing exploitation of RNA for groundbreaking therapies needs a precise delivery system to effectively target cells. This study focuses on the development of pH-responsive nanoparticles as intelligent carriers, with a particular emphasis on investigating the pH-responsiveness of the poly(2-(diisopropylamino)ethyl methacrylate)) (PDPA) block for encapsulating and delivering small RNAs (sRNA) to cancer cells. The block copolymers, composed of poly(oligo(ethylene oxide) methyl ether methacrylate) (POEOMA) and PDPA, or POEOMA-*b*-PDPA, demonstrated impressive complexation efficiencies of approximately 89% for sRNAs and 91% for pre-miRNA. Dynamic Light Scattering (DLS) analysis revealed particle sizes ranging from 76 to 1375 nm. Interestingly, the morphology of the polyplexes was found to be pH-dependent, resulting in spherical but polydispersed particles at lower pH values, and nanoparticles with a more uniform size yet altered morphology at higher pH values. Furthermore, the polyplexes exhibited notable capability in shielding RNA from RNases, and the transfection of A549 and fibroblast cells demonstrated no cytotoxic effect. Notably, the polyplexes enabled efficient transfection of the A549 cell line with pre-miRNA-29b and miRNA-29b, leading to a significant reduction (approximately 51% and 47%, respectively) in expression levels of the DNMT3B target gene. Overall, this research significantly contributes to the advancement of pH-sensitive nanoparticles, presenting a promising avenue for responsive delivery systems with potential implications in diseases such as cancer.

Acknowledgements:
This work was supported by the FCT through the project UIDB/00709/2020 and UIDB/00285/2020. Baptista B. acknowledges a fellowship within the project PUREmiRSILs and the fellowship BID/ICIFC/CICS/Santander Universidades-UBI/2021 within the Santander Universidades-UBI/2021 Protocol. Andreia S. R. Oliveira acknowledges FCT for her doctoral grant (SFRH/BD/144989/2019).

P3.27 - SYNTHESIS AND CYTOTOXICITY EVALUATION OF SUPPORTED IONIC LIQUIDS FOR THE PURIFICATION OF P53-MINICIRCLE DNA BIOPHARMACEUTICALS

***João Vasco Valente*[1], *Ana I. Valente*[1], *Augusto Q. Pedro*[1], *Mara G. Freire*[1]**

[1] *CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Portugal.*

(*) *e-mail:* valente.joao@ua.pt

Keywords: Biopharmaceuticals; minicircle DNA (mcDNA); Suported Ionic Liquids (SILs); Toxicity.

ABSTRACT

In an aging society, cancer is the second cause of death worldwide with projections of 28.4 million new cases by the year of 2040. Nucleic acids-based biopharmaceuticals, among which the non-viral vector minicicle DNA (mcDNA), are emerging as groundbreaking therapeutic agents for cancer, primarily because of their enhanced therapeutic efficacy, specificity, and reduced occurrence of side effects. [1] Current mcDNA downstream processing methodologies are not as efficient as required, mostly due to the complexity of the biological medium in which mcDNA is produced. To overcome this limitation, innovative materials for the isolation of p53-mcDNA were prepared by covalent attachment of ionic liquids in a solid support (SILs, supported ionic liquid) were prepared, and their potential cytotoxicity was evaluated towards two human cell lines (Caco-2 and HepG2). SILs materials were prepared by the immobilization of different imidazolium- and ammonium-based ILs in spherical silica and characterized using elemental analysis, zeta potential and NMR. The studied materials exhibit low cytotoxic potential with a decrease in cell viability lower than 10% for Caco-2 and 30% for HepG2, respectively. These promising results open the possibility of supported ionic liquids for the purification of p53-minicircle DNA biopharmaceuticals with application in oncology, currently under investigation.

References:

[1] Walsh, G. Biopharmaceutical benchmarks 2018, Nature Biotechnology 2018, 36, 1136-1145, doi:10.1038/nbt.4305.

Acknowledgements:

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). This work was also developed within the framework of the project "PureDNA - Development of cost-effective platforms based on ionic liquids for the purification of p53-minicircle DNA biopharmaceuticals with application in oncology", 2022.03394.PTDC, financially supported by national funds (OE), through FCT/MCTES. Augusto Q. Pedro acknowledge FCT for the research contract CEECIND/02599/2020 under the Scientific Stimulus – Individual Call.

P3.28 - PH RESPONSIVE TAXIFOLIN-LOADED DELIVERY SYSTEMS FOR CERVICAL CANCER THERAPY

Miguel Neto [1(*)], ***Miguel Ferreira*** [1], ***Diana Gomes*** [1,2,3], ***Luís Passarinha*** [1,2,3,4], ***Diana Costa*** [1], ***Ângela Sousa*** [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *Associate Laboratory i4HB—Institute for Health and Bioeconomy, Faculdade de Ciências e Tecnologia, Universidade NOVA, Caparica, Portugal*

[3] *UCIBIO—Applied Molecular Biosciences Unit, Departamento de Química/Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal*

[4] *Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal*

(*) *e-mail: miguel.leite.neto@ubi.pt*

Keywords: cervical cancer; flavonoids; HPV; polymeric delivery systems; taxifolin.

ABSTRACT

Human Papillomavirus (HPV) is the main causative agent for the development of cervical cancer since its E6 oncoprotein targets p53 tumor suppressor protein for degradation. Flavonoids have been described as potential anticancer agents, due to their interaction with E6 oncoprotein, avoiding the latter to bind to p53.Flavonoids, such as taxifolin, have very low bioavailability, limiting its efficacy. In this way, the development of nanoformulations is essential to effectively deliver taxifolin to cancer cells. Thus, this work explored natural polymer-based (chitosan and gellan gum) nanoparticles to encapsulate taxifolin, aiming for an increase in taxifolin loading capacity and solubility. The systems were prepared through polyelectrolyte complexation technique and were characterized regarding their size, polydispersity index, zeta potential, morphology, FTIR, encapsulation efficiency, *in vitro* release studies at pH 5.8, representative of the tumoral microenvironment, and pH 7.4, representing the bloodstream, along with cell internalization, viability, and western blotting. Several ratios between chitosan, gellan gum and taxifolin were tested. The most favorable formulation showed a size of 276.23±30.68 nm, a PdI of 0.36±0.06, a zeta potential of +30.80±5.66 mV, 65.56% of encapsulation efficiency and a taxifolin release of 70% at pH 5.8 and 25% at pH 7.4. This work brings valuable information concerning the encapsulation of taxifolin into delivery systems and its relationship with drug bioavailability. In addition, our work represents a step forward in the design/development of flavonoids-based delivery systems for cancer therapy applications.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Miguel Ferreira and Diana Gomes acknowledge the doctoral fellowships from FCT ref: 2023.01957.BD and 2020.06792.BD. Diana Costa acknowledges FCT her Assistant Researcher Contract 2021.03946.CEECIND. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2021.

P3.29 - RECOVERY OF PROTEIN-BASED BIOPHARMACEUTICALS FROM CULTURE MEDIA USING POLYMER-BASED AQUEOUS BIPHASIC SYSTEMS

Leonor S. Castro [1(*)], ***Augusto Q. Pedro*** [1], ***Mara G. Freire*** [1]
[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal*

[(*)] *e-mail: l.castro@ua.pt*

Keywords: aqueous biphasic systems; polymers; biopharmaceuticals; Komagataella pastoris.

ABSTRACT

The rise in life expectancy, combined with climate change and growing urbanization, has led to an increase in the prevalence of chronic and infectious diseases. A promising strategy to mitigate the negative impact in human health is by using biopharmaceuticals, biological products obtained from living organisms highly effective in the prophylaxis and treatment of a wide range of disorders. Despite their numerous benefits over traditional synthetic products, their application is not as widespread, due to their complex manufacturing process that remains time-consuming and expensive, making biopharmaceuticals not widely available worldwide, particularly in low- and middle-income nations. In this work, polymer-based aqueous biphasic systems (ABS) are presented as an alternative for the recovery of interferon α-2b (IFNα-2b), representative of protein-based biopharmaceuticals, manufactured resorting to a bioprocess-based on *Komagataella pastoris* X-33. The recombinant yeast strain harbors the pPICZα vector containing the desired gene and coupled with the alpha-mating factor signa, deemed essential for the secreted expression of IFNα-2b. In this sense, ABS composed of polyethylene glycol (PEG) with different molecular weights and polypropylene glycol 400 g/mol (PPG400) were applied for the recovery of IFNα-2b directly from the culture media. The results demonstrate that the PEG molecular weight and the respective mixture compositions greatly affect the IFNα-2b partitioning behavior. Using PEGs with lower molecular weight, the protein is selectively partitioned with high yield to the phase enriched in PEG, but it precipitates at the interface if using high molecular weight PEGs. Overall, this work highlights the need to adjust both the nature of ABS components and the corresponding mixture compositions to optimize biopharmaceutical recovery, thus offering novel and customized routes for the recovery of protein-based biopharmaceuticals.

Acknowledgements:
This work was developed within the scope of the projects CICECO-Aveiro Institute of materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Leonor S. Castro and Augusto Q. Pedro acknowledge FCT, respectively, for her PhD grant 2020/05090/BD and for the research contract CEECIND/02599/2020 under the Scientific Stimulus – Individual Call.

P3.30 - TARGETED DELIVERY OF MINICIRCLE DNA VACCINE AGAINST COVID-19 TO ANTIGEN-PRESENTING CELLS USING MANNOSYLATED POLYETHYLENIMINE- CHOLESTEROL-BASED NANOPARTICLES

Dalinda Eusébio [1(*)], Milan Paul [2], Swati Biswas [2], Zhengrong Cui [3], Diana Costa [1], Ângela Sousa [1]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[2] Nanomedicine Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science-Pilani, Hyderabad Campus. Jawahar Nagar, Medchal, Hyderabad, India
[3] The University of Texas at Austin, College of Pharmacy, Division of Molecular Pharmaceutics and Drug Delivery, Austin, Texas, USA

(*) e-mail: dalinda.eusebio@ubi.pt

Keywords: COVID-19, Minicircle DNA Vaccine, Polyethylenimine, Cholesterol, Mannose.

ABSTRACT

Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Globally, there have been 762 million confirmed cases of COVID-19, including approximately 7 million deaths, reported to the World Health Organization (WHO). DNA vaccines can be a potential solution to protect global health, triggering both humoral and cellular immune responses. Therefore, DNA vaccines can be considered powerful tools against COVID-19. Minicircle DNA (mcDNA) is an innovative vector completely devoid of bacterial-derived sequences, which are usually associated with safety concerns when compared with conventional plasmid DNA. This work explored different nanosystems based on polyethylenimine (PEI), functionalized with cholesterol (CHOL) and mannose (MAN) to deliver parental plasmid (PP) and mcDNA vectors encoding the receptor-binding domain (RBD) of SARS-CoV-2 to antigen-presenting cells (APCs). For comparative purposes, three different systems were evaluated: PEI, PEI-CHOL and PEI-CHOL-MAN. The systems were prepared at various nitrogen-to-phosphate group (N/P) ratios and characterized in terms of encapsulation efficiency, surface charge, size, polydispersity index (PDI), morphology and stability over time. Fourier transform infrared spectroscopy (FTIR) was applied to investigate the functional groups present on the surface of the nanoparticles. Moreover, *in vitro* transfection studies of dendritic cells (JAWS II) and human fibroblast cells were performed. Viability studies assured the safety of all nanocarriers. Confocal microscopy studies confirmed the intracellular localization of nanosystems, resulting in enhanced cellular uptake using PEI-CHOL and PEI-CHOL-MAN nanosystems when compared with the PEI alone. Regarding the RBD expression, PEI-CHOL-MAN was the system that displayed higher levels of transcripts and protein in JAWS II cells. Furthermore, nanosystems were effective in inducing dendritic cell maturation, with significantly increased production of pro-inflammatory cytokines. This work constitutes a significant advance in the development of a suitable platform for the effective targeted delivery of DNA vaccines to APCs, which can potentially lead to enhanced immune responses.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Dalinda Eusébio acknowledges the doctoral fellowship from FCT (Reference:

2020.10201.BD). Diana Costa acknowledges FCT her Assistant Researcher Contract 2021.03946.CEECIND. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under the scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2021.

P3.31 - MANNOSYLATED CHITOSAN-OCTA-ARGININE-BASED NANOPARTICLES FOR TARGETED DELIVERY OF MINICIRCLE DNA VACCINE AGAINST COVID-19 TO ANTIGEN-PRESENTING CELLS

Dalinda Eusébio [1(*)], Milan Paul [2], Swati Biswas [2], Zhengrong Cui [3], Diana Costa [1], Ângela Sousa [1]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[2] Nanomedicine Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science-Pilani, Hyderabad Campus. Jawahar Nagar, Medchal, Hyderabad, India
[3] The University of Texas at Austin, College of Pharmacy, Division of Molecular Pharmaceutics and Drug Delivery, Austin, Texas, USA

(*) e-mail: dalinda.eusebio@ubi.pt

Keywords: COVID-19; Minicircle DNA Vaccine; Chitosan; Octa-arginine; Mannose.

ABSTRACT

Coronavirus disease (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 21 September 2023, confirmed COVID-19 infections numbered over 762 million individuals worldwide and have resulted in nearly 7 million deaths, reported to the World Health Organization (WHO). Nucleic acid vaccines emerged as a novel approach to induce efficient and safe immune responses. Nevertheless, DNA vaccines exhibit superior stability and greater simplicity in manufacturing compared with messenger RNA (mRNA) vaccines. This work explored different nanosystems to deliver parental plasmid (PP) and mcDNA vectors encoding the receptor-binding domain (RBD) of SARS-CoV-2 to antigen-presenting cells (APCs). The delivery systems were based on two different chitosan polymers, the high molecular weight chitosan (HMW) and 5 kDa chitosan. Nanosystems were formulated using an ionotropic gelation technique, which allowed the conjugation of different molecules, specifically chitosan, tripolyphosphate (TPP,) octa-arginine (R8) and R8-mannose. Nanosystems were characterized in terms of encapsulation efficiency, size, polydispersity index (PDI) and surface charge. Scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) were performed to investigate the morphology and the functional groups present on the surface of the nanoparticles, respectively. Dendritic cells (JAWS II) and human fibroblast cells were used in *in vitro* transfection studies. Viability studies demonstrated that nanosystems did not elicit cytotoxic effects. Fluorescence confocal microscopy studies demonstrated that mannosylated systems had a significant effect on dendritic cellular uptake, although HMW-TPP-R8-MAN system performance stood out. Moreover, mannosylated systems showed higher levels of RBD transcripts. This work demonstrated that mannosylated nanosystems promoted an effective targeted delivery and cellular uptake of the DNA vaccine to APCs, which may strengthen the induction of humoral and cellular immune responses against SARS-CoV-2. This knowledge can contribute to the development of improved methods concerning the immunogenicity of DNA vaccines.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Dalinda Eusébio acknowledges the doctoral fellowship from FCT (Reference: 2020.10201.BD). Diana Costa acknowledges FCT her Assistant Researcher Contract 2021.03946.CEECIND. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under the scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2021.

P3.32 - DEVELOPMENT OF A NOVEL T-CELL ENGAGER TRISPECIFIC ANTIBODY FOR ENHANCED IMMUNE TARGETING OF NON-HODGKIN LYMPHOMA

Margarida Ferreira-Silva [1,2(*)], ***Inês Rosa*** [1,2], ***Afonso P. Basto*** [1,2], ***Pedro Bule*** [1,2], ***Sara Nogueira*** [1,2], ***Rainer Storb*** [3], ***Luís Tavares*** [1,2], ***Frederico Aires da Silva*** [1,2], ***Joana N. R. Dias*** [1,2]

[1] *Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal*
[2] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal*
[3] *Fred Hutchinson Cancer Center and the University of Washington School of Medicine, Washington, USA*

[(*)] *e-mail: amsilva@fmv.ulisboa.pt*

Keywords: Trispecific antibody; T-cell engager; Immunotherapy; Non-Hodgkin lymphoma; Canine animal model.

ABSTRACT

Advances in T-cell-based immunotherapies resulted in unprecedented and durable clinical responses. Nevertheless, most cancer patients remain unresponsive to immunotherapy. Bispecific antibodies that engage tumor targets and interact with cytotoxic T-cells are considered one of the most promising immunotherapies. However, providing co-stimulatory signals may improve antitumoral T-cell responses. Herein, we aim to develop a trispecific antibody (trimAb) that interacts with canine CD20, CD3 and CD28 for improved activation and sustained T-cell response against canine non-Hodgkin lymphoma (cNHL), a relevant animal model of human NHL. An anti-CD20 sdAb library was constructed from a rabbit immunized with HEK293T cells transfected with canine CD20 vector. Selection of specific sdAbs for canine CD20 receptor was performed using a subtractive cell phage display. Clone binding and expression were tested by ELISA. Epitope mapping, co-IP and flow cytometry assays were performed to validate and characterize sdAbs binding properties. To construct the trimAb, the anti-CD20 sdAb was engineered into a dog Fc silenced IgG2 with the variable region of canine anti-CD28 and anti-CD3 scFv using a knob-in-hole approach. Optimization of canine T-cell isolation and *in vitro* assays for evaluation of trimAb ability to promote T-cell activation and cNHL cell cytotoxicity were conducted.

A sdAb library was successfully developed and highly specific sdAbs for canine and human CD20 were selected. ELISA screening allowed the selection of sdAbs targeting canine and human CD20. Target validation and characterization studies confirmed sdAbs specificity against canine CD20, selecting a lead sdAb candidate. A trimAb targeting canine CD20, CD3 and CD28 was engineered. T-cells were isolated from healthy dogs' blood. *In vitro* assays to evaluate the trimAb ability to promote sustained T-cell activation and cytolytic activity on cNHL cells were optimized.

Overall, this work paves the way for the development of multispecific T-cell engager immunotherapies for cNHL treatment.

Acknowledgements:
This study was funded by the Portuguese Funding Agency, Fundação para a Ciência e Tecnologia, FCT through the project EXPL/CVT-CVT/1285/202. Center for Interdisciplinary Research in Animal Health (CIISA) and the Associated Laboratory for Animal and Veterinary Science (AL4AnimalS) has provided support via Project UIDB/00276/2020 and LA/P/0059/2020-AL4AnimalS, respectively, both funded by FCT.

P3.33 - TARGETED TAXIFOLIN DELIVERY TO CERVICAL CANCER CELLS THROUGH ALBUMIN-BASED DELIVERY SYSTEMS

Miguel Ferreira [1(*)], ***Diana Gomes*** [1,2,3], ***Milan Paul*** [4], ***Swati Biswas*** [4], ***Diana Costa*** [1], ***Ângela Sousa*** [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[2] *Associate Laboratory i4HB—Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA, Portugal*
[3] *UCIBIO—Applied Molecular Biosciences Unit, Departament of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal*
[4] *Nanomedicine Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science-Pilani, Hyderabad campus. Jawahar Nagar, Medchal, Hyderabad, India*

(*) *e-mail: miguel.r.ferreira@ubi.pt*

Keywords: Albumin, Cervical cancer, Lipid nanoparticles, Taxifolin, Targeted delivery.

ABSTRACT

Cervical cancer induced by human papilloma virus (HPV) remains a significant global health concern, claiming over 340,000 lives annually. For this reason, new therapeutic approaches are being developed, with the use of natural compounds presenting promising results. Taxifolin is a natural compound known for its anti-cancer properties and have demonstrated a significant potential to inhibit HPV E6 oncoprotein in the cervical cancer. Nevertheless, its bioavailability is very limited due to its easy degradation at acidic pH, weak solubility, and low stability. Therefore, new delivery systems are being developed to encapsulate taxifolin and effectively deliver it in cervical cancer cells, enhancing its therapeutic impact.

The application of albumin-DOPE lipoprotein nanoparticles emerges as a promising strategy to enhance the effectiveness of taxifolin administration, capitalizing on the synergy between these carriers to precisely target overexpressed albumin receptors on cervical cancer cells. The taxifolin-loaded nanoparticles have shown a size and a surface charge of 101.23±1.05 nm and -22.5±3.24 mV, respectively. The taxifolin content was evaluated and an encapsulation efficiency of 79.26±3.61 % and a loading efficiency of 21.26% were determined. Additionally, in vitro release studies and hemolytic studies were performed and have shown no hemolytic effect and a significant release over 48 h, ideal for therapeutic application. Furthermore, viability assays conducted on HPV-positive cancer cells (HeLa) demonstrated an IC50 of 68.27 uM, while showing no toxicity towards healthy cells (human fibroblasts).

Altogether, these findings illuminate a promising pathway for the development of precision therapies in cervical cancer treatment, emphasizing the significance of targeted drug delivery strategies in improving therapeutic outcomes.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Miguel Ferreira and Diana Gomes acknowledge the doctoral fellowships from FCT ref: 2023.01957.BD and 2020.06792.BD. Diana Costa acknowledges FCT her Assistant Researcher Contract 2021.03946.CEECIND. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2201.

P3.34 - AN IMMUNOTOXIN DERIVED FROM *PSEUDOMONAS AERUGINOSA* AS A PROMISING APPROACH FOR THE TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER

Rafaela Marimon [1,2(*)], ***Isa Moutinho*** [1,2], ***Ana André*** [1,2], ***Ana Leonardo*** [1,2], ***Luís Tavares*** [1,2], ***Frederico Aires-da-Silva*** [1,2]

[1] *Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Portugal*
[2] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)*

[(*)] *e-mail: rafaela.marimon@gmail.com*

Keywords: TNBC; recombinant antibodies; TROP-2; phage display; *Pseudomonas aeruginosa*.

ABSTRACT

There were about 2.3 million new cases of breast cancer and 685.000 deaths from this disease in 2020. Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer that represents 20% of all breast cancers and is characterized by the absence of three molecular targets, limiting treatment options to chemotherapy and radiotherapy. Targeted therapies offer a more precise approach, focusing on the specific molecular characteristics of cancer cells and reducing off-target effects. There has been a great effort to identify new biomarkers and therapeutic molecules that can be used to develop effective therapies for TNBC, overcoming the problem of nonspecificity of current therapies. This work aimed to develop a promising type of targeted therapy, an immunotoxin, for the treatment of TNBC targeting the TROP-2 protein, which is overexpressed in TNBC cells.

A rabbit was immunized with TROP-2 and a single-domain antibody (sdAb) library was constructed and subjected to phage display selection. In this process, specific sdAbs against TROP-2 were selected. The most promising sdAb was conjugated with PE38 toxin derived from *Pseudomonas aeruginosa* through overlap PCR, constructing the final immunotoxin. Finally, its cytotoxicity was evaluated in TNBC cells.

The serum obtained from the immunized rabbit presented antibodies that specifically recognize and bind to TNBC cells. Two sdAbs libraries were efficiently constructed, one derived from the bone marrow and other from spleen. Both libraries were representative and diverse. The selected sdAb showed high binding and specificity to TROP-2. The selected sdAb was successfully conjugated with PE38 toxin and the final immunotoxin showed promising cytotoxic activity in TNBC cells.

Our promising results demonstrate that the produced immunotoxin is specific to TROP-2 and shows activity against TNBC cells. Our study provides a proof-of-concept for the development of targeted antibody-based therapies for TNBC using toxins derived from *Pseudomonas aeruginosa* and recombinant antibodies.

Acknowledgements:
CIISA has provided support via Project UIDB/00276/2020, funded by FCT, and LA/P/0059/2020-AL4AnimalS.

P3.35 - SEEKING FOR FUNGAL SIALIDASE INHIBITORS

Gonçalo Conde de Oliveira [1,2], Chantal Fernandes [1], Luís Rodrigues [1,3], Rita Domingues [1], Daniela Calheiros [1], Michael Brad Strader [4], Aishwarya L. Saha [4], Kavita Sharma [4], Christian Hadiwinarta [4], Jay E. Slater [4], Dina Murtinho [2], Elisa da Silva Serra [2], Teresa Gonçalves [1,3]

[1] CNC—Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, Coimbra, Portugal

[2] CQC and Department of Chemistry, University of Coimbra, Coimbra, Portugal

[3] FMUC—Faculty of Medicine, University of Coimbra, Coimbra, Portugal

[4] Laboratory of Immunobiochemistry, Division of Bacterial, Parasitic and Allergenic Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA

(*) e-mail: goncalo-conde@hotmail.com

Keywords: *Alternaria alternata*; sialidase; sialidase inhibitors; respiratory allergies.

ABSTRACT

Respiratory allergic diseases are a major public health problem globally. Fungi may cause these allergies and *Alternaria alternata* is considered one of the most important sources of fungal allergens worldwide and is associated with severe asthma that can be fatal. *A. alternata* proteomic analysis showed high expression of sialidase, an enzyme responsible for cleaving sialic acids from glycoconjugates in cell membranes, present in great abundance in the human airways. In several pathogenic agents, sialidases participate in host colonization and infection ~~of tissues~~, constituting an important therapeutic target. This work aims to determine *A. alternata* sialidase function, localization, predicted 3D-structure and its specificity for sialic acid and for the derivative 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN), as well as the synthesis of specific inhibitors.

In vitro sialidase activity was measured at 560 nm. Chalcone derivatives were obtained from aldehydes (benzaldehyde or 4-chlorobenzaldehyde) and ketones (acetophenone or 4-chloroacetophenone). Some steps in the synthesis of the inhibitors 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (Neu5Ac2en) and 2,3-didehydro-2,3-dideoxy-D-glycero-D-galacto-nonulosonic acid (KDN2en) were optimized from Hong et al., 2006 (10.1002/anie.200601555). The sialidase activity was higher in *A. alternata* spores than in hyphae and in intracellular fractions. The sialidase activity was also observed in extracellular vesicles, proving that these structures transport the sialidase out of the fungal cell. The inhibitor of *Influenza* virus sialidase, Oseltamivir showed a IC50 of 4.6 mM. Four synthesized chalcone derivatives did not show inhibitory activity on *A. alternata* sialidase. Some of the intermediates in the synthesis of the specific inhibitor Neu5Ac2en and KDN2en were obtained in low yields; therefore, the process is under optimization. *A. alternata* sialidase was studied for the first time, and great progress was made in optimizing the chemical synthesis of specific inhibitors, which may have a relevant application in the prevention of airways colonization by *A. alternata* and consequently, prevention of allergies.

Acknowledgements:
This work was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER- 000012- HealthyAging2020 and CENTRO-01-0145-FEDER- 022095: ViraVector; the COMPETE 2020–Operational Programme for Competitiveness and Internationalisation, and the Portuguese national funds via FCT– Fundação para a Ciência e a Tecnologia, I.P.:UIDB/04539/2020 and UIDP/04539/2020.

P3.36 - DEVELOPMENT OF A FAST METHOD FOR THE EARLY DETECTION OF *PAENIBACILLUS LARVAE* SPORES IN BEEHIVES - A STRATEGY TO CONTROL AMERICAN FOULBROOD

Renato Mota [1], ***Paulo Carvalho*** [1], ***Diana Rodrigues*** [1], ***Artur Ribeiro*** [1], ***Sílvio B. Santos*** [1], ***Ana Oliveira*** [1]

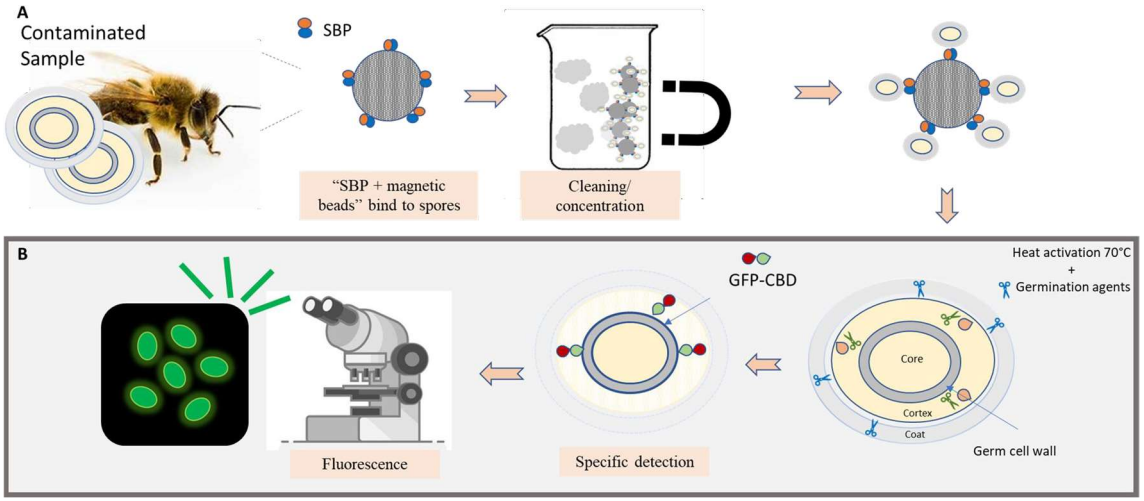
[1] *CEB – Centro de Engenharia Biológica, Universidade do Minho, Gualtar, Braga, Portugal*

[(*)] *e-mail:* renatovelosomota@gmail.com | anaoliveira@deb.uminho.pt

Keywords: American Foulbrood; *Paenibacillus larvae* detection; spores capture; cell wall binding domain; bee rescue; prophylaxis.

ABSTRACT

American Foulbrood (AFB) is a highly devastating bacterial disease from honeybees, caused by the spore-forming bacterium *Paenibacillus larvae*. The innocuous resistant spores are carried by honeybees, but when they germinate inside bee larvae, the disease sets in. As antibiotics are not an option, incinerating contaminated hives is mandatory, profoundly impacting the ecological balance and beekeeping economy. Clinical symptoms in hives only become apparent after the infection has progressed and cannot be managed, but if discovered early, preventive, and prophylactic measures can be put in place. Current detection methods provide late responses preventing beekeepers from monitoring the health status of the hive in real time. This work aimed to develop a method for a fast and in situ detection of *P. larvae* spores, even in the pre-clinical stages of the infection. For capturing spores from artificially infected bee samples, we tested an upstream bead-based magnetic separation, employing a spore-binding protein (γD-crystallin). Then, for *P. larvae*-specific detection, a bacteriophage-derived cell wall binding domain (CBD), PlyPl23_CBD, was used after pre-germinating spores. A fluorescent probe (GFP) fused to PlyPl23_CBD enabled the detection of *P. larvae* spores by fluorescence microscopy. A bead-based method for spore-capturing was developed, resulting in a spore recovery rate of approximately 80%. Spore germination assays revealed that, after 210 minutes of germination, spores could be efficiently detected by fluorescence microscopy, using GFP-PlyPl23_CBD. All combined, the sequential steps of the developed methodology enabled spore detection in 5 hours. The outcomes of this study lay the foundation for a quick on-site detection method, capable of early detection of *P. larvae* spores in beehives, that can prevent hives destruction and mitigate a potential Ecological imbalance.



(A) Use of magnetic beads coated by a spore-binding protein (MB-SBP) to effectively recover and concentrate *P. larvae* spores from bee samples –
rate recovery of ~80%;
(B) Use of PlyP23_CBD (fused to GFP) to specifically bind to *P. larvae* spores after induced spore germination – detection after 210 minutes of germination.

P3.37 - FAST AND SENSITIVE DETECTION OF *PSEUDOMONAS AERUGINOSA* IN CLINICAL SETTINGS USING ENGINEERED REPORTER PHAGES

Maria João Costa [1,2(*)], ***Luciana Meneses*** [1,2], ***Joana Azeredo*** [1,2], ***Sílvio Santos*** [1,2], ***Diana Priscila Pires*** [1,2]

[1] *CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal.*
[2] *LABBELS – Associate Laboratory, Braga, Guimarães, Portugal*

[(*)] *e-mail: maria.joao.costa@ceb.uminho.pt*

Keywords: phage engineering; *Pseudomonas aeruginosa*; detection; bioluminescence.

ABSTRACT

P. aeruginosa is a bacterial pathogen responsible for a wide range of infections. As a result, the World Health Organization identified it as one of the top priority pathogens that urgently calls for the development of novel treatments. Bacteriophages have emerged as a promising therapeutic approach and their properties can be enhanced by phage-engineering. This opens an extensive variety of possibilities, allowing to assemble chimeric phages with new functions. Considering the slow turnover of conventional diagnostic methods and the problems associated with the molecular and immunogenic methods, this study aimed at assembling a bioluminescence-based reporter phage for the fast and sensitive detection of *P. aeruginosa* in clinical care.

Using the yeast-based phage engineering platform, the phage vB_PaeP_PE3 was genetically modified by removing genes with unknown function (*g*1-*g*12) and then used as a scaffold for the insertion of the NanoLuc® luciferase gene that was swapped with gene *g*53. The assembled reporter phage (vB_PaeP_PE3Δgp1-*gp*12,*gp*53:NLuc) was then used for sensitivity and specificity assays. The detection limit was evaluated through the infection of serial dilutions of *P. aeruginosa* suspensions with the reporter phage, and subsequent quantification of luminescence.

Our data showed that the assembled reporter phage was capable of reliably detect 500 CFU/mL within 7h or an average 1 CFU/mL after 24h, and no false positives were observed. Similar results were also obtained when the reporter phage was tested in blood, being capable of detecting an average of 8 CFU/mL within 24 hours.

Overall, compared to culture-dependent methods, the NanoLuc-based reporter phage allows a fast and sensitive detection of *P. aeruginosa* cells using a simple protocol. Therefore, this phage-based detection system is a promising alternative to the common methods for the accurate detection of *P. aeruginosa* in clinical settings.

Acknowledgements:
The authors acknowledge the financial support from the Portuguese Foundation for Science and Technology (FCT) under the scope of the project EXPL/EMD-EMD/1142/2021. LM also acknowledges the support from FCT through the PhD grant SFRH/BD/07494/2020.

P3.38 - RAPID DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH DRUG RESISTANCE IN MALARIA PARASITES USING ISOTHERMAL AMPLIFICATION APPROACH

Mariana Pinto [1(*)&], ***Ana Tavares*** [1&], ***Mayreli Ortiz*** [2], ***Vasoula Skouridou*** [2], ***Miriam Jauset- Rubio*** [2], ***Miguel Viveiros*** [1], ***Ciara O'Sullivan*** [1,2], ***Ana Paula Arez*** [1], ***Márcia M. Medeiros*** [1]

[1] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal*

[2] *Departament d'Enginyeria Química, Universitat Rovira i Virgili, Tarragona, Espanha*

[&] *both authors contributed equally for this work*

[(*)] *e-mail: a21002042@ihmt.unl.pt*

Keywords: malaria; diagnostic; drug resistance; isothermal-amplification; biosensors.

ABSTRACT

Despite efforts to reduce malaria transmission worldwide, no major progress has been made in the past decade. Drug resistance is recognized as one of the major obstacles to malaria control, with key drivers of resistance associated with specific single nucleotide polymorphisms (**SNPs**). The lack of rapid, cost-effective, and simple diagnostic technologies to detect antimalarial drug resistance is a major cause of the spread of drug-resistant parasites in endemic countries.

We aim to develop a rapid, reliable, and portable biosensor using an isothermal amplification approach for the detection of SNPs associated with resistance of *Plasmodium falciparum* parasites to antifolates (sulfadoxine-pyrimethamine) used in various chemoprevention regimens for children and pregnant women in endemic areas. The test is based on the optical detection of the isothermal extension of primers in the solid phase. 5'-end thiolated primers containing the SNP of interest at the 3'-end nucleotide hybridize to target DNA. Primer extension with peroxidase-labeled dinucleotides occurs only from the primer containing the 3'-end nucleotide complementary to the SNP of interest[1].

To achieve the objective, the following procedures have been performed: i) selection of SNPs associated with pyrimethamine (N51I+C59R+S108N) and sulfadoxine (A437G+K540E) resistance located in the dihydrofolate reductase (*pfdhfr*) and dihydropteroate synthetase (pfdhps) *P. falciparum* genes, respectively, ii) design of synthetic DNA templates and primers, iii) preparation of DNA templates from in vitro cultures of *P. falciparum* clones containing the SNPs of interest, iv) optimization assays using synthetic DNA templates (ii) and appropriate control DNA from parasite clones (viii). The methodology will then be validated using DNA templates from field-collected *P. falciparum* isolates from Equatorial Guinea[2]. At the end of the project, we expect to have a proof-of-concept for all SNPs with the aim of adapting the isothermal amplification detection of drug resistance in malaria parasites to a portable device.

References:

1. Ortiz, M., Jauset-Rubio, M., Skouridou, V., Machado, D., Viveiros, M., Clark, T. G., Simonova, A., Kodr, D., Hocek, M., & O'Sullivan, C. K. (2021). Electrochemical Detection of Single-Nucleotide Polymorphism Associated with Rifampicin Resistance in Mycobacterium tuberculosis Using Solid-Phase

Primer Elongation with Ferrocene-Linked Redox-Labeled Nucleotides. ACS Sensors, 6(12). https://doi.org/10.1021/acssensors.1c01710
2. Guerra, M., Neres, R., Salgueiro, P., Mendes, C., Ndong-Mabale, N., Berzosa, P., de Sousa, B., & Arez, A. P. (2017). Plasmodium falciparum genetic diversity in continental Equatorial Guinea before and after introduction of artemisinin based combination therapy. Antimicrobial Agents and Chemotherapy, 61(1). https://doi.org/10.1128/AAC.02556-15

Acknowledgements:
Fundação para a Ciência e a Tecnologia for funds to GHTM - UID/04413/2020 (RESMALDETECT project) and LA-REAL – LA/P/0117/2020.

P3.39 - IMPROVED PURIFICATION STRATEGIES FOR RNA AFFORDED BY THE USE OF BIOBASED IONIC LIQUIDS AS CONSTITUENTS OF AQUEOUS BIPHASIC SYSTEMS

Ana F. Pereira [1(*)], Augusto Q. Pedro [1], Leonor S. Castro [1], Maria J. Quental [1], Ana P. M. Tavares [1], Luís C. Branco [2], João A. P. Coutinho [1], Fani Sousa [3], Mara G. Freire [1]

[1] CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal

[2] LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal

[3] CICS-UBI – Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal

(*) e-mail: anafilipa98@ua.pt

Keywords: Biopharmaceuticals; Aqueous biphasic systems; Amino acid ionic liquid; Purification; Stability.

ABSTRACT

RNA has dominated scientific community attention due its emergence as a promising biopharmaceutical, paving the way for innovative medicines with broad therapeutic and prophylactic efficiencies. Notwithstanding its clinical relevance, some challenges in RNA bioprocessing still limit its widespread application, such as its easy degradability coupled with the laborious and costly methods required for its purification.

In this context, amino-acid-based ILs (AA-ILs) may play a significant role on this field, by virtue of the high affinity between amino-acids and RNA and the favorable nucleic acids-stabilization properties exhibited by AA-ILs, thus arising as alternatives platforms for purifying RNA. From the exposed, this work aims to develop more competitive and sustainable purification-preservation platforms for RNA, with the ultimate goal of purifying RNA from complex recombinant lysates.

AA-ILs comprising L-arginine, L-lysine and L-histidine as cations combined with chloride or DL-aspartate, were synthesized and characterized, and their ability to form aqueous biphasic systems (ABS) was investigated. All AA-ILs in study formed ABS with polypropylene glycol with a molecular weight of 400 $g.mol^{-1}$. Extraction studies were then performed with a complex sample containing RNA and gDNA, existing two systems allowing an almost complete separation of RNA from gDNA without compromising its integrity. Overall, the approach herein developed represents a promising strategy to surpass the critical demand of RNA with high-quality, envisaging its potential use as biotherapeutics.

Acknowledgements:
This work was developed within the scope of the projects CICECO-Aveiro Institute of materials, UIDB/50011/2020, UIDP/50011/202 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC), and within the scope of the EIC-Pathfinder YSCRIPT project with reference 101047214, supported by the budgets of the Horizon Europe Program. Ana Pereira, Leonor Castro, Augusto Pedro and Ana Tavares acknowledge FCT, respectively, for the PhD grants 2022/13247/BD and 2020/05090/BD and the research contracts CEECIND/2020/02599 and CEECIND/2020/01867 under the Scientific Stimulus – Individual Call.

P3.40 - IMPLEMENTATION OF A *GALLERIA MELLONELLA* INFECTION MODEL AT GHTM/IHMT-NOVA

Telma Rodrigues [1], Mariana Andrade [1], Joana Marques [1], Liliana Rodrigues [1], Miguel Viveiros [1], Henrique Silveira [1], Isabel Couto [1], Sofia Santos Costa [1(*)]

[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisbon, Portugal

[(*)] e-mail: scosta@ihmt.unl.pt

Keywords: Infection model; *Galleria mellonella*; pathogenicity assays; bacterial pathogens.

ABSTRACT

Galleria mellonella represents a sustainable invertebrate infection model that is gaining increasing interest, particularly for pathogenicity and infection assays, host-pathogen interaction studies, and pharmaco-toxicological studies.

This work describes the implementation of a *G. mellonella* colony at GHTM/IHMT-NOVA to provide standardized larvae for the research community. Infection protocols for relevant bacterial pathogens were optimized.

G. mellonella at the last larval stage were acquired from a commercial house specialized in exotic animals and the species confirmed by PCR. The larvae were reared, in the dark, at 27-28 °C and 60-80% relative humidity with a high protein diet. Infection assays were optimized with reference strains *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27583, *Neisseria gonorrhoeae* ATCC49226 and *Escherichia coli* ATCC25922.

A *G. mellonella* colony was established with parameters such as diet and routine maintenance tested. In-house reared *G. mellonella* presented a life cycle between 31 to 34 days (from egg to adult). Each female laid on average > 1,500 eggs with a rate of egg hatching of ≈34%. Compared to the commercially acquired, in-house reared larvae were healthier and more resistant to infection by *S. aureus* ATCC25923. The optimization of infection assays included pre-incubation conditions and bacterial inoculum size, among other parameters. By applying the optimized infection assays, at equivalent inoculums, the four bacterial pathogens tested could be differentiated and ranked according to their virulence potential, as follows: *P. aeruginosa* >> *S. aureus* ≈ *E. coli* >> *N. gonorrhoeae*.

This work represents a build-up on the animal infection models available at GHTM/IHMT-NOVA for collaborators and the research community. Ongoing work focus on expanding this model to fungal and parasite pathogens, as well as additional functional assays, such as pharmaco-toxicological studies.

Acknowledgements:
Fundação para a Ciência e a Tecnologia (FCT, Portugal) through funds to Project DREBI Ref. 2022.07931.PTDC, to GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020) and grant UI/BD/154472/2022 awarded to MA.

P3.41 - NEAR INFRARED LIGHT RESPONSIVE THIOL-MALEIMIDE HYDROGELS AIMED FOR BREAST CANCER CHEMO-PHOTOTHERMAL THERAPY

Francisco J.P. Costa [1], Micaela Nave [1], Rita Lima-Sousa [1], Cátia G. Alves [1], Bruna L. Melo [1], Ilídio J. Correia [1,2(*)], Duarte de Melo-Diogo [1(**)]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.

[2] CIEPQPF – Departamento de Engenharia Química, Universidade de Coimbra, Coimbra, Portugal.

[(*)] email: icorreia@ubi.pt | demelodiogo@fcsaude.ubi.pt[(**)]

Keywords: Chemo-photothermal therapy; Graphene family nanomaterials; Macroscale delivery systems; Thiol-Maleimide hydrogels.

ABSTRACT

Nanomaterials have gained considerable attention for application in cancer therapy due to their ability to encapsulate chemotherapeutic drugs. However, the biological barriers are challenging elements that compromise the ability of systemically administered nanomaterials to efficiently reach the tumour site. In this regard, hydrogels prepared through Thiol-Maleimide Michael type additions have been showing promising results for the local delivery of nanomaterials due to their chemical-selectivity and simple preparation. Still, the majority of the hydrogels prepared using this crosslinking chemistry are based on synthetic polymers, which may not be optimal due to their weak biodegradability. In this work, a novel Thiol-Maleimide hydrogel derived from natural polymers was developed for application in breast cancer chemo-photothermal therapy. Hyaluronic acid was functionalized with cysteine and deacetylated Chitosan was reacted with 3-Sulfo-*N*-succinimidyl 4-(maleimidomethyl)-cyclohexane-1-carboxylate sodium salt in order to attain natural polymers compatible with the Thiol-Maleimide chemistry. In parallel, Dopamine-reduced graphene oxide was loaded with Doxorubicin for obtaining a near-infrared (NIR) light-responsive nanomaterial with chemo-photothermal capabilities. The combination of the two functionalized polymers and Doxorubicin loaded Dopamine-reduced graphene oxide enabled the preparation of a Thiol-Maleimide crosslinked hydrogel incorporating this nanomaterial (termed as DOX/DOPA-rGO@TMgel). In *in vitro* studies, when breast cancer cells were exposed to DOPA-rGO@TMgel and NIR light (hydrogels' photothermal therapy), their viability was reduced to about 59%. Closely, the use of DOX/DOPA-rGO@TMgel (hydrogels' chemotherapy) diminished the cancer cells' viability to 50%. Notably, the combined treatment mediated by DOX/DOPA-rGO@TMgel and NIR light (hydrogels' chemo-photothermal therapy) prompted a decrease in breast cancer cells' viability to only 21%. Overall, the produced Thiol-Maleimide hydrogels are promising for the chemo-photothermal therapy of breast cancer cells.

Acknowledgements:
This work was supported by the projects/grants UIDB/00709/2020, UIDP/00709/2020, POCI-01-0145-FEDER-031462, PTDC/BTABTA/0696/2020, 2022.06320.PTDC, 2021.00590.CEECIND (Duarte de Melo-Diogo), SFRH/BD/144922/2019 (Rita Lima-Sousa), SFRH/BD/145386/2019 (Cátia G. Alves), 2021.06044.BD (Bruna L. Melo).

P3.42 - MESOPOROUS SILICA-COATED GOLD NANOCLUSTERS FOR ENHANCED CANCER CHEMO-PHOTOTHERMAL THERAPY

Natanael F. Fernandes [1(*)]***, Soraia Borges*** [1]***, Carolina F. Rodrigues*** [1]***, Duarte de Melo-Diogo1, Ilídio J. Correia*** [1, 2]***, André F. Moreira*** [1,3]

[1] *CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique, Portugal.*

[2] *CIEPQPF — Departamento de Engenharia Química, Universidade de Coimbra, Rua Sílvio Lima, Portugal.*

[3] *CPIRN-UDI/IPG - Centro de Potencial e Inovação em Recursos Naturais, Unidade de Investigação para o Desenvolvimento do Interior do Instituto Politécnico da Guarda, Guarda, Portugal.*

(*) *email: natanael.f.f.fernandes@gmail.com*

Keywords: Cancer; gold core silica shell nanoparticles; photothermal therapy; nanoclusters.

ABSTRACT

Combinatorial therapeutic strategies that combine chemotherapy and photothermal treatments through nanomaterials have demonstrated remarkable potential in pre-clinical cancer research. Among these, gold core silica shell (AuMSS) nanomaterials have gained prominence due to their ability to combine the photothermal potential of gold with the high loading capacity of mesoporous silica loading capacity with efficient photothermal properties. We developed a novel and simplified methodology for producing gold nanoclusters coated with mesoporous silica, termed AuMSS nanoclusters. In this process, glutathione (GSH) was employed as a mediator in the formation of these gold nanoclusters. Our approach precisely regulates the aggregation of gold nanospheres through the addition of GSH, resulting in enhanced light absorption capacity within the 700-1100 nm spectral range. Subsequently, acridine orange loading was successfully achieved, reaching a maximum of 30 µg of acridine orange per mg of nanoparticle. Upon near-infrared (NIR) laser irradiation, the AuMSS nanoclusters exhibit robust photothermal capabilities, generating a substantial temperature rise (ΔT) of 20 °C. In *in vitro* experiments involving both healthy fibroblasts and cervical cancer cells, the AuMSS nanoclusters demonstrated excellent biocompatibility at concentrations of up to 200 µg/mL. Encouragingly, preliminary cytotoxicity assessments revealed that the NIR light-triggered photothermal effect of the AuMSS nanoclusters resulted in a remarkable reduction in the viability of cervical cancer cells, with values approaching 20%. Our findings underscore the immense potential of AuMSS nanoclusters as a dual-purpose chemo-photothermal therapeutic agent. When combined with gold's inherent bioimaging capabilities, these nanoclusters hold the promise for further advancement in the development of multifunctional nanomedicine with enhanced anticancer properties. This study represents a significant step towards using the full potential of combinatorial nanotherapies in the fight against cancer.

Acknowledgements: This work was supported by the projects/grants UIDB/00709/2020, UIDP/00709/2020, POCI-01-0145-FEDER-031462, PTDC/BTABTA/0696/2020, 2022.06320.PTDC, 2021.00590.CEECIND (Duarte de Melo-Diogo), Carolina F. Rodrigues (SFRH/BD/144680/2019). Natanael Fernandes (2022.14608.BD).

MICRO BIOTEC 23

Poster Session

Topic 4

P4.1 - PHAGE THERAPY AGAINST *PSEUDOMONAS AERUGINOSA* BIOFILMS IN URINE

Inês Martinho [1], Márcia Braz [1]*, Carla Pereira [1], Adelaide Almeida [1]

[1] Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

[(*)] e-mail: marciabraz96@ua.pt

Keywords: *Pseudomonas aeruginosa;* Multidrug resistance*;* Phage therapy; Biofilm; Antibiotics.

ABSTRACT

Pseudomonas aeruginosa stands out as one of the most challenging pathogens in healthcare settings, namely because of its ability to resist to antibiotics, form biofilms and cause numerous infections such as urinary tract infections. In this sense, new alternatives to conventional antimicrobials are needed. Phage therapy emerges as a promising alternative to antibiotics. This therapy involves the use of bacteriophages (also called phages), viruses that only infect and kill bacteria. In this study, phage therapy was applied to reduce *P. aeruginosa* biofilms in urine. Previous planktonic assays in Tryptic Soy Broth (TSB), were carried out in order to choose the most effective phage and multiplicity of infection (MOI, ratio between the number of phages and bacteria) for the biofilm assays. The most effective phage selected to reduce *P. aeruginosa* biofilms was a phage isolated from the combined preparation "Intesti bacteriophage" created by the Eliava BioPreparations company with a maximum *P. aeruginosa* reduction of approximately 5 log colony forming units (CFU)/cm^2, after 8 h at 37 °C (MOI of 1). On biofilm assays, a bacterial control containing only the *P. aeruginosa* biofilm, a phage control containing only phage and a sample containing both the biofilm and phage were included. A maximum biofilm reduction of about 5 log CFU/cm^2 after 4 h at 37 °C was observed, with a MOI of 1. These results were compared with the ones obtained with the ciprofloxacin antibiotic. Ciprofloxacin was able to reduce the *P. aeruginosa* biofilm in about 4 log CFU/cm^2, after 4 h at 37 °C. Phages have been shown to be effective against *P. aeruginosa* biofilms with even better results than those obtained with antibiotic. In a world, where antibiotic resistance is a serious public health problem, phage therapy appears as a viable alternative to antibiotics.

Acknowledgements:
The authors acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020). Márcia Braz thanks FCT for the PhD grant (2020.0657.BD). Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Thanks are also due to the Biology Department and the University of Aveiro, where this research work was performed.

P4.2 - VIRULENCE TRAITS OF *CUTIBACTERIUM ACNES*: DIFFERENCES AMONG DIFFERENT PHYLOTYPES

Ana Sofia Oliveira [1,2], Carlos Gaspar [1,2,3], Joana Rolo [1,2], Rita Palmeira-de-Oliveira [1,2,3], João Paulo Teixeira [4,5], José Martinez-de-Oliveira [1,2], Ana Palmeira-de-Oliveira [1,2*]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal
[2] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[3] Labfit–Health Products Research and Development Lda, UBImedical, Covilhã, Portugal
[4] National Institute of Health, Environmental Health Department, Porto, Portugal
[5] EPIUnit - Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal.

[(*)] e-mail: apo@fcsaude.ubi.pt

Keywords: *acne vulgaris;* antibiotic susceptibility; biofilm; lipase; porphyrin.

ABSTRACT

Among the factors that contribute to *acne vulgaris* (AV), *Cutibacterium acnes* bacterium is reported to play a role in the disease. As recent investigation has interchanged the role of *C. acnes* in skin microbiota, importance has been given to differences in the virulence profile of strains. In this work we evaluated the virulence traits of strains belonging to phylotypes associated either with AV (IA_1) or healthy skin (II).

One collection-type strain (DSM1897) and seven clinical isolates collected from healthy volunteers were used. Strains were classified into different phylotypes using a multiplex-touchdown PCR protocol. Minimum inhibitory concentrations (MICs) for antibiotics with EUCAST resistance classification (clindamycin and benzylpenicillin) or clinical relevance (tetracycline and erythromycin) were determined by microdilution method. Biofilm production capacity was determined using crystal-violet staining. Quantification of extracellular lipase activity was performed by measuring the levels of 4-methylumbelliferone released from 4-methyl umbelliferyl oleate. The amount of porphyrin production was evaluated by porphyrin extraction from bacterial supernatants and quantified by absorbance measuring.

No major differences were found in antibiotic susceptibility of strain, all being susceptible to antibiotics with EUCAST classification. Lower MIC values were still found for tetracycline and erythromycin in phylotype II strains. Regarding biofilm formation capacity, strains from type IA_1 were stronger biofilm formers, producing approximately twice the biofilm of type II strains, with exception of DSM1897. They were also significantly stronger lipase producers (approximately 3-fold). For porphyrin production, a trend for higher production was present for IA_1 strains, still with no statistical significance, as the production within phylotype II was strain dependent.

While some virulence traits were increased in strains from phylotype IA_1, others appear to be strain related, highlighting the multifactorial nature of the disease. As clinical strains were collected from healthy volunteers, this highlights the relevance of additional factors in AV progression.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Financial support from FCT was also provided to ASO [SFRH//BD/136192/2018] and JR [SFRH/BPD/115145/2016].

P4.3 - FACTORS ASSOCIATED WITH PSEUDOMONAS AERUGINOSA INFECTION IN A UROLOGY AND KIDNEY TRANSPLANTATION UNIT IN CENTRAL PORTUGAL: DETECTION OF ANTIMICROBIAL RESISTANCE

Mariana Duarte Almeida [1,2], Belmiro Parada [1,3,4], Sarah M. Johansson [5], Pedro Valada [2,6], João Pereira [7,8], Rita Leal [1,9], João Frade [10], Catarina Chaves [10], Ana Rita Rebelo [5], Teresa Gonçalves [1,2], Rene S. Hendriksen [5], Célia Nogueira [1,2*]

[1] FMUC – Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[2] CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra
[3] iCBR - Coimbra Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[4] CACC - Clinical Academic Centre of Coimbra, Coimbra, Portugal
[5] EURL-AR, European Reference Laboratory for Antimicrobial Resistance, Technical University of Denmark (DTU), National Food Institute, Lyngby, Denmark
[6] FCTUC – Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal
[7] Urology and Kidney Transplantation Unit, CHUC – Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[8] Urology Unit, Instituto Português de Oncologia do Porto Francisco Gentil, Porto, Portugal
[9] Nephrology Unit, CHUC – Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[10] Microbiology Laboratory, Pathology Unit, CHUC – Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

[(*)] e-mail: cnogueira@fmed.uc.pt

Keywords: *Pseudomonas aeruginosa,* Healthcare infection, Risk factors, Antimicrobial Resistance, Colistin Resistance

ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen responsible for severe infections in immunocompromised patients. This study aimed to identify risk factors associated with *P. aeruginosa* infection in a Urology/Kidney transplant unit and to analyze its antimicrobial resistance patterns, and investigate the presence of genes linked to colistin resistance. One-year case-control study was carried out comparing clinical features of 92 patients with *P. aeruginosa* infection with 92 patients without infections. Multivariable regression analysis was employed to determine the risk factors contributing to *P. aeruginosa* infections.Antimicrobial susceptibility reports were obtained for the 92 clinical isolates of *P. aeruginosa*, and molecular mechanisms of colistin resistance were studied in 31 of them, focusing on *mcr* and *pmrA/pmrB* genes. Our results show that the length of current hospital stay (OR=1.139, 95% CI [1.055- 1.230]), previous surgery (OR=41.225, 95% CI [4.355-390.230]), and other infections within the preceding 90 days (OR=4.944, 95% CI [1.989-12.285]) were identified as independent risk factors for *P. aeruginosa* infection. Notably, a high degree of antibiotic resistance was observed among *P. aeruginosa* isolates, with 46.7% of them classified as multi or extensively drug-resistant. However, no significant association was found between the presence of *mcr* and *pmrA*/*pmrB* genes and colistin resistance. Addressing the risk factors is likely to reduce the incidence of *P. aeruginosa* infections and mitigate associated morbidity and mortality and impair the spread of antimicrobial resistance.

P4.4 - DECIPHERING THE ANTIBACTERIAL MODE OF ACTION OF CURCUMIN- ANTIBIOTIC COMBINATIONS UNDER PHOTODYNAMIC ACTIVATION AGAINST STAPHYLOCOCCUS AUREUS

Ariana Gonçalves [1,2], José Fernandes [3], Maria José Saavedra [4,5,6], Cristiana Pereira [7], Manuel Simões [1,2,8], Anabela Borges [1,2,8(*)]

[1] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

[2] ALICE—Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Portugal

[3] Centro de Química - Vila Real, Escola de Ciências e Tecnologia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

[4] Department of Veterinary Sciences, Antimicrobials, Biocides & Biofilms Unit (A2BUnit), University of Trás-os-Montes and Alto Douro, Portugal

[5] CECAV-Veterinary and Animal Research Center and Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Trás-os-Montes and Alto Douro, Portugal

[6] CITAB-Centre for the Research and Technology of Agro-Environmental and Biological Sciences and Institute for Innovation, Capacity Building, and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

[7] Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal

[8] DEQ—Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

[(*)] email: apborges@fe.up.pt

Keywords: Antibiotic Resistance; Antibacterial Mode of Action; Antimicrobial Photodynamic Inactivation; Curcumin-Antibiotic Combinations; Phytochemicals; *Staphylococcus aureus* infections.

ABSTRACT

The treatment of bacterial infections is currently a major challenge due to increasing antibiotic resistance. Despite the threatening nature of this emerging problem, antibiotics remain the only available strategy to combat bacterial infections. To restore the efficacy of antibiotics, alternative approaches have been explored, including the use of phytochemicals as antibiotic boosters. Indeed, plant secondary metabolites are known for their antibacterial properties and potent adjuvant potential, and some of them like curcumin (Cur), also have photodynamic properties that make them useful for antimicrobial photodynamic inactivation (aPDI). In this way, the present study focused on evaluating the antibacterial activity and mode of action of the synergistic antimicrobial photodynamic activation of Cur-tobramycin (Cur-Tob) against two clinical strains of *Staphylococcus aureus* (CECT 976: methicillin-susceptible; MJMC568-B: methicillin-resistant). The targets of antibacterial action were investigated by assessing Cur-Tob combined effects on various bacterial physiological indices, namely: reactive oxygen species (ROS) generation, membrane integrity alteration (propidium iodide uptake, potassium leakage), bacterial surface charge changes, DNA damage, motility inhibition, and virulence factors production interference (proteases, gelatinases, and lipases). When compared with non-photoactivated Cur-Tob combinations, photoactivated ones demonstrated increased antibacterial activity against both clinical strains. Moreover, photoactivated Cur-Tob combinations were able to efficiently increase the levels of ROS and thus membrane

permeability and DNA damage. The present strategy also showed moderate activity in inhibiting the sliding motility of *S. aureus* strains and the reduction of proteases, gelatinases, and lipases production. Overall, this study highlights the remarkable potential of the synergy of aPDI in combinatorial antibiotic therapy as a sustainable manner to overcome bacterial resistance. Furthermore, the mode of action behind the antibacterial effect was demonstrated for the first time.

Acknowledgements:
This work was supported by LA/P/0045/2020 (ALiCE), UIDP/00511/2020 (LEPABE) and FCT – Portuguese Foundation for Science and Technology through the Scientific Employment Stimulus—Individual Call— [CEECIND/00823/2021] and PhD fellowship (2022

P4.5 - EVALUATION OF *IN VITRO* COMPATIBILITY OF BACTERIAL CANDIDATES AS BIOMODULATORS FOR INTESTINAL WOUND HEALING CONSORTIA

Diana Almeida [1]; Sónia Mendo [2]; José Andrade [3]; Ana Maria Gomes [1]*

[1] Universidade Católica Portuguesa (UCP), Centro de Biotecnologia e Química Fina (CBQF) – Associate Laboratory, School of Biotechnology (ESB), Porto, Portugal
[2] Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal
[3] 1H-TOXRUN – One Health Toxicology Research Unit, University Institute of Health Sciences, CESPU, G*andra, Portugal*

(*) e-mail: amgomes@ucp.pt

Keywords: consortia, compatibility, antagonism, biofilm.

ABSTRACT

Enteropathogenic infections and inflammation are known to compromise intestinal barrier's integrity, often resulting in chronic disruptions and recurrent gastrointestinal infections and inflammatory conditions. Emerging research indicates *Akkermansia muciniphila* as a key player within a microbial community, facilitating mucosal healing and promoting beneficial microenvironmental changes enhancing mucosal regeneration and barrier function. Exploiting these bacterial networks holds promise for therapeutic interventions; however, selection of effective consortia faces compatibility challenges, requiring evidence of coexistence among consortium members. To evaluate possible incompatibilities issues arising when harnessing a functional microbial consortium a literature-selected set of lactobacilli and bifidobacteria strains based on intestinal pro- regenerating and antimicrobial attributes were tested for their antagonistic activity against *A. muciniphila* (DSM 22959) using the well-diffusion technique performed using live cultures and cell- free supernatants (CFS) (untreated, neutralized and catalase- and trypsin-treated). The impact of dual-strain coculturing on biofilm formation was evaluated by the crystal violet colorimetric method while investigating potential stimulating effects of added bile salts.

Lactobacilli live cultures exhibited significant inhibitory zones, ranging from 18.7 ± 0.7 to 19.7 ± 1.5 mm (mean ± SEM) when compared to the positive control (16.18 ± 0.06 mm). Untreated CFS also inhibited *A. muciniphila* considerably (12.5 ± 0.71 to 14.8 ± 0.9 mm), and these effects remained unaffected by catalase and trypsin treatments. However, inhibition was completely inactivated with pH neutralization suggesting a pH-dependent (e.g., production of lactic acid)/ specific enzymatic mode of action. Conversely, mainly active bifidobacteria cultures inhibited *A. muciniphila growth* (13.8 ± 0.4 to 18.8 ± 1.2 mm). Concerning biofilm formation while cocultures did not increase production, introduction of bile salt stress stimulated its formation.

Our findings underline the presence of compatibility challenges between these probiotic strains and *Akkermansia muciniphila*, which require alternative strategies for harnessing the beneficial attributes of all strains within the consortia, while preserving their functional integrity.

Acknowledgements:
This work was supported by national funds through Foundation for Science and Technology, I.P. (FCT)/MEC (PIDDAC), for doctoral project reference 2021.05046.BD. We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.

P4.6 - *RHODOTORULA MUCILAGINOSA*: A NEW PLAYER IN VULVAR INFECTIONS

Cátia F. Caetano [1,2], Mariana Z. Fernandes [1,2], Carlos Gaspar [1,2,3], Ana S. Oliveira [1,2], Rita Palmeira-de-Oliveira [1,2,3], José Martinez-de-Oliveira [1], Ana Palmeira-de-Oliveira [1,2,3], Joana Rolo [1,2]*

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.
[2] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal;
[3]HPRD: Research and Development of Health Products, Lda, Covilhã, Portugal

(*) e-mail: joanarolo@fcsaude.ubi.pt

Keywords: vulvovaginal candidosis; women's health; fungi and clinical mycology

ABSTRACT

Vulvovaginal candidosis is a frequent infection affecting millions of women worldwide. It is caused mostly by yeast of the genus *Candida*, of which the most frequent is *Candida albicans*. Yeasts of the genus Rhodotorula are an emerging yeast pathogen, being recently involved in skin and medical-device associated infections, particularly in immunocompromised hosts. In this study we aim to assess the significance of the presence of *Rhodotorula* spp. in vulvar samples, as well as its ecological relationship with *Candida albicans.*

Forty-seven patients seeking a gynecological consultation have been involved in the study. A vulvar swab was routinely collected from which yeast isolates have been isolated and identified with Vitek. Antifungal susceptibility testing to two azole compounds (fluconazole and clotrimazole) was assessed with the microdilution broth assay. Ability to form biofilms of yeast isolates was assessed with the microtiter plate assay using cristal violet staining. In addition, mixed biofilms of *R. mucilaginosa* and *C. albicans* were performed, as well as growth curves in co- cultures. Finally, the ability of *C. albicans* to form germ tubes in-vitro in the presence of *R. mucilaginosa* was also assessed.

We were able to isolate 38 *Rhodotorula* spp. isolates from the vulva of the patients (38/47, 81%). Most of the patients carrying these isolates were asymptomatic at time of sampling, but all were sampled after treatment with antifungals for vulvovaginal candidosis. Twelve of these patients (12/38, 32%) had a co-carriage of *Candida* spp. isolates in their vulva. Antifungal susceptibility testing showed that the great majority of Rhodotorula spp. isolates were resistant to fluconazole (90%, MIC>64 µg/mL), but susceptible to clotrimazole (70%, MIC<2 µg/mL). All *Rhodotorula* spp. isolates were great biofilm formers. The growth curve of *C. albicans* was showed to be slower in the presence of *R. mucilaginosa*, and there was inhibition in the amount of biofilm biomass and germ tubes in co-culture.

Yeast isolates belonging to the *Rhodotorula* genus were frequently isolated from the vulva of asymptomatic women, particularly following treatment for vulvovaginal candidosis, which might be related with their apparent intrinsic resistance to fluconazole. Our results revealed that these two yeasts have antagonistic behaviours in co-culture.

P4.7 - EVALUATION OF YEAST SURFACE DISPLAY OF THE ENDOLYSIN PLY511 AGAINST *LISTERIA MONOCYTOGENES*

David Sáez Moreno [1,2] **, *Joana Cunha*** [1,2] **, *Luís D.R. Melo*** [1,2]**, *Joana Azeredo*** [1,2*]**, *Lucília Domingues*** [1,2*]

[1] *Universidade do Minho, Centre of Biological Engineering, Braga, Portugal*
[2] *LABBELS associate laboratory, Braga / Guimaraes, Portugal*

[(*)] *e-mail: luciliad@deb.uminho.pt | jazeredo@deb.uminho.pt*

Keywords: Yeast surface display, endolysin, flow cytometer, *Listeria monocytogenes.*

ABSTRACT

Yeast surface display, also known as yeast display, is a protein engineering technique that uses the expression of recombinant proteins of interest, fused with native cell wall proteins of yeast. Ply511 endolysin is a cell wall hydrolase with N-acetylmuramoyl-L-alanine amidase activity.

Ply511 is naturally produced by the Listeria phage A511 to lyse bacterial cells and has been proposed as an alternative to antibiotics.

Listeria monocytogenes is a Gram-positive pathogen causative of human infections, resulting in febrile gastroenteritis, perinatal infection, and central nervous system infections. These infections are frequently acquired through the ingestion of contaminated food.

In our study, we employed CRISPR-Cas9 to genetically modify Saccharomyces cerevisiae to display the listeria endolysin Ply511 on its surface. Flow cytometer analyses confirmed the expression of the genetic construction integrated in the recombinant yeast. Also, the enzymatic peptidoglycan-degrading activity of the engineered yeast was confirmed by using heat-killed L. monocytogenes cells. In killing assays, the recombinant yeast did not show CFU reduction of Listeria in comparison with the wild-type. These results suggest that yeast display of endolysins needs to be improved to be used as an antimicrobial strategy in the context of engineered probiotics, to assure that bacterial killing is achieved.

Acknowledgements:
Portuguese Foundation for Science and Technology (FCT) - strategic funding of UIDB/ 04469/2020; David Saez PhD scholarship (UI/BD/151411/2021).

P4.8 - EXPLORING THE ANTIMICROBIAL ACTIVITY OF BLACK SCORPIONFISH (*SCORPAENA PORCUS*) VENOM

Rita Magalhães [1,2]***, José Neto*** [1,2]***, Eva Cunha*** [3,4]***, Joana Robalo*** [1,2]***, Manuela Oliveira*** [3,4,5]***, Frederico Almada*** [1,2]

[1] *MARE – Marine and Environmental Sciences Centre, Lisbon, Portugal.*
[2] *ISPA – Instituto Universitário de Ciências Psicológicas, Sociais e da Vida, Lisbon, Portugal.*
[3] *CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.*
[4] *Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS).*
[5] *cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.*

Keywords: *Scorpaenidae;* Venomics; Antimicrobial resistance; ESKAPE pathogens

ABSTRACT

Venomous species have evolved to produce bioactive molecules able to act as natural defences against other organisms. Nowadays, the dissemination of antimicrobial resistant bacteria continues to increase, prompting the World Health Organization to prioritise the development of innovative antibacterial strategies. Accordingly, this project aims to evaluate the composition and properties of fish venoms, exploring their potential as antimicrobial agents. The venom was extracted from the spines of *Scorpena porcus* individuals (n=3) using the sponge- in-a-tube method, and aliquots with concentrations ranging from 2.1 to 3.3 mg/mL were prepared in potassium phosphate buffer for further characterization. The protein composition of *S. porcus* venom was assessed by SDS-PAGE electrophoresis and toxicity towards eukaryote cells was tested using rabbit erythrocytes. The antimicrobial potential of venom samples was evaluated by a spot-on-lawn assay towards a $1.5x10^8$ CFU/mL bacterial suspensions of the following ESKAPE pathogens: *Acinetobacter baumannii* CCUG 57250, *Enterobacter hormaechei* CCUG 58962, *Enterococcus faecium* CCUG 58548, *Klebsiella pneumoniae* CCUG 60138, *Pseudomonas aeruginosa* CCUG 51971 and *Staphylococcus aureus* CCUG 35603. The reference strain *Escherichia coli* ATCC 25922 was also tested as a control. Protein venom profile presents a distinct band pattern between 75-120 KDa and bellow 14 KDa. A protein concentration of 0.1g/mL results in 80% haemolysis while 0.01g/mL has no haemolytic ability. Regarding antimicrobial ability, samples with concentrations above 2.5 mg/mL were able to inhibit *S. aureus*, while all samples were able to partially inhibit the growth of *E. hormaechei*. Even though more than half of venomous vertebrates are fish, research on the bioactivity of their venom is scarce, making them ideal candidates for venomic research. These results provide preliminary information on the antimicrobial properties of these bioactive compounds and reinforce the need for further research in this area.

Acknowledgements:
This work was supported by MARE–ISPA FishVen Project 2022.08756.PTDC through the strategic project UIDB/04292/2020 awarded to MARE and through the project LA/P/0069/2020 granted to the Associate Laboratory ARNET (Funded by FCT). F.A. (Frederico Almada) was supported by a research contract DL57/2016/CP1339/CT0003 and J.N. (José Neto) through the PhD scholarship UI/BD/150955/2021.

P4.9 - EFFECTS OF BIOCHAR SUPPLEMENTATION ON ANTIMICROBIAL RESISTANCE AND VIRULENCE FACTORS OF ENTEROCOCCI ISOLATED FROM RECYCLED MANURE SOLIDS

Ana José Pires [1,2*], Catarina Geraldes [1,2], Joana Fernandes Guerreiro [1,2], Lélia Chambel 3, David Fangueiro [4], Ricardo Bexiga [1,2], Manuela Oliveira [1,2,5]

[1] CIISA—Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2] AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal
[3] BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[4] LEAF - Research Center, Terra Associate Laboratory, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal
[5] cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

[(*)] e-mail: apires@fmv.ulisboa.pt

Keywords: Recycled Manure Solids (RMS), Biochar, *Enterococcus,* Antibiotic Resistance, Virulence Factor

ABSTRACT

Recycled Manure Solids (RMS) are increasingly being used as cow bedding, making it crucial to develop viable strategies to control potential health hazards associated with their use. The present study aimed to evaluate the effect of RMS supplementation with Biochar, an inorganic char coproduct, on bacteria antimicrobial resistance and virulence expression, focusing on enterococci as a model. A total of 109 isolates presumptively identified as enterococci were collected from a 30-day incubation experiment in two periods, April-May and June-July, at four timepoints each, from five test groups: negative-RMS and positive- RMS+10%H2SO4 controls, RMS+2.5%Biochar, RMS+5%Biochar, and RMS+10%Biochar. Isolates' identification was confirmed through PCR and clonality established by (GTG)5 fingerprinting. The antimicrobial susceptibility profile of 40 representative isolates was determined by disk diffusion using Ampicillin, Vancomycin, Oxytetracycline, High-Dose Gentamicin, Enrofloxacin and Amoxicillin-Clavulanic Acid, and their phenotypic virulence profile established using specific media to evaluate the production of hemolysin, gelatinase, biofilm, DNase, proteinase and lecithinase. The susceptibility profile of isolates from RMS+5%Biochar samples presented the lowest resistance overall. While the negative control isolates were resistant to ampicillin (8%) and enrofloxacin (5%), RMS+5%Biochar isolates were only resistant to oxytetracycline (8%) and susceptible to all other antimicrobials tested. The negative control isolates showed the highest virulence profiles, with 15% expressing both hemolysin and proteinase and 13% biofilm. RMS+5%Biochar isolates also showed the lowest virulence ability, with the exception for proteinase expression, since 10% of the RMS+5%Biochar isolates were able to express this enzyme, in comparison with 8% of the RMS+2.5%Biochar isolates. No isolates were positive for gelatinase, DNase and lecithinase, independently of their origin. Concluding, 5% biochar seems to be the most adequate for RMS supplementation. Results support the hypothesis that biochar supplementation has beneficial effects on RMS, prompting further studies to evaluate if these effects apply to other relevant bacterial species.

Acknowledgements:

This research was funded by CIISA—Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, Project UIDB/00276/2020; and by Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), LA/P/0059/2020–AL4AnimalS (funded by FCT)

P4.10 - TOWARDS A SIMPLE AND COST-EFFECTIVE PROTOCOL FOR VIRAL SURVEILLANCE IN WASTEWATERS

Sofia G. Seabra [1]*, Mónica Nunes [2], Victor Pimentel [1], Aires Moura [1], Filipe Tomaz [1], Gonçalo Seixas [1], Inês M. Alves [1], Mafalda N. S. Miranda [1], Daniela Alves [1], Diocreciano M. Bero [3], Sádia Pereira [3], Ana Paula Abílio [3], Carlos São José [4], Ana Domingos [1], Marta Pingarilho [1], Ana B. Abecasis [1], Ricardo Parreira [1]

[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal.
[2] Faculty of Sciences, University of Lisbon, Lisbon Portugal.
[3] Instituto Nacional de Saúde, Mozambique.
[4] iMed.ULisboa, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

(*) e-mail: sgseabra@ihmt.unl.pt

Keywords: Magnetic-beads-traps; Viral monitoring; Wastewater Viral-surveillance.

ABSTRACT

Viral monitoring on wastewaters is being increasingly used worldwide as a cost-effective, non-invasive approach to inform about pathogens circulating in the population from the catchment area of those wastewaters. Viral isolation from wastewater samples has several technical challenges due to their complex nature and their low viral concentrations. Usually, this process involves labor-intense filtration, centrifugation, and flocculation procedures that require specialized equipment. In this study we addressed these challenges by spiking synthetic wastewater with varying concentrations of different viruses and assessing an alternative extraction method. This approach involved water absorption using a hygienic tampon followed by viral capture using nanotraps. The viruses utilized in our study included a non-enveloped dsDNA phage SPP1 siphovirus, as well as four non-enveloped ssRNA viruses: Hazara Orthonairovirus, West Nile virus, HIV, and SARS-CoV-2. Prior to spiking, the last three viruses, which are pathogenic, were heat-inactivated. They were tested at three different concentrations in the synthetic wastewater. After DNA/RNA extraction, we amplified and quantified some of the viruses by qPCR to evaluate the efficacy of the method in capturing diverse virus types, even after heat inactivation. The adoption of this approach, which combines absorption material with nanotraps for viral capture, would contribute to a simpler and more cost-effective procedure that could be incorporated in a wastewater-based viral surveillance plans, not only for wastewater treatment facilities, but also for drainage points and septic tanks in various settings, including hospitals, schools and other institutions.

Acknowledgements:
This work was funded by Fundação para a Ciência e Tecnologia (FCT) through funds to GHTM - UID/04413/2020 and LA-REAL – LA/P/0117/2020. S.G.S. was funded by FCT, Portugal, through contrato-programa 1567 (CEECINST/00102/2018).

P4.11 - FULL SURVIVAL OF GALLERIA MELLONELLA INFECTED WITH *STAPHYLOCOCCUS AUREUS* AFTER TREATMENT WITH NISIN Z

Isa Serrano [1,2]*, Dalila Mil-Homens [3], Joana F. Guerreiro [1,2], Eva Cunha [1,2], Sofia S. Costa [4], Luís Tavares [1,2], and Manuela Oliveira [1,2]

[1] *CIISA—Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*
[2] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal*
[3] *iBB-Institute for Bioengineering and Biosciences, and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal.*
[4] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisbon, Portugal*

(*) *e-mail: iserrano@fmv.ulisboa.pt*

Keywords: Diabetes mellitus; Diabetic foot infections; *Galleria mellonella; Nisin Z; Pseudomonas aeruginosa; Staphylococcus aureus.*

ABSTRACT

Diabetes mellitus affects nearly 6.4% of the worldwide population, and this number may double by 2030. Up to 25% of diabetic patients may develop diabetic foot ulcers (DFUs). Among DFU patients, 80% will suffer lower-limb amputations due to diabetic foot infections (DFIs), which are generally colonized by polymicrobial biofilms. *Staphylococcus aureus* is the DFIs' predominant pathogen, frequently found together with *Pseudomonas aeruginosa* in chronic and severe infections. Due to their high virulence and antibiotic resistant profile, it is crucial to find alternatives to conventional antibiotics for DFI treatment. Previous studies showed that Nisin Z supplemented with EDTA (0.4%) had higher antibacterial, antibacteriostatic, and antibiofilm efficiency towards *S. aureus* and *P. aeruginosa* DFI isolates. Therefore, we aimed to confirm these data in a *Galleria mellonella* model.

G. mellonella wax moth larvae were reared at 25 °C in the dark, and worms of the final- instar larval stage were selected (10 larvae for each experiment). The larvae were injected with a lethal dose of each bacterium via the hindmost left proleg. After approximately 1 hour, the larvae were injected with Nisin Z (200 µg/ml) in the penultimate right proleg. Then, they were kept in Petri dishes and maintained in the dark at 37 °C for 120 hours. Each larva was scored daily on the *G. mellonella* health index: survival, melanization, mobility, and cocoon formation. Experiments were performed with three independent replicates.

Nisin Z treatment led to 100% survival of the larvae infected with *S. aureus* but had no antibacterial activity against *P. aeruginosa*. Unexpectedly, EDTA supplementation did not increase antipseudomonal activity. Nisin Z was not cytotoxic to the larvae.

Nisin Z may be used as a complement to conventional antibiotic therapy against *S. aureus* in DFI. *G. mellonella* is a valuable model before proceeding to preclinical studies in mammals.

Acknowledgements:
Authors would like to acknowledge: CIISA—Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisbon, Portugal (Project UIDB/00276/2020); AL4AnimalS-Laboratório Associado para a Ciência Animal e Veterinária (LA/P/0059/2020); iBB Institute for Bioengineering and Biosciences (UIDB/04565/2020 and UIDP/04565/2020), i4HB Associate Laboratory Institute for Health and Bioeconomy (LA/P/0140/2020); and GHTM - (UID/04413/2020 and LA-REAL – LA/P/0117/2020).

P4.12 - IDENTIFICATION AND GENOMIC ANALYSIS OF THE FIRST *STAPHYLOCOCCUS ARGENTEUS* ISOLATE CAUSING INFECTION IN A COVID-19 PATIENT IN PORTUGAL

Teresa Conceição [1]*, Mafalda Felgueiras [2], Valquíria Alves [2], Hermínia de Lencastre [1,3]

[1]Laboratório de Genética Molecular, Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa (UNL), Oeiras, Portugal;
[2]Unidade de Saúde Local de Matosinhos – Hospital Pedro Hispano, Matosinhos, Portugal;
[3]Laboratory of Microbiology & Infectious Diseases, The Rockefeller University, New York, USA

(*) e-mail: teresagc@itqb.unl.pt

Keywords: *Staphylococcus argenteus* infection, COVID-19, Portugal

ABSTRACT

The species *Staphylococcus argenteus* was first described in 2015 and is phylogenetically divergent from *Staphylococcus aureus*. *S. argenteus* infections have been steadily increasing worldwide, although it remains indistinguishable from *S. aureus* by standard microbiological methods. *S. aureus* is endemic in Portugal with 25% of methicillin resistance reported in 2021, when the first *S. argenteus* pulmonary infection was detected. We describe here the phenotypic profiling and genomic characterization of this *S. argenteus* isolate.

In December 2021, a 71-year-old man was admitted to the intensive care unit due to respiratory failure associated with COVID-19. A putative *S. aureus* isolate was initially identified in tracheal aspirate, but a *S. argenteus* species was later confirmed by amplification of nonribosomal peptide synthetase (NRPS) gene. Further characterization of *S. argenteus* ULSM26 included phenotypic profiling, antibiotic susceptibility testing (disc diffusion and Vitek®2), *spa* typing, and whole-genome sequencing. Genomic analysis enabled *in silico* identification of antimicrobial resistance, virulence factors, plasmids and phages using ResFinder_v3.1, VirulenceFinder_v2.0, PlasmidFinder_v2.0, and Phaster. MLST and cgMLST were determined using Ridom Seqsphere_v8.

ULSM26 isolate was characterized by *spa* type t5078 and ST2250 (clonal complex 75), suggestive of *S. argenteus*, which was confirmed by NRPS gene amplification and 99.96% average nucleotide identity with *S. argenteus* MSHR113. ULSM26 exhibited phenotypic resistance to penicillins and tetracyclines encoded by *blaZ*, *tet(L)* and *tet(38)* genes. ULSM26 harbored several virulence genes (*hld*, *sak*, *scn*) related to infection and immune evasion, as well as genes for colonization and biofilm formation. Additionally, ULSM26 carried an Inc18 plasmid including the *blaZ* operon, and three bacteriophage-associated regions. cgMLST associated ULSM26 (13 allelic differences) to two Southeast Asiatic *S. argenteus* isolated in 2011.

Detection of virulent *S. argenteus* in Portugal is of concern and highlights the need for updated laboratory protocols to prevent misidentification and curb the potential spread of *S. argenteus* infections.

P4.13 - TRACKING ANTIMICROBIAL RESISTANCE PATTERNS IN CLINICAL AND HEALTHCARE-ASSOCIATED *ENTEROCOCCUS SPP.*

Joana Monteiro Marques [1,2¥*], ***Mariana Coelho*** [1,2¥], ***Teresa Barreto-Crespo*** [3,4], ***Teresa Semedo- Lemsaddek*** [1,2]

[1] *CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.*
[2] *AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal.*
[3] *iBET - Institute of Experimental Biology and Technology, Oeiras, Portugal.*
[4] *ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.*
¥ Equal contributors to the work.

(*) *e-mail: jmarques@fmv.ulisboa.pt*

Keywords: *Enterococcus,* healthcare settings, antimicrobial resistance, surveillance.

ABSTRACT

Enterococcus spp. are commensals that have emerged as frequent cause of infections in healthcare settings*, E. faecalis and E. faecium* being considered the main pathogens. Over 5 months, enterococci were isolated from urine (n=79), pus (n=51), blood (n=10) and other sources (n=8) at a hospital and a clinical analysis laboratory, in Lisbon. Healthcare-associated surface sampling (n=135) was carried out at the same hospital, including inpatient facilities of several services (beds, bathroom handles and faucets, common tables), emergency room (reception desks, offices, medical instruments) and common areas (elevators, waiting room chairs, vending machines). Each sample was spread onto enterococci selective media (with/without vancomycin supplementation), and around 20% of characteristic colonies were selected from countable plates for genus confirmation and selection of representative isolates, based on RAPD- PCR. A total of 115 enterococci, namely from urine (n=44), pus (n=25), blood (n=8), other sources (n=8) and healthcare-associated surfaces (30), were evaluated regarding antimicrobial resistance against 11 clinically relevant antibiotics by the disk diffusion method, following the breakpoint criteria defined by CLSI and EUCAST guidelines.Overall, high levels of resistance to quinupristin-dalfopristin, erythromycin and vancomycin were observed in both clinical and healthcare-associated isolates, following CLSI criteria, and to quinupristin-dalfopristin, teicoplanin and streptomycin according to EUCAST. Some levels of resistance to other clinically relevant antimicrobials were detected, including β-lactams and aminoglycosides. Resistance to the last resort antibiotic linezolid was detected in 4% of clinical isolates, only according to CLSI criteria. A considerable percentage of vancomycin resistance was observed in healthcare-associated surfaces (40%), being higher than that of clinical isolates (27%), when considering CLSI guidelines. In addition, nine isolates (8%) were considered multidrug- resistant according to CLSI, and seven (6%) following EUCAST criteria.

In conclusion, this study highlighted the importance of antimicrobial resistance surveillance, mainly focusing on the dissemination of resistance determinants between patients and healthcare settings.

Acknowledgements:
This study was supported by FCT—Fundação para a Ciência e Tecnologia IP Portugal, through projects, UIDB/00276/2020 (CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon) and LA/P/0059/2020-AL4ANIMALS (AL4AnimalS). Teresa Semedo-Lemsaddek is financially supported by national funds through FCT under the Transitional Standard—DL57/2016/CP1438/CT0004, Joana Monteiro Marques holds a doctoral fellowship – BIPM CIISA 2/2022.

P4.14 - COMPARISON OF THE ANTIMICROBIAL ACTIVITY OF INTRACANAL MEDICATION PASTES USING AN EX VIVO MODEL OF ENDODONTIC INFECTION BY *ENTEROCOCCUS FAECALIS*

Eva Cunha [1,2,#*], ***Ana Margarida Sousa*** [3,#], ***Luís Tavares*** [1,2], ***Manuela Oliveira*** [1,2,4], ***António Ginjeira*** [3]

[1] *CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*
[2] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal*
[3] *School of Dental Medicine, Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal.*
[4] *cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal*
[(#)] Both authors contributed equally to this work

[(*)] *e-mail:* evacunha@fmv.ulisboa.pt

Keywords: Intracanal medication*; Enterococcus faecalis;* Endodontic infection

ABSTRACT

Endodontic infections are caused by bacterial invasion of the root canal, with enterococci being a major cause of such infections, due to their ability to form biofilms. Endodontic treatment is performed using intracanal medication, like combined antibiotic and calcium hydroxide- based pastes, but there is no consensus regarding the best approach. The main goal of this study was to compare the antimicrobial activity of four intracanal medication pastes using an *ex vivo* model of endodontic infection caused by *Enterococcus faecalis*.

A total of 48 single rooted human teeth were distributed into six groups: A–Calasept Plus®; B–UntraCalXS®; C–Pure calcium hydroxide [$Ca(OH)_2$]; D–Double antibiotic paste (ciprofloxacin and metronidazole); E–negative control (with no treatment and no induced infection); F–positive control (with simulated infection). First, the *in vitro* antimicrobial activity of each intracanal medication was evaluated by spot-on-lawn technique. Second, to simulate an endodontic infection, root canals were inoculated with *E. faecalis* ATCC51299 and incubated (37°C, 24h). Then, each intracanal treatment was applied accordingly with the test group, followed by incubation (37°C,1 week). After, medication was removed and the presence of bacteria was assessed at 24, 48 and 72h by qualitative turbidity evaluation. Differences between groups were analysed using Fisher's exact test (p-value<0.05 as statistically significant).

The double antibiotic paste showed a higher inhibition ability *in vitro* when compared with $Ca(OH)_2$ based pastes. Using the *ex vivo* model, statistically significant differences were observed between groups A, B and C when compared with group D. In fact, $Ca(OH)_2$ based pastes were ineffective in eliminating *E. faecalis*, in contrast with the double antibiotic paste.

The double antibiotic paste presented the higher antimicrobial activity against the simulated endodontic infection. However, the selection of other intracanal medication should be performed individually focusing on the maximum benefit for each patient.

Acknowledgements:
This work was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal (Project UIDB/00276/2020); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

P4.15 - CAN WILD BOARS BE RESERVOIRS OF RELEVANT RESISTANT BACTERIA?

Manuela Oliveira [1,2,3], Raquel Abreu [1,2], Zita Martins Ruano [2,4,5], Madalena Vieira-Pinto [2,4,5], Ricardo Dias [3,6], Luís Tavares [1,2], Eva Cunha [1,2*]

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal
[3] cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[4] CECAV – Animal and Veterinary Research Centre, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[5] Departamento de Ciências Veterinárias, Escola de Ciências Agrárias e Veterinárias, Universidade de Trás-os-Montes e Alto (UTAD), Vila Real, Portugal
[6] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

[(*)] e-mail: evacunha@fmv.ulisboa.pt

Keywords: Wild Boars; One Health; ESBL; MRSA

ABSTRACT

The One Health concept recognizes that the health of humans, animals and the environment are linked. Several pathogens can cross species barriers and threat global health, leading to emergent infectious diseases (ID). Wild boar's numerous population, wide distribution and multispecies communication potential, make them relevant keys in the spread of ID by serving as reservoirs of different pathogens, including bacteria. High risk pathogens, such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum Beta-lactamase-producing Enterobacteriaceae (ESBL), have already been identified in wild pigs. The main goal of this study is to evaluate the prevalence of MRSA and ESBL strains in nasal samples from wild boars.

A total of 21 individual swab nasal samples (AMIES®) were collected from wild boars in the north region of Portugal. Each Swab was inoculated in Brain and Hearth Infusion (BHI) broth and incubated for 24h at 37ºC. Then, each suspension was inoculated in two selective chromogenic mediums, one for ESBL-positive bacteria isolation (chromID® ESBL) and another for MRSA isolation (chromID® MRSA SMART) for 24h at 37ºC. Then, suspicious MRSA and ESBL isolates were subcultured and phenotypically characterized through gram staining, catalase and oxidase production, and in case of ESBL isolates, lactose fermentation ability on MacConkey agar.

From a total of 21 nasal swabs, no MRSA suspicious colonies were detected. However, 95% (n=20/21) of the samples revealed suspicious ESBL, combining a total of 28 isolates, with 7 isolates potentially belonging to the genus *Klebsiella*, *Enterobacter*, *Serratia* or *Citrobacter* spp., 2 to *Proteus*, *Providencia* or *Morganella* spp., 8 to other Enterobacteriaceae and 11 oxidase positive bacteria.

ESBL are a major global public health concern, being wild boars' relevant reservoirs of these pathogens. Further analysis will allow to identify and understand the clonal relationship of these isolates, in order to reduce their potential zoonotic risk.

Acknowledgements:
This work was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal (Project UIDB/00276/2020); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

P4.16 - GUT BACTERIAL EVOLUTION DURING PREGNANCY

Joana Bom [1], Elsa Seixas [1], Nelson Frazão [1*]

[1] Instituto Gulbenkian de Ciência, Oeiras, Portugal.

[(*)] e-mail: nfrazao@igc.gulbenkian.pt

Keywords: Bacteria, Evolution, Pregnancy.

ABSTRACT

The dynamics of microbial communities in the gut undergo intricate changes during pregnancy, potentially affecting overall host-microbe interactions. In this study, our objective was to elucidate the molecular evolution of *Escherichia coli* (*E. coli*) within the gut of pregnant mice. Employing a gut colonization approach, we isolated *E. coli* populations from the gut of both pregnant and non-pregnant mice, subsequently subjecting them to whole-genome sequencing.

Our analysis revealed that the rate of *E. coli* evolution is identical in pregnant and non-pregnant animals, with both *E. coli* populations showing signs of adaptation (dN/dS > 1). The number of *E. coli*-specific mutations was more variable among pregnant animals, in line with the dysbiotic gut environment during pregnancy, attributed to higher levels of inflammation.

A unique adaptive mutation emerged exclusively within *E. coli* populations isolated from pregnant mice. This intergenic mutation was associated with the import of cysteine, a crucial amino acid with potential bacterial cytotoxic properties. Given the anticipated increase in cysteine availability in the gut due to elevated food intake during pregnancy, we hypothesize that this mutation confers a selective advantage by mitigating excessive cysteine uptake. Such an adaptive mutation could play a pivotal role in safeguarding *E. coli* from cysteine-induced toxicity during pregnancy and subsequent transmission to newborns.

This study provides novel insight into the adaptive mechanisms of *E. coli* populations in response to the distinct physiological conditions imposed by pregnancy. The identification of a pregnancy-associated mutational target related to cysteine transport sheds light on the intricate interplay between host physiology, diet, and microbial evolution within the gut ecosystem. Further research on the functional implications of this mutation will deepen our understanding of the microbial strategies that contribute to the stability and resilience of the gut microbiota during pregnancy and are transmitted to the newborn's gut.

Acknowledgements:
We thank the Instituto Gulbenkian de Ciência Rodent and Genomic Facilities for their assistance.

P4.17 - SHARED EVOLUTIONARY PATH IN SOCIAL MICROBIOMES

Nelson Frazão [1]*, Isabel Gordo [1]

[1] Instituto Gulbenkian de Ciência, Oeiras, Portugal.

(*) e-mail: nfrazao@igc.gulbenkian.pt

Keywords: Bacteria, Evolution, Social interactions.

ABSTRACT

Social networks can influence the ecology of gut bacteria, shaping the species composition of the gut microbiome in humans and other animals. Gut commensals evolve and can adapt at a rapid pace when colonizing healthy hosts. Here, we aimed at assessing the impact of host-to-host bacterial transmission on *Escherichia coli* evolution in the mammalian gut.

Using an *in vivo* experimental evolution approach in mice, we found a transmission rate of 7% (±3% 2× standard error [2SE]) of *E. coli* cells *per* day between hosts inhabiting the same household. Consistent with the predictions of a simple population genetics model of mutation–selection–migration, the level of shared events resulting from within host evolution is greatly enhanced in cohoused mice, showing that hosts undergoing the same diet and habit are not only expected to have similar microbiome species compositions but also similar microbiome evolutionary dynamics. Furthermore, we estimated the rate of mutation accumulation of *E. coli* to be 3.0×10^{-3} ($\pm 0.8 \times 10^{-3}$ 2SE) mutations/genome/generation, irrespective of the social context of the regime. Our results reveal the impact of bacterial migration across hosts in shaping the adaptive evolution of new strains colonizing gut microbiomes.

Acknowledgements:
We thank the Instituto Gulbenkian de Ciência Rodent and Genomic Facilities for their assistance.

P4.18 - VIRULENCE AND ANTIBIOTICS RESISTANCE IN ENTEROCOCCUS ISOLATED FROM A VETERINARY BIOLOGIC ISOLATION AND CONTAINMENT UNIT (BICU)

Catarina Geraldes [1,2*], ***Mónica Afonso*** [1,2], ***Eva Cunha*** [1,2], ***Raquel Abreu*** [1,2], ***Luís Tavares*** [1,2], ***Solange Gil*** [1,2,3], ***Manuela Oliveira*** [1,2,4]

[1] *CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon*
[2] *Associate Laboratory for Animal and Veterinary Science (AL4AnimalS)*
[3] *Biological Isolation and Containment Unit (BICU), Veterinary Hospital, Faculty of Veterinary Medicine, University of Lisbon*
[4] *cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa*

[(*)] *e-mail: cgeraldes@fmv.ulisboa.pt*

Keywords: *Enterococcus,* Virulence, Antibiotic Resistance, Veterinary, Isolation Units

ABSTRACT

Enterococci are ubiquitous microorganisms, known for their intrinsic resistance to several antibiotics, their ability of environment permanence and biofilm formation capacity, are of major relevance in hospital settings, especially in Biological Isolation and Containment Units (BICU). 73 *Enterococcus* spp. (60 *E. faecium,* 9 *E. hirae,* and 4 *E. faecalis*, identified through PCR), were collected from the Veterinary Hospital BICU. Isolates were evaluated for phenotypic production of virulence factors using different mediums - Columbia agar +5% sheep blood (cytolysin), gelatin agar (gelatinase), DNase agar (DNAse), skim milk agar (proteinase), tryptic soy agar +egg yolk (lecithinase) and Red Congo agar (biofilm). Antibiotic resistance was asserted through disk diffusion. It was observed that 93.2% of the isolates were able to produce hemolysin, 95.9% biofilm, 16.4% proteinase and 2.7% gelatinase. No isolates were positive for DNAse or lecitinase production. Also, 72.6% of the isolates presented resistance to ampicillin, 68.5% to tetracycline, 54.8% to ciprofloxacin, 6.85% to gentamicin and 5.5% to doxycycline. No resistance to vancomycin or chloramphenicol was detected. Additionally, 31% of isolates was classified as multidrug resistant (MDR), all identified as *E. faecium*. According to the pathogenicity classification by Singh et al., 2017, based on the multidrug resistance and virulence indexes, 5 isolates were classified as high threat, 5 as moderate, 26 as low and 37 as no threat. The presence of MDR isolates, the high percentage of biofilm and hemolysin producers, and the isolation of five high-threat pathogens could be associated to the high number of animals with gastrointestinal disease and under antibiotic pressure present in isolation unit, leading to a shift in the intestinal microbiome and selection of resistant microbial strains. The monitorization of these microorganisms in these units, but also in veterinary hospitals and clinics, is imperative to prevent the spread of these pathogens and hospital associated infections.

Acknowledgements:
This research was funded by CIISA—Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, Project UIDB/00276/2020; and by Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), LA/P/0059/2020–AL4AnimalS (funded by FCT).

P4.19 - THE INFLUENCE OF COVID-19 PANDEMIC IN ANTIMICROBIAL RESISTANCE DISSEMINATION IN VETERINARY MEDICINE

Diana Gomes [1,2#*], ***Leonor Macedo*** [3#], ***Luis Tavares*** [1,2], ***Manuela Oliveira*** [1,2,4]

[1] *Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.*
[2] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal*
[3] *Instituto Universitário de Ciências Psicológicas, Sociais e da Vida (ISPA), Lisbon, Portugal.*
[4] *cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustaina-bility Institute, Faculdade de Ciências, Universidade de Lisboa*
[#] *Both authors contributed equally to this work.*

[(*)] *e-mail: dgomes@fmv.ulisboa.pt*

Keywords: antibiotics, bacterial resistance, COVID-19, urinary tract infection (UTI)

ABSTRACT

The COVID-19 pandemic had multiple consequences worldwide, including contributing for the increase of bacterial resistance dissemination, well established regarding human medicine. The main aim of this work was to evaluate the impact of COVID-19 in the spread of resistance in the veterinary setting, focusing on Urinary Tract Infections (UTI) in dogs and *Escherichia coli* as infection and bacterial models, respectively. UTI is one of the most frequent infections in companion animals, including dogs, and one of the leading causes for antibiotherapy in veterinary medicine. Around 14% of these animals suffer from UTI at least once in their life, being observed that *E. coli* is the most frequent microorganism responsible for UTIs in dogs, including resistant strains. In this work, the susceptibility pattern of *E. coli* isolates obtained from urine samples of dogs with UTI, including 52 isolates collected in 2019 (pre-pandemic) and 119 isolates collected in 2022 (pos-pandemic) towards several classes of antimicrobials (namely beta-lactams, quinolones, aminoglycosides, macrolides, tetracyclines, nitrofurans and sulphamides), was determined and compared (Mann-Whitney test). As expected, an increased bacterial resistance was observed towards the majority of the antibiotics tested. Results revealed significant differences between the susceptibility of isolates from 2019 and 2022 towards amikacin, clavulanic acid-amoxicillin, ampicillin, ciprofloxacin, cefotaxime, enrofloxacin, gentamycin, nitrofurantoin, and trimethoprim-sulfamethoxazole, while no significant differences were observed for cephalexin and tetracycline. The increased bacterial resistance observed is probably associated with the reduction of face-to-face consultations during lockdown, which may have led to an increase in the prescription and misuse of antibiotics. As observed for human medicine, results show that the pandemic contributed for resistance spread in veterinary settings, prompting the need for the establishment of effective preventive measures.

Acknowledgements:
This work was supported by CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal (Project UIDB/00276/2020); and by the Associate Laboratory for Animal and Veterinary Sciences (LA/P/0059/2020 - AL4AnimalS).

P4.20 - MACROPHAGE'S GENE EXPRESSION RESPONSE TO OXIDATIVE STRESS IMPOSED BY LYME BORRELIOSIS' SPIROCHETES

Mónica Franco [1], Maria Luísa Vieira [2], Ana Armada [2]*

[1] Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal.
[2] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal.

(*) e-mail: aarmada@ihmt.unl.pt

Keywords: *Lyme Borreliosis, Borrelia garinii, Borrelia lusitaniae,* macrophages, pathogenesis, oxidative stress, reactive oxygen species, antioxidant defenses

ABSTRACT

Lyme borreliosis (LB), also known as Lyme disease, is a zoonotic, multisystemic, and emerging pathology caused by spirochetes of the complex *Borrelia burgdorferi* sensu lato (s.l.), whose transmission occurs through the bite of infected ticks, primarily of the *Ixodes* genus.
In humans, LB manifests in a wide spectrum of symptoms and severity, affecting the skin, heart, musculoskeletal system, and central nervous system (CNS).
Since its pathogenicity is predominantly associated with the immune response triggered by the host following contact with these bacteria, and not provoked by their direct action, it is essential to carry out more studies in this area.
Macrophages (MΦs) play a critical role in the control and elimination of this agent, which is why this investigation was conducted to study the innate immune response mediated by MΦs, namely in terms of oxidative stress (imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses).
For this, the human monocytic cell line THP-1 was used, which, after differentiation, was exposed to two species with distinct virulence: *B. garinii*, with tropism for the CNS and *B. lusitaniae*, isolated from a human skin biopsy.
By RT-qPCR, the relative expression of several MΦs' genes was assessed: *nfkb* (NF-kB), *casp-3, sod-2, nfe2l2* (Nrf2), *gpx-1, nox-2, il-10* and *gapdh* (endogenous gene) at 3, 24 and 48 hours after infection. The production of ROS was also evaluated through fluorescence microscopy.
Preliminary results suggest alterations in the gene expression of the infected MΦs. It was observed a decrease in the expression of the *gpx-1* and *nfe2l2* genes, which both exert antioxidant- promoting functions. By promoting an oxidative stress environment, these results showcase the possibility of a modulated effect of *Borrelia* on MΦs' oxidative stress, possibly enhancing their survival by compromising MΦs' homeostasis.

References

Steere AC *et al*. 2016. Nat Rev Dis primers, 2(1): 1-19
Mantle D *et al*. 2022. Antioxidants, 11(4), 667

P4.21 - FERRITIN RELEASE DURING MYCOBACTERIAL INFECTION – A ROLE FOR EXTRACELLULAR VESICLES?

Leonor T. S. Gomes [1,2,3], Óscar Fonseca [1,3], José Pires [1,4], Gabriel Oliveira [1,3,5,6], Ana S. Ramos [1,2,3], Isabel Oliveira [1,2,3], Tânia Silva [1,3], Daniela Freitas [1,5], M. Salomé Gomes [1,3], Ana C. Moreira [1,3,7]

[1] i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

[2] FCUP - Faculdade de Ciências, Universidade do Porto, Porto, Portugal

[3] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

[4] IPATIMUP - Instituto de Patologia e Imunologia Molecular, Universidade do Porto

[5] BiotechHealth – International Doctoral Program in Molecular and Cellular Biotechnology Applied to Health Sciences

[6] FFUP – Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

[7] IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto

[(*)] *e-mail:* leonorg@i3s.up.pt

ABSTRACT

During infection there is a dispute for iron, as both the host and the pathogen need this element. In fact, due to its properties of transition element, iron participates in many biological processes, being crucial to almost all living beings. However it can also be toxic, damaging the organism. Thus, iron levels must be tightly regulated. Infection results in altered host´s iron homeostasis, which can contribute to pathology. One of the important proteins in mammalian's iron metabolism is ferritin. Ferritin is composed of L and H subunits, the ratio of which depends on the tissue and iron requirements. The main function of the protein is to store iron inside the cells, protecting them against the iron's damaging properties. Moreover, it has been described that upon Mycobacterium avium infection, macrophages release H-ferritin into circulation, possibly as a way of redistributing the protein and the iron it contains. Furthermore, upon iron overload, ferritin is secreted by fibroblasts in CD63-positive extracellular vesicles (EVs). Thereby, we hypothesize that upon M. avium infection, macrophages release ferritin-loaded EVs and these may be important for host-pathogen interaction.

To test this hypothesis, we analyzed the effects of M. avium infection, in vivo, on ferritin redistribution. Additionally, we infected bone marrow-derived macrophages with the mycobacteria and evaluated the presence of ferritin in the secreted EVs. Based on our preliminary data, H-ferritin is redistributed upon M. avium infection in vivo. Furthermore, in vitro macrophages release EVs with L-ferritin, and this secretion is increased in case of infection. The identification of the mechanism of ferritin release into circulation during infection may open new strategies to fight against infection and other inflammatory processes.

Acknowledgements:
This project was financed by Portuguese National Funds through FCT – Fundação para a Ciência e Tecnologia, I.P. within the project EXPL/BIA-BQM/1170/2021.

P4.22 - PROTEASOME MUTATION PROMOTES RESISTANCE TO ARTEMISININ IN *PLASMODIUM FALCIPARUM*

***Adriana Gonçalves*; Miguel Teixeira; Ana Rita Pinheiro; Pedro Cravo; Pedro Ferreira**

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; InnovPlantProtect, Elvas, Portugal; Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal;*
[2] *Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT-NOVA), Portugal Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal.*

(*) *e-mail: id10896@alunos.uminho.pt*

ABSTRACT

Malaria parasites increasingly develop resistance to all drugs available in the market, hampering the goal of reducing malaria burden and deaths. Therefore, understand the mechanisms behind this is crucial to develop treatments with improved and sustainable efficacy in the future.

Clinical artemisinin resistance has been associated with mutations in the k13 gene, nevertheless, mutations in different parts of the ubiquitin-proteasome pathway modulate artemisinin susceptibility in malaria parasites. The ubiquitin-proteasome system is essential to eukaryotic cells as it is responsible for the degradation or recycle of proteins, influencing several cellular processes, being critical for the rapid cellular modifications of malaria parasites during its life cycle progression between the two hosts, especially in the stages with high replication rates. These pathways involve protein posttranslational modification, named ubiquitination, which attaches proteins to a polyubiquitin chain that is, subsequently, recognized by the 26S proteasome. The type of ubiquitination defines if proteins are recycled or degraded by the proteasome. The first step of substrate processing by the proteasome is recognizing a ubiquitylated substrate, mediated by the ubiquitin receptors within the base subcomplex of the 19S RP, including the rpn2.

A single-nucleotide variant (SNV), E738K, in the 26S proteasome regulatory subunit rpn2 gene was found in *P. chabaudi* artemisinin resistant parasite line. To study this variant, two plasmids were constructed bearing the E738 and 738K variants, placing the part of the *P. chabaudi* rpn2 gene where the variant is, in the P. falciparum gene. These chimeric genes were created due to a 78.6% homology between the proteins of both species and the impossibility to culture *P. chabaudi* parasite in vitro. We demonstrated that the 738K variant confer DHA resistance to the parasites being the proteasome involved in this resistance. Moreover, we demonstrated that the ubiquitin-proteasome pathway plays an important role in the DHA mechanism of action.

P4.23 - PREVALENCE OF EXTENDED-SPECTRUM B-LACTAMASE (ESBL)-PRODUCING ENTEROBACTERIACEAE IN DOGS OF CAPE VERDE AND SÃO TOMÉ AND PRÍNCIPE

Raquel Abreu [1,2*], ***Alice Matos*** [3], ***Luís Capela*** [1,2,3], ***Rita Jorge*** [3], ***Eva Cunha*** [1,2], ***Luís Tavares*** [1,2], ***Manuela Oliveira*** [1,2,3,4]

[1] *CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*
[2] *AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, Lisbon, Portugal*
[3] *Associação Veterinários Sem Fronteiras, Portugal*
[4] *cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE– Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa*

(*) *e-mail: rmsilva@fmv.ulisboa.pt*

Keywords: ESBL; antimicrobial resistance; dogs; Cape Verde; São Tomé and Príncipe; public health

ABSTRACT

The rise of antimicrobial resistance is a major global health concern, responsible for an increase in morbidity and mortality worldwide. Cape Verde and São Tomé and Príncipe are African countries in which antimicrobial-resistant bacteria constitute an important cause of death. Our aim was to investigate the potential role of dogs as reservoirs and spreaders of ESBL-producing Enterobacteriaceae, classified as critical priority pathogens in the WHO priority list for research and development of new antibiotics. To investigate the role of dogs as reservoirs for the dissemination of resistant bacteria, 198 rectal AMIES swab samples were collected from confined and non-confined dogs in São Nicolau (n=19) and Praia (n=45), Cape Verde, and São Tomé (n=35) and Príncipe (n=99) Islands, and transported to the Laboratory of Microbiology and Immunology, Faculty of Veterinary Medicine, Lisbon, for further processing. Samples were subjected to a pre-enrichment in Brain Heart Infusion (BHI) broth at 37°C for 24h, inoculated on chromID® ESBL and incubated at 37°C, 24h. Isolates were characterized regarding their macro and microscopic morphology, gram staining and oxidase reaction. It was possible to observe that 77.3% (n=153) of the samples displayed ESBL-positive bacteria (São Nicolau 57.9%; Praia 77.1%; São Tomé 62.2% and Príncipe 87.9%), from which 357 ESBL- producing isolates were obtained. Most isolates were presumptively identified as *Escherichia coli* (n=357; 58.5%), followed by *Klebsiella* spp./*Enterobacter* spp./*Citrobacter* spp. (17.6%), *Proteus* spp. (12.9%) and others (10.9%). Results obtained demonstrate a high frequency of ESBL-producing Enterobacteriaceae in the gastrointestinal tract of dogs from these countries, indicating that they may act as reservoirs and spreaders of resistant bacteria, which can constitute a zoonotic hazard. Due to the contact of dogs with humans and other wild animals, they may constitute a public health concern, and should be monitored in the context of resistance dissemination in these countries, in a One-Health perspective.

Acknowledgements:
Authors would like to acknowledge to CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UIDB/00276/2020, Funded by FCT) and AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences (Project LA/P/0059/2020).

P4.24 - MONITORING OF ANTIBIOTIC RESISTANCE AND CONTAMINANTS OF EMERGING CONCERN IN SMALL-SCALE WETLAND-BASED MUNICIPAL TREATMENT SYSTEMS

A. Margarida Teixeira [1]*, Diana Matos [2], Lahiruni Halwatura [3], Diana Aga [3], Célia M. Manaia [1]

[1] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

[2] Águas de Portugal

[3] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY 14260, United States

(*) e-mail: s-anmariteixeira@ucp.pt

Keywords: monitoring, antibiotic resistance, constructed wetlands, cultivation methods, qPCR

ABSTRACT

Human sewage is one of the major sources of antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and chemical contaminants of emerging concern (CECs). Wastewater treatment is a crucial barrier to prevent environmental contamination. This study aimed to assess the efficacy of three constructed wetlands (CWs) (<200 p.e.) located in small villages, in Northern Portugal dedicated to the treatment of domestic effluents. Twenty-four hour composite samples of influent and effluent were collected over four campaigns in the winter (March), spring (May), summer (July) and autumn (October) during the year of 2023. Triplicate total DNA extracts from 50-250 ml of sample were used to measure the abundance of biomarkers associated with anthropogenic contamination (*intI1; uidA; sul1*; crAssphage; *ermB, ermF, qacEΔ1, tetX, mefC and aph(3´´)-ib*)[1] and the bacterial load through 16S rRNA gene quantification by qPCR. Cultivable *Escherichia coli* and total coliforms were quantified on Chromogenic Coliform Agar (CCA). CECs extracted by solid-phase extraction (SPE) were quantified by liquid chromatography- mass spectrometry (LC–MS). Total coliforms ranged from 4.5 – 6.1 log UFC/mL in influent samples and 1.7 – 3.8 log UFC/mL in effluent samples. Total bacterial abundance, assessed based on the 16S rRNA gene, ranged between 8.0 – 8.9 log-units gene copy/mL in influent and 6.3 – 7.6 log-units in effluent. The biomarkers tested showed removal values of up to 3 log-units gene copy/mL. The chemical analysis of 119 compounds showed that pain killers as acetaminophen, illicit drugs as cocaine, antihyperlipidemic as fenofibric-acid, antihypertensives as irbesartan or psychoactive drugs as oxazepam were present in all samples (1st and 2nd campaigns), persisting after treatment. The results obtained so far suggest that the three CWs have good treatment capacity, with an important role of macrophytes, although dependent on the growth stage along the year, and with limited capacity to remove CECs.

1 Teixeira, A. M., Vaz-Moreira, I., Calderón-Franco, D., Weissbrodt, D., Purkrtova, S., Gajdos, S., Dottorini, G., Nielsen, P. H., Khalifa, L., Cytryn, E., Bartacek, J., Manaia, C. M. Candidate biomarkers of antibiotic resistance for the monitoring of wastewater and the downstream environment. Under Review.

Acknowledgements:
National Funds from FCT – Fundação para a Ciência e a Tecnologia (UID/Multi/50016/2019). AMRT was supported by the FCT PhD grant UI/BD/151388/2021 and FSE (Fundo Social Europeu). Scientific and technological cooperation protocol between Águas do Norte and Universidade Católica Portuguesa within the scope of ARQUIMEDES doctoral scholarships.

P4.25 - VIRULENCE PROFILE OF *ESCHERICHIA COLI* FROM CAPTIVE BIRDS OF PREY USED IN AVIFAUNA CONTROL AND AS EDUCATIONAL AMBASSADORS

Rita Magalhães [1,2]***, Raquel Abreu*** [1,2]***, Catarina Geraldes*** [1,2]***, Eva Cunha*** [1,2]***, Luís Tavares*** [1,2]***, Manuela Oliveira*** [1,2,3]

[1] *CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal.*
[2] *Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS).*
[3] *cE3c—Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

Keywords: One Health; Virulence Factors; Biofilms; *Escherichia coli;* Falconry.

ABSTRACT

In Portugal, captive birds of prey are used for pest control in many urban centers, while also participating in falconry exhibitions as educational ambassadors. Travelling across the country, they may represent a Public Health concern, as they can be hosts of pathogenic zoonotic bacteria. These strains may express virulence factors, responsible for their pathogenicity and ability to stimulate host defences, which can be disseminated to other bacteria affecting humans and other animals. Therefore, it is of major importance to monitor the presence of these bacteria in raptors.

Cloacal samples from 27 healthy captive birds of prey used in avifauna control and falconry exhibitions were collected using AMIES swabs, transported to the Laboratory of Bacteriology, Faculty of Veterinary Medicine, University of Lisbon, Portugal, where they were processed for *Escherichia coli* isolation and identification using conventional techniques. Next, isolates' ability to phenotypic express several virulence factors (DNase, lecithinase, protease, gelatinase and biofilm production) was assessed using specific media, and their virulence index determined.

From the samples tested, 77.8% were positive for *E. coli*, allowing to obtain 84 isolates. Most of these isolates presented gelatinase activity (89.2%); almost half produced biofilms (48.8%); and 28.6% produced hemolysins. Finally, none of the isolates expressed lecithinase, DNase or protease. Isolates' virulence index ranged between 0.17 and 0.5, with just one isolate not expressing any of the virulence determinants tested.

To our knowledge, this study represents the first evaluation of the virulence potential of faecal *E. coli* from raptors, allowing to observe that most isolates were gelatinase and biofilm producers. Gelatinase activity is involved in tissue damage, while biofilms are bacterial communities associated with chronic infections and treatment failure. In conclusion, the pathogenic potential of faecal bacteria of captive birds of prey should be characterized to improve management and housing practices, aiming to avoid their environment dissemination.

Acknowledgements:
This work was supported by CIISA–Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Project UIDB/00276/2020 (Funded by FCT) and by Laboratório Associado para Ciência Animal e Veterinária (LA/P/0059/2020 - AL4AnimalS).

P4.26 - COMPARING SALIVA OF ADULTS AND CHILDREN AS A PROBIOTIC SOURCE

Manuel Cardoso-Baptista [1]*, Carla Campos [2,3], Teresa Correia-de-Sá [4,5], Joana Ferreira-Gomes [4,5], Benedita Sampaio-Maia [1,5], Carolina Costa [5,6]

[1] Faculdade de Medicina Dentária da Universidade do Porto, Portugal
[2] Instituto Português de Oncologia do Porto Francisco Gentil, Portugal
[3] Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal
[4] Faculdade de Medicina da Universidade do Porto, Portugal
[5] INEB – Instituto Nacional de Engenharia Biomédica / I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[6] Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Portugal

(*) e-mail: manelcardosobaptista@gmail.com

Keywords: Probiotics, Oral cavity, Gut

ABSTRACT

Probiotics, beneficial bacteria related with host well-being, have gained prominence as promoters of human health. These microorganisms are isolated from food, the environment, and, most frequently, the gut. Saliva as a probiotic source remains relatively unexplored.
Therefore, this study aimed to assess the prevalence of bacteria with probiotic potential in saliva from adults and children, exploring the presence of different species throughout life stages.
Saliva samples stored at -80ºC in glycerol-infused Brain Heart Infusion (BHI) broth from 79 children, aged 6 to 10 years old, and 66 adults (>18 years old) were directly cultured in De Man Rogosa and Sharpe (MRS) agar in order to isolate bacteria with probiotic potential. After 48 hours of anaerobic incubation at 37ºC, distinct colonies were reisolated and identified by MALDI-TOF MS. The results showed that children's saliva had a higher richness of bacteria with probiotic potential ($p<0.001$) when compared to adults, in particular a greater prevalence of species belonging to the *Streptococcus mitis* group ($p<0.05$). Within our participants (children and adults), we identified individuals with 0 (16.2%), 1 (51.4%), 2 (30.5%), or 3 (1.9%) distinct species of probiotic oral isolates. *Streptococcus salivarius* was the most prevalent species, present in 50% of the population, followed by *Streptococcus mitis* and *Streptococcus oralis* (not discriminated by MALDI-TOF MS, 15%), *Streptococcus parasanguinis* (10%), *Lactobacillus plantarum* (6%), and *Limosilactobacillus fermentum* (6%). Many other species of the genera *Bifidobacterium, Lactobacillus, Levilactobacillus, Lacticaseibacillus, Limosilactobacillus*, among others, were present in a prevalence lower than 5%.

Bacteria with probiotic potential were isolated from the oral cavity of more than 80% of the participants, suggesting probiotics as common oral colonizers and suggesting saliva as an interesting source of probiotics, particularly from children, due to a higher richness in probiotic species in comparison to adults.

Acknowledgements:
Carolina Costa's fellowship was supported by FCT/MCTES scholarship with the reference 2020.08540.BD

P4.27 - EXPLORING THE ANTIFUNGAL POTENTIAL OF YUCATAN PENINSULA MARINE ORGANISMS AGAINST MEDICALLY RELEVANT CANDIDA SPECIES

Diana Grilo, Dawrin Pech-Puch, Susana Eunice Calva-Pérez, Andreia Pedras, Harold Villegas-Hernández, Sergio Guillén-Hernández, Raúl Díaz-Gamboa, Mateo Forero Tunjano, Jaime Rodríguez, Carlos Jiménez, Oscar A Lenis-Rojas and Catarina Pimentel

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.

[2] Departamento de Biología Marina, Universidad Autónoma de Yucatán, Mexico.

[3] Escuela Nacional de Estudios Superiores Unidad Mérida, Universidad Nacional Autónoma de México, Mexico.

[4] Centro de Interdisciplinar de Química e Bioloxía, Facultade de Ciencias, Universidade da Coruña, Spain.

[(*)] e-mail: diana.grilo@itqb.unl.pt

Keywords: Candida; Yucatan Peninsula; antifungal; marine natural products.

ABSTRACT

Invasive fungal infections pose a major threat to public health due to their association with high mortality and morbidity rates. The widespread occurrence of infections refractory to current antifungals has further exacerbated this scenario, making the search for new and more effective antifungals an urgent medical concern. In this study, 65 marine extracts from the Yucatan Peninsula, Mexico, were screened for antifungal activity against Candida albicans and Candida glabrata, two of the most prevalent fungal species responsible for nosocomial invasive fungal infections worldwide.

Nine crude extracts exhibited potent antifungal activity against *Candida spp*. Bioassay-guided fractionation of the sponge Monanchora arbuscula extract revealed extraordinary fungicidal activity of several fractions. Mirabilin B and penaresidin B were found in one of the most active fractions after chemical analysis using UHPLC-HRMS and NMR, and their role in the antifungal activity is discussed. Overall, the marine species of the Yucatan Peninsula are highlighted as significant sources of natural compounds with promising fungicidal properties.

P4.28 - ASSESSING THE VIRULENCE POTENTIAL OF DIFFERENT *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES WITH *GALLERIA MELLONELLA* LARVAE MODEL

Mariana Sousa [1(*)]***, Sónia Silva*** [2,3]***, Daniela Araújo*** [2]***, Joana Castro*** [2,3]***, Gonçalo Almeida*** [2]***, Catarina Ventura*** [1]***, Rui Magalhães*** [1]***, Vânia Ferreira*** [1]***, Paula Teixeira*** [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[2] *National Institute for Agrian and Veterinary Research (INIAV, I.P.), Vairão, Vila do Conde, Portugal*

[3] *Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga, Portugal*

[(*)] *e-mail: marsousa@ucp.pt*

Keywords: bacterial pathogenesis; infection; nonvertebrate animal model; foodborne.

ABSTRACT

Listeria monocytogenes is a highly diverse species, exhibiting differential virulence potential within strains from different clonal complexes (CCs). Hypervirulent CCs strains tend to be associated with higher frequency in clinical cases and severe outcomes, while hypovirulent CCs are characterized by a reduced level of virulence and are often associated with food-related contamination. Recently, researchers have employed *Galleria mellonella* larvae as an *in vivo* model to characterize these variable virulence patterns among *Listeria* strains. Although it has only been utilized once to study CC-related virulence thus, there is still uncertainty about its relevance as an *in vivo* model. Infection studies with *G. mellonella* larvae were performed to evaluate the virulence potential of 16 *Lm* strains from CC1, CC2, CC4, CC6, CC388, CC87, CC9 and CC121. Hence, the survival rate and health index scores of larvae were used to quantify the virulence capacity of this pathogen. Results obtained indicate that: the CC2 strain exhibited a hypovirulent phenotype in the larvae with the highest survival rate and health index scores, followed by two strains from CC1 and CC6. In contrast, another CC6 strain exhibited reduced larvae survival rates, followed by the CC4 strain. Furthermore, strains from CC9, which is considered hypovirulent, caused around 47% mortality (Figure 1). Our findings revealed clear variations in virulence patterns that were previously determined with other *in vitro* and *in vivo* models. Moreover, it was observed a strain-dependent intra-clonal complex virulence difference in the infection of *G. mellonella* larvae. Additionally, by eliminating the dependence of *L. monocytogenes* strains on the *inlA* gene for host cell invasion, it was observed that hypovirulent clones demonstrated an infection potential equal to or greater than that of hypervirulent strains. Hence, there are still virulence markers that need to be characterized to improve the genotypic distinction of these CCs.

Acknowledgements:
This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GenoPhenoTraits4Persitence - Genomic and phenotypic traits contributing to persistence of Listeria monocytogenes in food processing environment (PTDC/BAA-AGR/4194/2021).

P4.29 - DEVELOPMENT OF ANTIMICROBIAL COATINGS BASED ON POLYURETHANE DISPERSIONS INCORPORATED WITH UV-LIGHT STABILIZERS AND INVESTIGATION OF THEIR MECHANISM OF ACTION

Tiago Costa [1,2(*)], Belém Sampaio-Marques [1,2], Nuno M. Neves [2,3], Helena Aguilar [4], Alexandra G. Fraga [1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.*

[3] *3B's Research Group on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, Portugal*

[4] *Têxtil Manuel Gonçalves - Tecidos Plastificados e Outros Revestimentos Para a Indústria Automóvel, S.A. (TMG Automotive), Guimarães, Portugal*

[(*)] *e-mail: id7790@alunos.uminho.pt*

Keywords: antimicrobial coatings; polymeric additives; reactive nitrogen species; Tinuvin 770 DF; UV-stabilizers.

ABSTRACT

Inhibition of polymer degradation is crucial to extend the lifespan of polymeric materials, while preserving their properties and function over-time [1-3]. UV-stabilizers are currently used as additives in the polymeric industry to increase durability and resistance to UV-degradation. Interestingly, antimicrobial properties are being attributed to some UV-stabilizers, which is a significant feature since they could contribute to the reduction of microbial-induced polymer biodegradation and prevent the spread of pathogens through surfaces [4,5]. This work aims to: (1) develop antimicrobial lacquer-films based on polyurethane dispersions incorporating a UV-stabilizer and (2) investigate the antimicrobial mechanism of action of a specific UV-stabilizer. The UV-stabilizer, commercialized by BASF, Tinuvin 770 DF - a hindered amine light stabilizer (HALS) - was incorporated in polyurethane formulations to produce lacquer-films and its antimicrobial activity was tested against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Lacquer-films incorporated with Tinuvin 770 DF showed strong antimicrobial performance against gram-positive (>4.5 Log reduction) and gram-negative bacteria (>6 Log reduction), as well as against fungi (>2 Log reduction). Importantly, these lacquer-films showed good cytocompatibility with L-929 fibroblast cell line. Regarding the mechanism of action, we demonstrated a positive correlation between the production of reactive nitrogen species (RNS), Tinuvin 770 DF concentration, and microbial death. Moreover, in the presence of an RNS-scavenger (copper(II) chloride dihydrate), the antimicrobial properties of Tinuvin 770 DF were affected, resulting in an increase of the IC50. Our findings suggest that RNS produced during autoxidation of Tinuvin 770 DF could be responsible for the antimicrobial properties of lacquer-films incorporated with this UV-stabilizer. We conclude that Tinuvin 770 DF has high potential not only to prevent photo- and biodegradation of polymers, but also contributes to public health by reducing the spread of potentially dangerous microorganisms.

References:
[1] C. Wang, et al., J. Ind. Ecol., vol. 25(5), 2021. [2] J. P. Candlin, Comprehensive Analytical Chemistry, vol. 53(8), 2008. [3] J. D. Gu, Int. Biodeterior. Biodegrad., vol. 59(3), 2007. [4] J. Olczyk et al., Molecules, vol. 25(23), 2020. [5] M. D. Samper, et al. J. Appl. Polym. Sci., vol. 129(4), 2012.

P4.30 - EXPLORING GENOMIC SIGNATURES OF *STAPHYLOCOCCUS AUREUS* BEYOND COMPARTMENTS: LINKS AT THE ANIMAL-HUMAN INTERFACE

Beatriz Ramos [1,2(*)], ***Mónica V. Cunha*** [1,2]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE–Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.* [2] *Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal.*

(*) *e-mail:* beatriz.g.ramos@gmail.com

Keywords: Antimicrobial resistance; Whole genome sequencing; Mobile genetic elements; Human-livestock-wildlife interfaces; Phylodynamics

ABSTRACT

Staphylococcus aureus thrives at animal-human-environment interfaces. A large-scale work from our group has shown that antimicrobial resistance (AMR) in commensal *S. aureus* strains from wild ungulates in Portugal is driven by agricultural land cover and livestock farming[1], raising the hypothesis that AMR in wildlife strains may originate from humanized landscapes, either from contact with humans (via unknown pathways) or through direct/indirect interactions with livestock. In this work, we generate the largest available dataset of *S. aureus* draft genomes from wild animals in Portugal to begin addressing these hypotheses and understanding the origin of AMR among wildlife commensal strains. Multi-locus sequencing typing based on 98 polished genome assemblies underlined sequence types (ST) specific of red deer or wild boar. Despite the number of AMR and virulence determinants varied according to the database used for homology searches, there was consistency between AMR genotypes and phenotypes. Specific STs significantly harbored fewer virulence determinants than others, although significant differences across host species were not apparent. Host specificity determinants were screened, disclosing the unexpected presence, in wildlife, of the immune evasion cluster (IEC) encoded in a φSa3 prophage, which is a human-specific virulence determinant. Reconstruction of host transitions with map-to-reference and discrete ancestral trait mapping (DATM) strategies was carried out for relevant STs, after enrichment of the dataset with publicly available genomes from livestock and humans and regional extension to Spain. DATM analyses focused on ST398 suggest that *S. aureus* retrieved from wildlife may have transitioned from livestock. Altogether, our findings support the spillover hypothesis of human and livestock strains into free- ranging wild animals, with livestock bridging the movement of *S. aureus* strains from human to wildlife hosts.

References:
[1] https://doi.org/10.1016/j.envpol.2022.119116

Acknowledgements:
This work was supported by Fundação para a Ciência e a Tecnologia, IP [project OCELOT (2022.01539.PTDC) and institutional funding (UIDB/00329/2020; UIDB/04046/2020; LA/P/0121/2020)].

P4.31 - ANTAGONISTIC ACTIVITY OF *LACTIPLANTIBACILLUS PLANTARUM* AGAINST *ALIARCOBACTER BUTZLERI*

Alexandre Vieira [1(*)], Cristiana Mateus [1], Fernanda Domingues [1], Mónica Oleastro [2], Susana Ferreira [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal*

[(*)] *e-mail:* alexandre.vieira@ubi.pt

Keywords: *Aliarcobacter butzleri*; *Lactiplantibacillus plantarum*; Growth inhibition; Adhesion and invasion; competition.

ABSTRACT

Aliarcobacter butzleri is a Gram-negative bacterium that can be found in a variety of environments and hosts, for which the main route of transmission is suggested as the consumption of contaminated food and water. This species is considered an emergent enteropathogen, which to establish gastrointestinal infections, must be able to tolerate various adverse conditions, including the inhibition by probiotics, such as *Lactiplantibacillus plantarum*. The aim of this study was to explore the potential antagonistic activity of *L. plantarum* ATCC 8014 against *A. butzleri.* To this end, the ability to survive the gastrointestinal tract was first evaluated considering the survival at acidic pH and the minimum inhibitory concentration of bile salts. Following this, a co-culture assay was performed to assess if there was a growth inhibition of *A. butzleri* in the presence of *L. plantarum*. In addition, the effect of the culture-free supernatant (CFS) of *L. plantarum* on growth and biofilm formation by *A. butzleri* was evaluated, as well the evaluation of the *in vitro* competition effect of *L. plantarum* against *A. butzleri* using Caco-2 cells. The results indicate that *L. plantarum* can survive to acidic conditions and physiological bile salt physiological concentrations, while *A. butzleri* was more susceptible to acid environment. Regarding co-culture, *A. butzleri* did not survive after 24 hours in the presence of *L. plantarum*. Also, the CFS from *L. plantarum* inhibited growth and biofilm formation by *A. butzleri*. In the adhesion and invasion tests, *A. butzleri* and *L. plantarum* competed to adhere to epithelial cells, but *A. butzleri* seems to not suffer any changes in growth while the same cannot be stated for *L. plantarum*. In short, these results suggest that *L. plantarum* affects the growth and virulence of *A. butzleri*, but more assays are needed to ascertain its effect on virulence.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Cristiana Mateus is recipient of a doctoral fellowship (UI/BD/151023/2021) under the scope of the CICS-UBI Programmatic Funding (UIDP/00709/2020). Susana Ferreira acknowledges UBI and FCT by the contract of Scientific Employment according to DL57/2016.

P4.32 - *N. MENINGITIDIS* CAPSULE MODULATES THE HOST EGR1 SIGNALING AND CONTRIBUTES TO *N. MENINGITIDIS* MEDIATED PATHOGENESIS

Sunil D. Saroj [1(*)]**, *Riya Joshi*** [1]
[1] *Symbiosis School of Biological Sciences, Symbiosis International (Deemed University) Lavale, Pune 412115, Maharashtra, India*

[(*)] *e-mail:* sunil.saroj@ssbs.edu.in

Keywords:

ABSTRACT

Neisseria meningitidis, a Gram-negative opportunistic pathogen, expresses a plethora of virulence factors to aid infection and evade host's immune response. The molecular mechanisms governing host responses to *N. meningitidis* infection are still unknown. Early growth response 1 (EGR1) is a transcription factor that regulates host inflammatory responses and can be rapidly activated by a variety of environmental stimuli. Virulence factors expressed by meningococci can alter the epithelial cell gene expression and modulate this response. One of the most important aspects determining pathogenesis and virulence in *N. meningitidis* is the capsule. This study focused on determining the role of two major *N. meningitidis* virulence factors, capsule, and adhesins, on EGR1 induction. *N. meningitidis* harvested at A600 ≈ 0.5, 1.0 and 2.0 were used to infect A549 nasopharyngeal epithelial cell line and the J774A.1 murine macrophage cell line at MOI of 100. PD153035 (125 nM) and PD184352 (125 nM) are EGFR and ERK1/2 chemical inhibitors, respectively. The differential expression of EGR1 and pilins were analysed using qPCR. India Ink capsule staining was used to study capsule formation. We show that *N. meningitidis* harvested at different stages of growth differentially regulates expression of EGR1. It was deduced that changes capsule expression was responsible for growth related phenomenon. Also we demonstrate that wild-type *N. meningitidis* induces EGR1 though EGFF and ERK1/2 pathway and mutant in the expression of capsule induces EGR1 only through EGFR pathway. PilC and PilW are too required for induction of EGR1 though ERK1/2 pathway. We demonstrate that EGR1 inhibition decreases invasion and survival of *N. meningitidis* and EGR1 is detrimental to host defense against *N. meningitidis* infection which may contribute to

N. meningitidis-mediated pathogenesis.

P4.33 - ROLE OF CARBON SOURCES IN THE ANTIMICROBIAL RESISTANCE AND VIRULENCE OF *NEISSERIA MENINGITIDIS*

Poonam Kanojiya [1(*)], **Sunil D. Saroj** [1]
1 Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), India

(*) e-mail: poonam.kanojiya@ssbs.edu.in

Keywords:

ABSTRACT

N. meningitidis is an opportunistic pathogen that resides asymptomatically in the nasopharynx of the human host exclusively. *N. meningitidis* causes fatal infections like meningitis and sepsis. Conditions inside the host might influence the transition from carriage to invasive. Various host and non-host factors like nutrient assimilation and desiccation can affect virulence. Within the host *N. meningitidis* encounters a range of stress including exposure to sub-inhibitory concentrations of antibiotics. This may result in the development of antimicrobial resistance (AMR). AMR is a threat to human welfare. Development of resistance plays a crucial role in enhancing bacterial virulence. Here we focus to gain insight on the different factors and molecular mechanisms that might be involved in the transition of *N.* meningitidis from asymptomatic to symptomatic behavior. *N. meningitidis* (ATCC 53416) grown on GC agar plates containing 1% Kellogg's supplement, and modified Kellogg's supplement till $A_{600} \approx 0.5$. MIC was performed using broth dilution method. *N. meningitidis* was subjected to desiccation at RT for 6 and 24h. TVC was performed. Quantitative PCR was performed. We observed that physiological concentrations of lactate and pyruvate supported the growth of *N. meningitidis*. *N. meningitidis* shows three-fold change in resistance against ciprofloxacin when grown in the presence of lactate and pyruvate respectively. However, no change in the resistance against erythromycin was observed. Additionally, when exposed to desiccation for 24h, the relative survival of *N. meningitidis* increases significantly ($P \leq 0.05$) in the presence of lactate and pyruvate. Here we report, physiologically relevant concentrations of lactate and pyruvate can aid in the development of antimicrobial resistance, desiccation tolerance, and virulence of *N. meningitidis*. These finding suggest that antibiotic exposure and desiccation (non-host environment) can assist in altering the virulence and pathogenesis of *N. meningitidis.*

P4.34 - THE ROLE OF THE LYME BORRELIOSIS LABORATORY (IHMT) *VS* THE COMMUNITY: A CASUISTIC ASSESSMENT OF THE DISEASE (2019 - 2023)

Ana Armada [1(*)]***, Maria Luísa Vieira*** [1(**)]

[1]*Microbiology Unit, Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal*

(*) *e-mail:* aarmada@ihmt.unl.pt | vieira@ihmt.unl.pt

Keywords: *Borrelia burgdorferi;* diagnosis; *Ixodes Ricinus*; Lyme borreliosis; immunoblot.

ABSTRACT

Lyme borreliosis (LB) is characterised by a multisystemic inflammatory disorder caused by the response to the pathogenic genomic species of *Borrelia burgdorferi sensu lato* (s.l.) which are transmitted by hard-bodied ticks of the *Ixodes* genus. LB diagnosis is based mainly on clinical signs and symptoms by assessing the risk of exposure to the vector, although in most cases, a laboratory approach is required, given the non-specific nature of some clinical manifestations. In Portugal, since 1999, LB is a compulsory notifiable disease. The recommended criterion for diagnosing LB includes a combination of clinical, epidemiological and laboratory data. This is not always followed consistently by doctors and laboratories, leading to persistent underreporting. Therefore, this retrospective study aimed to summarise and update the LB situation in Portugal between 2019 and September 2023, based on the caseload of the IHMT's Leptospirosis and Lyme Borreliosis Laboratory (LBL). Biological samples (serum, CSF, and whole blood) from 201 patients (106♀ e 95♂) from hospitals in the South, Lisbon and Tagus Valley and Centre regions, were analysed by serologic [IFA / immunoblot] and molecular [nested-PCR (5S- 23S intergenic space)] techniques, according to the ECDC guidelines, performing a total of 444 tests. Lyme Borreliosis was confirmed in 28.9% (n=58) patients (12 patients/year). These results showed an upward trend in positivity, particularly in the last three years: 17% (2019), 12% (2020), 38% (2021), 38% (2022) and 58% (2023) of positive cases. In recent decades, the incidence of LB has been increasing in several countries, and Portugal is no exception. In addition to the under-reporting of LB, there is a growing awareness of LB among health professionals, particularly doctors, who are requesting more confirmatory tests. The data presented here, although limited in time, shows the importance of early diagnosis of LB.

Acknowledgements:
Fundação para a Ciência e Tecnologia (FCT) for funds to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020).

P4.35 - UV-LED INACTIVATION KINETICS OF BACTERIA RESPONSIBLE FOR NOSOCOMIAL INFECTIONS

Beatriz Rito [1(*)], Leonor Matos [1], Diogo N. Proença [1], Paula V. Morais [1]

[1] University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Calcada Martim de Freitas, Coimbra, Portugal

(*) e-mail: Beatriz-Rito@hotmail.com

Keywords: UV-LED; disinfection; nosocomial infections.

ABSTRACT

Controlling the microbial load in the environment is a crucial strategy to reduce the spread of organisms. The continuous spread of nosocomial infections in hospital facilities and the emergence of the coronavirus disease (COVID-19) highlighted the importance of disinfection processes in health safety. This work aimed to evaluate the efficiency of an LED-based disinfection remotely controlled prototype on the ESKAPE group bacteria and virus phage in vitro inactivation to be applied in hospitals environments and health facilities disinfection. The tested microbial indicators *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and Bacteriophage lambda DSM 4499 were exposed to 275nm, 280nm (UVC), 310nm (UVB) and 340nm (UVA) to evaluate the effect of wavelength on the disinfection process. Exposure time (5min to 30min), exposure distance (0.25m and 0.5m) and surface materials (glass, steel, and polished wood) were evaluated on the disinfection efficiency. Recovery capacity of each species after UV damage was also determined. UVC-LED prototypes were able to inactivate 99.99% of microbial indicators after 20min exposure at a 0.5 m distance. The exposure time needed to totally inactivate *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and Bacteriophage lambda can be decreased by reducing the exposure distance. UVB-LED and UVA-LED prototypes were not able to promote a log reduction of 4 and were not effective on *B. subtilis* or bacteriophage lambda inactivation. Thus, only UVC-LED prototypes were tested on the decontamination of different surface materials, being successful. *P. aeruginosa* showed a slight ability to recover from UV damage and spores from *B. subtilis* were not totally inactivated. Nevertheless, the inactivation rate of these indicators remained at 99.99% with 24h incubation after UVC irradiation. The obtained results demonstrate that the UVC-LED 280nm prototype is the most indicated to disinfect surfaces from microorganisms usually found in hospital environments.

Acknowledgements

This research was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, under the project UIDB/00285/2020 and LA/P/0112/2020, and UCCCB University of Coimbra Bacteria Culture Collection. The authors would like to acknowledge to Lightenjing company, the developer and supplier of the tested UV-LED prototypes.

P4.36 - METAGENOMIC PROFILING OF WASTEWATER DURING THE FIRST WAVES OF COVID-19 HIGHLIGHTS SPECIFIC MICROBIOTA AND RESISTOME SIGNATURES

Beatriz Ramos [1], Nuno Brôco [2], Marta Carvalho [3], Rita Lourinho [4], João Vilaça [5], Silvia Monteiro [6,7,8], Ricardo Santos [6,7,8], Mónica V. Cunha [1,9(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[2] *AdP VALOR, Serviços Ambientais, S.A. Presently, Águas do Tejo Atlântico, Lisboa, Portugal.*

[3] *AdP VALOR, Serviços Ambientais, S.A. Presently, Ponto Verde Serviços, Lisboa, Portugal.*

[4] *Águas do Tejo Atlântico, Lisboa, Portugal.*

[5] *SIMDOURO, Canidelo, Portugal.*

[6] *Laboratório de Águas, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*

[7] *Civil Engineering Research and Innovation for Sustainability (CERIS), Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.*

[8] *Department of Nuclear Sciences and Engineering (DECN), Instituto Superior Técnico, Universidade de Lisboa, Bobadela, Portugal.*

[9] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[(*)] *e-mail: mscunha@ciencias.ulisboa.pt*

Keywords: wastewater surveillance; metagenomics; antimicrobial resistance; ESKAPE; Public Health.

ABSTRACT

Urban wastewater treatment plants (WWTPs), born as environmental protection systems, enable biomarker monitoring at the sewershed level. During the COVID-19 pandemic, wastewater-based epidemiology was implemented in Portugal as an early warning system of SARS-CoV-2 outbreaks. During COVIDETECT project roll out, we profiled the taxonomical and functional composition of raw wastewater beyond SARS-CoV-2. The DNA from 24-hour composite raw wastewater samples collected between April and November 2020 from two urban WWTP in the North and LVT regions (Portugal), serving one-million in equivalent population, were subjected to shotgun metagenomic sequencing. Raw wastewater from two hospitals, each located in the catchment areas of these WWTP, were also included. Assemblies were generated by SqueezeMeta, with homology searches against ResFinder/CARD hybrid, and VFDB databases. The resistome and virulome were first profiled without taxonomical constraint, and then specifically characterized for ESKAPE pathogens. We confirm that the urban and hospital wastewater resistome and microbiota exhibit specific signatures, with correlation network analyses highlighting frequent co-occurrence of the top bacterial genera. The most frequently found antimicrobial resistance (AMR) genes (ARGs) were classified in the multidrug, tetracyclines, and the Macrolides, Lincosamides, Streptogramines, classes. Links established between AMR determinants and bacterial hosts evidence that the diversity and abundance of ARGs is not restricted to ESKAPE, being also highly predominant among *Aliarcobacter, Aeromonas*, and *Acidovorax* genera. The *Aliarcobacter* genus particularly accumulated high abundance of sulfonamides and polymyxins ARGs, while *Acinetobacter* and

Aeromonas genera hosted the highest abundance of determinants conferring resistance to beta-lactams. Samples were highly enriched in virulence effectors within the immune modulation, adherence, and metabolic adaptation categories. Altogether, our results show that wastewater monitoring is a valuable component of infectious disease and AMR surveillance, providing a community-representative snapshot of public health trends.

Acknowledgements:
Sampling within COVIDETECT (048467) was supported by COMPETE, PORLisboa, PORNorte. Sequencing was supported by FCT IP (UIDB/00329/2020).

P4.37 - SARS-COV-2 S GENE SEQUENCING IN WASTEWATER SAMPLES ENABLES EARLY LINEAGE DETECTION AND UNCOVERS RARE MUTATIONS

Ana C. Reis [1,2], Daniela Pinto [1,2], Sílvia Monteiro [3,4,5], Ricardo Santos [3,4,5], João Vieira Martins [6], Mónica V. Cunha [1,2(*)]

[1] Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[2] Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

[3] Laboratório de Águas, Instituto Superior Técnico; Universidade de Lisboa, Lisboa, Portugal

[4] Civil Engineering Research and Innovation for Sustainability (CERIS), Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[5] Department of Nuclear Sciences and Engineering (DECN), Instituto Superior Técnico, Universidade de Lisboa, Bobadela, Portugal

[6] Directorate of Information and Analysis, Directorate-General of Health, Lisbon, Portugal

(*) e-mail: mscunha@ciencias.ulisboa.pt

Keywords: SARS-CoV-2; Wastewater-Based Epidemiology; Genomic variants; Spike gene sequencing.

ABSTRACT

As the COVID-19 pandemic peaked, many countries implemented genomic surveillance systems to track SARS-CoV-2 evolution and transmission. Transition from pandemic to endemic phase prioritized alternative testing strategies to maintain effective surveillance at population level, with reduced sequencing efforts. One promising approach is Wastewater-Based Epidemiology (WBE), offering non-invasive, cost-effective means of analyzing virus trends at sewershed level. From 2020 onwards, wastewater has been recognized as an instrumental source of information for public health, with national and international authorities exploring options to implement wastewater surveillance systems and increasingly relying on WBE as early outbreak warnings. In Portugal, pioneer projects joined academia, water utilities and Public Administration around WBE. To validate WBE as an effective genomic surveillance strategy, long term performance data collection is crucial. In this work, we present one year of systematic SARS-CoV-2 wastewater surveillance in Portugal, representing 35% of the mainland population. We employed two complementary methods for lineage determination - allelic discrimination by RT-PCR and S gene sequencing. This combination allowed us to monitor variant evolution in near-real-time and identify low-frequency mutations. Throughout this year-long study, from May 2022 to April 2023, we successfully tracked dominant Omicron sub-lineages, their progression and evolution, which aligned with concurrent clinical surveillance data. Our results underscore WBE's effectiveness in tracking virus variants, revealing mutations undetected via massive sequencing of clinical samples from Portugal, demonstrating WBE's ability of uncover new mutations and detect rare genetic variants. Our findings emphasize that combining routine clinical genomic surveillance with wastewater sequencing can greatly extend our understanding of SARS-CoV-2 genetic diversity at the population level.

Acknowledgements:
APA, DGS, Grupo Águas de Portugal (AdN, AdP VALOR, AdTA, AdCL, AdA, SIMDOURO), AGERE, Águas e Energia do Porto, SMAS Almada. Funding by EC through the Emergency Support Instrument [Grant Agreement No. 060701/2021/864489/SUB/ENV.C2], Fundo Ambiental, FCT IP [UIDB/00329/2020; UIDB/04046/2020; LA/P/0121/2020].

P4.38 - EFFECTS OF SHORT CHAIN FATTY ACIDS ON *ALIARCOBACTER BUTZLERI*'S VIRULENCE

Inês Fonseca [1(*)], Cristiana Mateus [1], Fernanda Domingues [1], Mónica Oleastro [2], Susana Ferreira [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal*

[(*)] *e-mail: ines.fonseca@ubi.pt*

Keywords: *Aliarcobacter butzleri*; Virulence Factors; Short Chain Fatty Acids; Survival.

ABSTRACT

Short chain fatty acids (SCFA) have a high significance in maintaining intestinal and metabolic health, with their production being important to the gut integrity and intestinal barrier function. SCFA are formed through the microbial fermentation of indigestible carbohydrates, which may be observed in increasing concentrations along the intestine. *Aliarcobacter butzleri* is recognized as an emerging enteropathogen, found mainly in food and water. Following ingestion, *A. butzleri* come into contact with SCFA in the intestine, its preferential colonization site, which may influence its virulence and survival. The main goal of this work was to understand the effects of SCFA in *A. butzleri*'s virulence. The bacterium growth, motility, biofilm formation and Caco-2 cells adhesion and invasion abilities were evaluated with different SCFA concentrations (30, 90 and 130 mM), considering a mixture of sodium acetate, sodium propionate or sodium butyrate. A total of eight *A. butzleri*' strains were used to perform these assays. In general, lower concentrations of SCFA had no significant effect on bacterial growth of most of the strains under study, while an increase of the lag phase or even growth inhibition was found with the higher concentrations of SCFA. Regarding the motility assay, there was a decrease in motility for most of the strains tested in the presence of SCFA. Also, generally, the formation of biofilms by this bacterium appears to be affected by SCFA, with the results obtained depending on the strain and concentration used. Concerning the cell adhesion and invasion assays, the results show a trend for the SCFA to impact the bacterium's ability to adhere to this cell line. In sum, SCFA may have a role in modulating *A. butzleri*'s survival and virulence.

Acknowledgements:
This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Cristiana Mateus is recipient of a doctoral fellowship (UI/BD/151023/2021) under the scope of the CICS-UBI Programmatic Funding (UIDP/00709/2020). Susana Ferreira acknowledges UBI and FCT by the contract of Scientific Employment according to DL57/2016.

P4.39 - DIETARY CAROTENOIDS AND THEIR MODULATION OF INTESTINAL MICROBIOTA

Helena R. Rocha [1(*)], Ana M. Gomes [1], Manuela E. Pintado [1], Marta C. Coelho [1(**)]

[1] *CBQF–Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto, Portugal*

(*) *e-mail: s-mhrocha@ucp.pt | mcoelho@ucp.pt*

Keywords: carotenoids; intestinal microbiota; modulation; metabolites; absorption.

ABSTRACT

Noncommunicable diseases (NCDs) are a major global health concern, causing 41 million deaths annually. Unhealthy dietary behaviours are closely linked to NCDs, the reason why nutrition and health organizations emphasize the importance of consuming fruits and vegetables regularly. Being the most common lipid-soluble phytochemicals in the human diet, carotenoids and their metabolites have been linked to various health benefits. The effectiveness of carotenoids is influenced by multiple factors, with the intestinal microbiota (IM) playing a pivotal role in their absorption and metabolism. Dietary choices significantly impact the IM composition, affecting microbial species' proliferation, which can have both protective and detrimental effects. Moreover, IM generates metabolites that enter the bloodstream, influencing the host's IM composition and function, and playing roles in certain diseases. The present work aimed to explore the interaction between carotenoids and the IM. An *in vitro* gastrointestinal digestion simulation was conducted with three carotenoids (β-carotene, lutein, and lycopene), a mixture of these pigments, and the algae *Osmundea pinnatifida*. After, digested samples were tested on human faeces to assess their impact on gut microbiota dynamics and metabolic activity. At the phylum level, the intestinal microbiota in all tested groups primarily consisted of *Bacteroidota, Bacillota, Pseudomonadota*, and *Actinomycetota*, consistent with prior research. In general, carotenoids promoted the growth of *Lachnospiraceae* family bacteria while reducing *Lactobacillus, Enterococcus, Streptococcus*, and *Bifidobacterium* populations. These bacteria used glucose as a carbon source and produced organic acids like succinic, acetic, butyric, propionic, and malic acids. Functionally, all groups exhibited antioxidant and anti-diabetic activity, with lutein and the Mix group showing the highest levels, respectively. None of the samples displayed mutagenicity, and some even exhibited an anti-mutagenic effect. Importantly, all samples with lower carotenoid concentrations were non-cytotoxic. This study reveals the complex relationship between carotenoids and the gut microbiota, underscoring their potential to positively influence human health.

Acknowledgements:
The authors would like to thank to the project Pacto da Bioeconomia Azul - Projeto Nº. C644915664-00000026 financiado pelo Plano de Recuperação e residência Nacional "PRR ". We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020.

P4.40 - THE HYDROGENOME OF NATURAL MINERAL WATERS: CONTRASTING CHARACTERISTICS AT HARVESTING SITES AND WATER STORAGE TANKS

Cátia Fidalgo [1(*)], Catarina Milho [1], Maria José Alves [1,2(*)]

[1] *AquaValor - Centro de Valorização e Transferência de Tecnologia da Água, Chaves, Portugal*

[2] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal*

(*) e-mail: maria.alves@ipb.pt | catia.fidalgo@aquavalor.pt

Keywords: Thermal Natural Mineral Water; Hydrogenome, Metagenomics; Thermal Water Treatments.

ABSTRACT

Thermal natural mineral water (TNMW) is valued for its health benefits, influenced by its physicochemical and microbial characteristics, which, in turn, are affected by factors such as temperature. Recent research has focused on characterising the microbiome (hydrogenome) of TNMW using 16S metagenomics, primarily in TNMW obtained from harvesting sites. However, in Portuguese thermal spas, TNMW is often temporarily stored in tanks, which, despite regular sanitisation, can impact the growth and diversity of microorganisms. This study aimed to compare the hydrogenome of TNMW samples collected from harvesting sites and water storage tanks in Northern Portugal. Five TNMWs sources were analysed, comprising five harvesting sites and nine tanks. From each site, 50L of TNMW were collected and filtered to isolate bacteria and total genomic DNA was extracted for 16S metagenomic sequencing library preparation, with sequencing performed on the Illumina MiSeq platform. The results indicated alterations in the hydrogenome of the analysed TNMWs, with higher levels of bacteria classification (phylum and class) and lower levels (genus) being affected. Proteobacteria emerged as the predominant phylum, exhibiting increased detection in harvesting sites compared to storage tanks, in four out of five TNMW sources. At the class level, an increase in Betaproteobacteria was observed in seven out of nine storage tanks. At the genus level, all samples exhibited distinct predominant bacterial groups. Consequently, the effects of TNMW on thermal spa treatments may diverge from predictions solely based on the hydrogenome composition of TNMW samples from harvesting points. These findings underscore the considerable bacterial diversity in all tested samples, emphasising the need for thermal spas to consider the role of TNMW storage in tanks when assessing the effects of thermal water treatments. Furthermore, this work highlights the potential impact of TNMW transfer and subsequent storage on its hydrogenome.

Acknowledgements:
Financial support from “AquaeVitae”-PROMOVE, MissãoInterface-PRR-AAC 01/C05-i02/2022 and “AquaValor”-NORTE-01-0246-FEDER-000053.

P4.41 - A NOVEL *LIMOSILACTOBACILLUS MUCOSAE* SUBSPECIES ISOLATED FROM THE FEMALE URINARY MICROBIOTA

Andreia Garcia [1,2(*)], ***Teresa Gonçalves Ribeiro*** [1,2,3], ***Filipa Grosso*** [1,2], ***Luísa Peixe*** [1,2,3]

[1] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal*

[2] *UCIBIO – Applied Molecular Biosciences Unit, Faculty of Pharmacy, University of Porto, Porto, Portugal*

[3] *CCP – Culture Collection of Porto, Faculty of Pharmacy, University of Porto, Porto, Portugal*

(*) *e-mail:* andreiasrgarcia@gmail.com

Keywords: New subspecies; *Limosilactobacillus mucosae;* Female urogenital microbiota; Urogenital health.

ABSTRACT

Extended culture-based isolation of bacteria from female urine samples, coupled with high-resolution taxonomic characterization, has facilitated the discovery of novel prokaryotic species by our research group[1,2,3]. In this study, we describe a novel *Limosilactobacillus mucosae* subspecies that compose the female urinary microbiota of a healthy reproductive-age woman. Strain c4Ub_87^T isolated from a female urine sample[4] was characterized based on morphological, physiological, biochemical, phylogenetic (16S rRNA gene), and whole-genome sequencing (Illumina NovaSeq) analysis. Genomic relatedness indices, including Average Nucleotide Identity (ANI)[5] and digital DNA-DNA hybridization (dDDH)[6], was determined. Strain c4Ub_87^T exhibits typical characteristics of the *Limosilactobacillus* genus[7]: Gram-positive rod-shaped; anaerobic/aerotolerant; catalase-/oxidase-negative; growth at 37 °C and 45 °C, but not at 15 °C; and very small genome (1.97 Mbp). Phylogenetic analysis of the 16S rRNA gene showed that strain c4Ub_87^T belongs to the genus *Limosilactobacillus*, and formed a monophyletic cluster with *L. mucosae* DSM 13345^T. The ANI value between strain c4Ub_87 ^{T} and the closest relative *L. mucosae* DSM 13345^T was 95.5%, slightly higher than the 95.0% ANI criterion for determining a novel species[8]. However, the unique biochemical characteristics (positive for L- arabinose, D-fructose, lactose, salicine, amygdalin and gentibiose) of strain c4Ub_87^T and the dDDH value of 67.6% between c4Ub_87^T and *L. mucosae* DSM 13345^T, lower than the cut-off for bacterial subspecies differentiation (79-80%)[9], support that strain c4Ub_87^T should be considered to represent a novel subspecies of *L. mucosae.* Phylogenetic, genomic, and phenotypic analysis provide support to consider two distinct lineages within *L. mucosae* as subspecies: *Limosilactobacillus mucosae* subsp. *mucosae* subsp. nov. (type strain S32^T =DSM 13345 ^{T} =CCUG 43179^T) and *Limosilactobacillus mucosae* subsp. *sanus* subsp. nov. (type strain c4Ub_87 ^{T}). Moreover, this study enhances our understanding of the diversity of *L. mucosae* populations in the healthy female urogenital tract.

References:

1 - Cappelli, E.A., et al., Expanding the Bacterial Diversity of the Female Urinary Microbiome: Description of Eight New Corynebacterium Species. Microorganisms, 2023. 11(2). https://doi.org/10.3390/microorganisms11020388

2 - Rocha, J., et al., Lactobacillus mulieris sp. nov., a new species of Lactobacillus delbrueckii group. Int J Syst Evol Microbiol, 2020. 70(3): p. 1522-1527. https://doi.org/10.1099/ijsem.0.003901

3 - Ksiezarek, M., et al., Limosilactobacillus urinaemulieris sp. nov. and Limosilactobacillus portuensis sp. nov. isolated from urine of healthy women. Int J Syst Evol Microbiol, 2019. 71(3).

https://doi.org/10.1099/ijsem.0.004726
4 - Ksiezarek, M., et al., Long-term stability of the urogenital microbiota of asymptomatic European women. BMC Microbiol, 2021. 21(1): p. 64. https://doi.org/10.1186/s12866-021-02123-3
5 - https://jspecies.ribohost.com/jspeciesws/#home
6 - https://tygs.dsmz.de/
7 - Ksiezarek, M., et al., Genomic diversity of genus Limosilactobacillus. Microb Genom, 2022. 8(7). https://doi.org/10.1099/mgen.0.000847
8 – Kim, M., Oh, H. S., Park, S. C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol, 64(Pt 2), 346-351. https://doi.org/10.1099/ijs.0.059774-0
9 Meier-Kolthoff, J.P., et al., Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of Escherichia coli, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci, 2014. 9: p. 2. 10.1186/1944-3277-9-2

Acknowledgements:
This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. A.G. is supported by national funds through FCT (UI/BD/151316/2021); T.G.R. by UCIBIO (UIDP/QUI/04378/2020), with the financial support of FCT/MCTES through national funds; F.G. by national funds through FCT in the context of the transitional norm (DL57/2016/CP1346/CT0034).

P4.42 - SIALIDASE-1 AS A KEY VIRULENCE FACTOR IN *SPOROTHRIX BRASILIENSIS* INFECTION

Tiago Cardoso [1], Ricardo Silvestre [1], Maria Horta [2], Gustavo H. Goldman [2], Agostinho Carvalho [1], Egídio Torrado [1], Cristina Cunha [1], Fernando Rodrigues [1(*)]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine; ICVS/3B's - PT Government Associate Laboratory, University of Minho, Portugal*

[2] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil.*

(*) *e-mail: frodrigues@med.uminho.pt*

Keywords: Fungi; Infections diseases; Sialidase-1; *Sporothrix brasiliensis*; *Sporothrix schenckii*.

ABSTRACT

Sporotrichosis is a fungal cutaneous infection, representing a major health problem for humans and animals. Over the last decades, *S. brasiliensis* has overtaken *S. schenckii* as the main cause of sporotrichosis causing large outbreaks with zoonotic transmission in Brazil. Owing the rise in the clinical relevance of *S. brasiliensis*, we recently performed a dual RNA sequencing analysis from human macrophages infected with *S. brasiliensis* or *S. schenckii*, to identify potential virulence factors that provide competitive advantage to *S. brasiliensis*. A specific transcriptome signature of *S. brasiliensis* absent in *S. schenckii* was identified, being sialidase-1, MFS quinate transporter, alpha glucoside:H+ symporter and vacuolar iron transporter on the top list of the up-regulated genes. Sialidases are important virulence factors in pathogens, including viruses and bacteria. Our data demonstrate that sialidase-1 upregulation in the context of infection was only observed with *S. brasiliensis* thereby supporting the hypothesis that *S. brasiliensis* might be taking advantage of sialidase-1 activity. Furthermore, the remodeling of plasma membrane transport systems suggests a key role of these transporters for intramacrophage survival. Our dual RNA-seq on macrophages infected with *S. brasiliensis* revealed a transcriptomic signature marked by an upregulation of a variety of cytokine and chemokine genes associated with immune cell recruitment and by a downregulation of genes associated with macrophage signaling as cytokine receptors, which was not observed in macrophages infected by *S. schenckii*. Overall, our data suggest that sialidase inhibitors – e.g. oseltamivir – may prove efficacy in inhibiting *S. brasiliensis* sialidase-1, and concomitantly revert the unprotective response of the macrophages during infection.

Acknowledgements:
This work was supported by National Funds (through the Portuguese Science Foundation, FCT, I.P.) by means of the research project Sporob_SiA (2022.03348.PTDC). Tiago Cardoso is a recipient of a master's fellowship from ICVS/FCT (ICVS_BI_2023_011(1)). Edígio Torrado was supported by the FCT Estímulo Individual ao Emprego Científico 2020.03070.CEECIND.

P4.43 - POPULATION STRUCTURE AND TRANSMISSION DYNAMICS OF *MYCOBACTERIUM BOVIS* AT THE LIVESTOCK-WILDLIFE INTERFACE

André C. Pereira [1,2], Ana C. Reis [1,2], José Lourenço [2], Gonçalo Themudo [1], Ana Botelho [3], Mónica V. Cunha [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[3] *Instituto Nacional de Investigação Agrária e Veterinária IP, Oeiras, Portugal.*

(*) *e-mail: mscunha@ciencias.ulisboa.pt*

Keywords: *Mycobacterium bovis*; livestock-wildlife interface; transmission; genomic epidemiology; phylodynamics.

ABSTRACT

Animal tuberculosis (TB) remains an impacting animal and public health problem worldwide. This disease, affecting a wide range of mammal species, is primarily caused by *Mycobacterium bovis*. National authorities tackle animal TB mainly through culling programmes focused on cattle, overlooking transmission in wildlife and infection via the environment. This work aimed at improving our understanding of *M. bovis* population structure and transmission processes at interfaces to support data-driven intervention. We developed eco-phylodynamic frameworks informed by pathogen whole genome sequencing data (WGS), representing a multi-host transmission system in Portugal, at the livestock-wildlife interface (bovine, red deer, wild boar). We found evidence for the co-circulation of *M. bovis* Eu1, Eu2, and Eu3 clonal complexes, enlightening Eu2 emergence and spread processes, showing most host transitions are intraspecific, while interspecific transmission between wildlife species, and between wild boar and cattle, are highly supported. We also report the first phylodynamic analysis of Eu3 in Iberia and, via ecological modelling, we show that most host transitions occurred toward higher temperature and precipitation and lower agriculture, road, and host density. Phylogeographic reconstruction evidenced frequent transmission of Eu3 strains between two ecological clusters, representing an ecological corridor of unrecognised importance. Our study indicates that *M. bovis* continues to spread at the cattle-wildlife interface within the animal TB hotspot area, possibly driven by the foraging behaviour of wild boar near agricultural lands. Red deer seems to be an important driver of TB within wildlife hosts, while the wild boar links the multi- host wildlife community and livestock. This work highlights the value of combining genomic epidemiology with ecological modeling and phylodynamic inference for epidemic response in an era of global changes.

Acknowledgements:
Fundação para a Ciência e a Tecnologia, IP [PTDC/CVT- CVT/29783/2017, UIDB/00329/2020 UIDB/04046/2020, LA/P/0121/2020], PORLisboa [LISBOA-01-0145-FEDER- 029783], COMPETE [POCI-01-0145- FEDER-029783].

P4.44 - IMPACT OF GUT MICROBIOTA ON THE PHENOLIC COMPOSITION AND BIOACTIVITY OF VIRGIN OLIVE OIL

Elsa Mecha [1,2], Claudia Santos [1,2], Ana Catarina Guerreiro [1,2], Rosário Bronze [1,2,3], Ana Teresa Serra [1,2(*)]

[1] *iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal.*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

[3] *Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal.*

(*) e-mail: tserra@ibet.pt

Keywords: gut microbiota; SHIME®; Virgin olive oil; Phenolic compounds; Volorectal cancer.

ABSTRACT

Colorectal cancer (CRC) is among the leading causes of mortality and morbidity throughout the world, thus representing a major public health problem. Higher consumption of olive oil has been associated with low incidence and prevalence of CRC. The anticancer activity of olive oil, and in particular virgin olive oil (VOO), has been attributed to the presence of some bioactive compounds including phenolics. However, this effect is mainly supported by epidemiologic data, cell-based and animal studies where the mechanisms of action of the main VOO compounds are poorly understood. Up to date there is no human intervention study carried out with VOO to support its protective effect against CRC. Recent findings from our group make clear that hydroxytyrosol, the most important phenolic of VOO, inhibits cell proliferation and may target cancer invasion and metastasis in cell spheroids of a human colorectal cancer cell line- HT29. However, the impact of VOO colonic metabolites on CRC cells is still unknown. In this work we used SHIME® technology to study the phenolic bioaccessibility of hydroxytyrosol and their metabolites during the digestion process. This approach simulated the digestion process throughout the gastrointestinal (GI) tract, including the colonic fermentation of the non-digestive fraction. The VOO extract, as well as the digested fractions (stomach, ST after 2h; intestinal, SI after 1.5h; colon after 6h and after 48h) were analysed by a Q ExactiveTM Focus Hybrid Q-Orbitrap for the untargeted metabolomic analysis. The data showed a huge diversity of metabolites that distinguish VOO from the digested fractions. If in VOO, the main metabolites were phenolic compounds, in the ST2h, SI1.5h and in colon, the furans, the lipid and lipid-like molecules and the dicarboxylic acids, were, respectively, the most abundant metabolites. Cell-based studies using CRC cell lines are being performed to study the anticancer potential of digested fractions.

Acknowledgements:
This study was funded by (i) iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020), a program financially supported by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES), through national funds and the Associate Laboratory LS4FUTURE (LA/P/0087/2020); ii) FCT/MCTES through the VOOmics project (PTDC/BAA- AGR/4732/2021); and iii) the Portuguese Mass Spectrometry Network (Rede Nacional de Espectrometria de Massa—RNEM; LISBOA-01-0145-FEDER-402- 022125). A.T. Serra also thanks FCT/MCTES for the individual Grant CEECIND/04801/2017.

P4.45 - UNEQUAL DISTRIBUTION OF ANTIFUNGAL SUSCEPTIBILITY AMONG *CANDIDA* SPP. ISOLATES OF THE FEMALE GENITAL TRACT

Filipa Oliveira Castro [1,2(*)], Francisca Bastos [1,2], Mariana Zagalo Fernandes [1,2], José Martinez-de-Oliveira [1], Ana Palmeira-de-Oliveira [1,2,3], Ana Rita Ferrão [1,2,4], Paula Gouveia Pestana [1,2,4], Joana Rolo [1,2]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[2] *Faculdade de Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal.*

[3] *Labfit-HPRD: Health Products Research and Development Lda, Covilhã, Portugal.*

[4] *CHUCB – Centro Hospitalar Universitário Cova da Beira, Covilhã, Portugal*

(*) *e-mail: filipa.daniela.castro@ubi.pt*

Keywords: *Clotrimazole; Fluconazole; Urinary infections; Vaginal infections; Yeasts infections.*

ABSTRACT

Among the known superficial mycotic infections, vulvovaginal candidosis is the second most common cause of vaginitis. However, there is still an incomplete picture of relationships between vulvovaginal and urinary infections, as well as the factors governing such associations. Theses relationships could be crucial for comprehensive understanding of the vaginal and urine mycobiome and its connection to global public health. In this study, the objective was to compare the species distribution and antifungal susceptibility profiles of *Candida* spp. isolates of the female genital tract. We collected 74 samples of 54 eligible child-bearing age women (20 from urinary infections; 54 from vulvovaginal infections - 20 were collected from vagina and 28 from vulva). Species identification was performed by automated identification by Vitek®-2. Antifungal susceptibility testing was performed for two antifungals (fluconazole and clotrimazole). We found that the great majority of yeasts were *Candida albicans* (70%), followed by *Nakaseomyces glabratus* (20%) and *C. parapsilosis* (10%). All species were present in the three niches, but their prevalence differed *Nakaseomyces glabratus* was more prevalent in urinary samples (30%) and *C. parapsilosis* was found in higher frequency in vulvogenital samples (14%). Regarding antifungal susceptibility, we found that the yeasts isolated from the urinary tract were susceptible to both antifungals, while in the other two niches we recovered resistant isolates (fluconazole resistance: 21% vulva, 26% vagina; clotrimazole resistance: 7% vulva, 15% vagina). Although the yeast species distribution in the female genital tract was similar, the antifungal susceptibility profiles were different. Vaginal isolates were found to be more resistant to antifungals, evidencing the higher pressure to develop resistance in this specific niche.

Acknowledgements: The work was developed within the scope of the CICS-UBI projects [UIDB/00709/2020] and [UIDP/00709/2020], financed by national funds through the Portuguese Foundation for Science and Technology/MCTES (FCT).

P4.46 - *GARDNERELLA* PANGENOME ANALYSIS: INSIGHTS INTO THE PHYLOGENY, RESISTOME, VIRULOME AND HOST ADAPTATION

Pedro Teixeira [1,2]***, Márcia Sousa*** [1,2]***; Filipa Grosso*** [1,2(*)]***, Teresa Gonçalves Ribeiro*** [1,2,3]***, Luísa Peixe*** [1,2,3]

[1] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal*

[2] *UCIBIO – Applied Molecular Biosciences Unit, Faculty of Pharmacy, University of Porto, Porto, Portugal*

[3] *CCP – Culture Collection of Porto, Faculty of Pharmacy, University of Porto, Porto, Portugal*

(*) *e-mail: filipagrosso@gmail.com*

Keywords: *Gardnerella;* Urogenital microbiome; Pangenome.

ABSTRACT

Gardnerella genus includes G. vaginalis (commonly associated with bacterial vaginosis (BV)), three new species - Gardnerella leopoldii, Gardnerella piotii, and Gardnerella swidsinskii – and ten genomospecies [1,2]. Gardnerella spp. has been associated with vaginal pathologies, but also detected in female urogenital microbiome of asymptomatic women [3]. We conducted an extensive comparative genomic analysis of Gardnerella genus to assess its taxonomic and genomic diversity in different human body sites and health statuses. Twenty-nine strains from urine (n=22) and vaginal swabs (n=7) of women [24 asymptomatic, 5 overactive bladder (OAB) donors] were identified by cpn60 and subjected to WGS (NovaSeq 6000; Illumina). A total of 152 Gardnerella genomes sequences [29 from this study and 123 from publicly available databases (urine-n=35, vaginal swab-n=84, blood-n=1, and unknown-n=3) were analysed: species identification was performed with TYGS; phylogenomic, pangenome and functional enrichment analyses were performed using anvi'o v7.1. Virulence factors (VFs) and antibiotic resistance genes (ARGs) content was predicted by VFDB and ResFinder 4.1 and analysed with R. We analysed 4 species and 10 genomospecies (G. vaginalis corresponded to 41%). Phylogenomic analysis revealed that >50% of the genomes available were misidentified or deposited as Gardnerella sp. Pangenome included 4537 gene clusters and the core genome 514. No association between the accessory genome and a particular body site or health status was observed, but one COG associated with defence mechanisms was enriched in strains from BV. ARG for aminoglycosides (aph(3')-Ia), macrolides (mefA, msrD, ermX), tetracyclines (tetM, tetL), lincosamide and streptogramins (lsaC) were detected in several isolates. We unveiled a remarkable genomic diversity within Gardnerella and the detection of ARGs in several isolates should be highlighted. While no conclusive link was established between the accessory genome and specific host conditions, particular sets of VFs and metabolic functions could be associated with certain Gardnerella species/genomospecies.

References:

1. Vaneechoutte et al. 2019 doi: 10.1099/ijsem.0.003200.
2. Putonti et al. 2021 doi: 10.1128/mSphere.00154-21.
3. Castro J et al., 2020, doi: 10.1016/j.tim.2019.10.002.

Acknowledgements:
This work is financed by FCT (UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020). MS, FG are supported by FCT (2020.05038.BD, DL57/2016/CP1346/CT0034, respectively), TGR by UCIBIO (UIDP/QUI/04378/2020), PT by the project NORTE-01-0145-FEDER-000052, "Healthy&ValorFOOD – Towards healthy and valorized foods, assuring their safety, authenticity and sustainability".

P4.47 - ANTIMICROBIAL BIOPROSPECTING OF ESSENTIAL OILS OF ALENTEJO' LAMIACEAE FLAVOURING PLANTS

M. Rosário Martins [1,2(*)], **Sílvia Macedo Arantes** [1], **A. Teresa Caldeira** [1,3,4]

[1] *HERCULES Laboratory & IN2PAST — Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, University of Evora, Evora, Portugal.*

[2] *Department of Medical and Health Sciences, School of Health and Human Development, University of Evora, Evora, Portugal.*

[3] *Chemistry and Biochemistry Department, School of Sciences and Technology, University of Evora, Evora, Portugal.*

[4] *City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, University of Evora, Evora, Portugal.*

(*) *e-mail: mrm@uevora.pt*

Keywords: Essential oils; Lamiaceae; Mediterranean herbs; Antimicrobial resistance; Synergistic activity.

ABSTRACT

Antimicrobial resistance is a significant global public health concern. Emerging resistance mechanisms threaten the treatment of nosocomial diseases, leading to prolonged and disability illnesses, with high morbidity and mortality and rising healthcare costs. The main objective of this study was to assess the antibacterial potential of essential oils (EOs) of some Lamiaceae of the genera *Calamintha*, *Lavandula*, *Mentha*, *Thymus* and *Origanum*, which grow naturally in the Alentejo region (Portugal) and used by local population either as flavouring herbs in regional dishes or in traditional medicine. EOs were obtained through hydrodistillation and their chemical compositions were analysed by GC-FID. The antimicrobial activity of these EOs was evaluated, against drug-resistant bacteria commonly found in healthcare settings, by solid disk diffusion and by broth microdilution assays, with evaluation of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Additionally, the potential synergistic effects of selected mixtures of EOs were evaluated by the checkerboard method. The chemical analysis revealed that the EOs of these flavouring plants are characterized by a high concentration of oxygenated monoterpenes. Most of these EOs demonstrated exceptional antimicrobial properties against a broad spectrum of microorganisms, including Gram-positive bacteria such as *S. epidermidis*, *E. faecalis*, and *S. aureus*, as well as Gram-negative Enterobacteriaceae strains. Notably, *Origanum* spp. EOs were the most effective against studied bacteria in agar diffusion assays and in liquid assays, which can be related to the high level in thymol and its derivates. Furthermore, certain combinations of two essential oils exhibited synergistic and/or additive effects, resulting in significantly reduced MIC/MBC values. These findings suggest that the essential oils from these flavouring plants and their combinations have potential as natural antibacterial agents in the food and pharmaceutical industries, namely as antimicrobial agents.

Acknowledgements:
To FCT - Fundação para a Ciência e a Tecnologia, under project UIDB/0444/2020.

P4.48 - Characterization of the differential antimicrobial activity of human endogenous fatty acids in pathogenic/commensal *STAPHYLOCOCCUS EPIDERMIDIS*

F Magalhães [1(*)]***, E Morais*** [1]***, LG Gonçalves*** [1]***, M Miragaia*** [1]***, AV Coelho*** [1]
[1] *ITQB NOVA, Oeiras, Portugal*

(*) e-mail: filipemaga97@gmail.com

Keywords: *Staphylococcus epidermidis,* pathogenicity, endogenous fatty acids, specific

ABSTRACT

Staphylococcus epidermidis (SE), despite many benefits to the host as a skin commensal, has emerged as an opportunistic pathogen. SE has 80% prevalence in medical device-associated infections which turning chronic lead to bacteraemia. Development of new preventive and therapeutic strategies are an urgent need. Human skin SE population consists of two main clonal lineages, A/C and B, presenting distinct genetic and phenotypic features associated to pathogenicity or commensalism, respectively. The antimicrobial activity of human endogenous antimicrobial fatty acids (AFAs) against clinical SE strains of both clonal lineages, was studied aiming their use as pathogenic specific antimicrobials. AFAs are key components of skin innate immunity, which minimizes the development of resistance and have potent in vitro antimicrobial activity against MDR strains and topically applied on the skin do not create irritation. Minimum inhibitory concentration (MIC) assays were performed resorting to liquid microdilution method with several AFAs. Lowest MICs against a SE pathogenic strain were determined when compared with the commensal. Furthermore, growth curves of both strains in liquid media with increasing gradient concentration of AFAs revealed a stronger growth inhibition effect against the pathogenic strain. Coincidently, growth inhibition assays with microdilution in agar plates also revealed lower antimicrobial activity against the commensal strain. In accordance with this experiment, colonies size was measured with the aim to study the differential growth with AFA concentration. At the moment, fluorescence spectrometry and fluorescence microscopy are being performed to study the effect of the promising AFAs in cell membrane fluidity and integrity, respectively.

Acknowledgements:
MOSTMICRO (LISBOA-01-0145-FEDER-007660); SymbNET project (952537) EU Horizon 2020

P4.49 - PLASMID PROFILING OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ASSOCIATED WITH SKIN AND SOFT TISSUE INFECTIONS IN COMPANION ANIMALS

Catarina Morais [1(*)], ***Sofia Santos Costa*** [1], ***Dennis Hanke*** [2,3], ***Henrike Krüger-Haker*** [2,3], ***Ana Santos*** [1], ***Constança Pomba*** [4], ***Andrea T. Feßler*** [2,3], ***Stefan Schwarz*** [2,3], ***Isabel Couto*** [1]

[1] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal*

[2] *Institute of Microbiology and Epizootics, Centre for Infection Medicine, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

[3] *Veterinary Centre for Resistance Research (TZR), School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

[4] *Laboratory of Antimicrobial and Biocide Resistance, CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*

[(*)] *e-mail: a21000758@ihmt.unl.pt*

Keywords: *Staphylococcus pseudintermedius*; plasmid; antimicrobial resistance; whole-genome sequencing.

ABSTRACT

The increasing antimicrobial resistance (AMR) of staphylococci causing skin and soft-tissue infections (SSTIs) in companion animals is a public health concern. In this study, we analyzed the plasmid content and the associated antimicrobial resistance profiles in relevant clonal lineages of *S. pseudintermedius* causing SSTIs in companion animals.

The study focused on 41 *S. pseudintermedius*, representing predominant and emerging clonal lineages associated with SSTIs in dogs and cats collected in Lisbon (Portugal), previously characterized regarding antimicrobial resistance and clonality[1]. Plasmid DNA was extracted, digested with XbaI restriction enzyme and restriction patterns analyzed in agarose gel electrophoresis. Plasmids were classified according to their predicted molecular weight as low (≤ 3 kb), medium (3 - 10kb), or high-molecular weight (≥ 23 kb) plasmids. Each unique restriction pattern was assigned to a plasmid profile. A subset of 15 strains was further analyzed by hybrid WGS (MinION, Illumina).

Twenty-five out of the 41 (61%) representative *S. pseudintermedius* isolates carried one or more plasmids, most of medium or high molecular weight, that corresponded to eleven plasmid profiles. Strains from relevant MLST sequence-types (ST) carried plasmids, namely ST71 (17/24), ST241 (3/3), ST157 (1/4), ST118, ST258, ST265 and ST551 (each 1/1). No plasmids were detected among ST45 strains (3/3). Ongoing WGS data analyses will allow deeper insight into S. *pseudintermedius* genomes and to establish relations between antimicrobial resistance phenotypes and the mobilome.

The rapid transfer of mobile genetic elements such as the plasmids studied here could boost the increasing AMR in *S. pseudintermedius* and other related species, such as *S. aureus*.

References:

1. Morais C, *et al*. (2023). *Front.Microbiol.*14:1167834. doi:10.3389/fmicb.2023.1167834

Acknowledgements:
Project BIOSAFE funded by FEDER and Fundação para a Ciência e a Tecnologia (FCT, Portugal), Grant LISBOA-01-0145-FEDER-030713, PTDC/CAL-EST/30713/2017. Further support by FCT to GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020), grant UI/BD/151061/2021 and Bilateral PT Germany (FCT/DAAD) mobility project 2022.15300.CBM.

P4.50 - COFFEE PULP AS A PREBIOTIC SUBSTRATE FOR *LACTOBACILLUS PARACASEI*: ASSESSING NUTRITIONAL COMPOSITION AND CHLOROGENIC ACID PROFILE

Marlene Machado [1], Sara Maia [1], Susana Machado [1], Liliana Espírito-Santo [1], M. Beatriz P. P. Oliveira [1], Helena Ferreira [2], Rita C. Alves [1(*)]

[1] REQUIMTE/LAQV, Dep. Chemical Sciences, Fac. Pharmacy, University of Porto, Porto, Portugal
[2] UCIBIO, Dep. Biological Sciences, Fac. Pharmacy, University of Porto, Porto Portugal

[(*)] e-mail: rcalves@ff.up.pt

Keywords: coffee by-products; 5-caffeoylquinic; dietary fiber; probiotic bacteria.

ABSTRACT

Currently, there is a growing demand for prebiotic ingredients due to their impact on intestinal health but also on bone and cognitive health. These substrates are resistant to gastrointestinal digestion and promote the growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacteria*. Recent evidence shows that components present in coffee and its by-products, such as chlorogenic acids, melanoidins, and oligosaccharides, can stimulate the growth of beneficial bacteria and suppress the growth of pathogens [1]. In this sense, the objectives of this study were to perform a chemical characterization of a coffee by-product, dried coffee pulp (also known as cascara), and to evaluate its prebiotic activity.

Dried samples were kindly provided by a Colombian producer through a national coffee importer and roaster company (JMV-José Maria Vieira, SA). The following parameters were determined: protein, lipids, dietary fiber, and ash contents by AOAC methods [3]; chlorogenic acid profile and caffeine by RP-HPLC-DAD [4]; evaluation of *Lactobacillus paracasei* growth after 24 and 48 hours of incubation with pulp (in different concentrations) by plating serial dilutions on MRS agar.

Overall, the results indicate that coffee pulp had a high dietary fiber content (46.12% dw), mostly represented by insoluble dietary fiber (36.99% dw). The main chlorogenic acid found in coffee pulp was 5-caffeoylquinic acid (2.21 mg/g dw). Coffee pulp stimulated the growth of *L. paracasei* in a dose-dependent manner. A decrease in the pH (Δ pH up to 2.20) of the medium was observed, indicating that *L. paracasei* can metabolize coffee pulp into acidic metabolites, such as short-chain fatty acids.

In conclusion, the chemical nature of coffee pulp, which is rich in dietary fiber and chlorogenic acids, makes it an excellent candidate for prebiotic ingredients. Coffee pulp can be metabolized by probiotic bacteria, fulfilling prebiotic requirements.

References:

[1] M. Machado, H. Ferreira, M.B.P.P. Oliveira et al. Crit. Rev. Food Sci. Nutr. (2023) 1-20.
[3] Official Methods of Analysis of AOAC International. AOAC. (21st edition), USA, 2019.
[4] M. Machado, L. Espírito-Santo, S. Machado, et al. Foods, 12 (2023) 2354.

Acknowledgements:

This work was funded by the project PTDC/SAU-NUT/2165/2021- COBY4HEALTH- Can coffee by-products decrease the risk of metabolic syndrome? A comprehensive approach to reduce waste and valorize health benefits, funded by Fundação para a Ciência e Tecnologia (FCT)/ Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), Portugal. Authors acknowledge FCT/MCTES for the national funds received

through the projects UIDB/50006/2020 and UIDP/50006/2020. Machado M thanks FCT/MCTES and ESF through NORTE 2020 for her PhD grant 2021.04907.BD. Alves R thanks FCT for funding through the Scientific Employment Stimulus - Individual Call (ref. CEECIND/01120/2017). The authors also thank to JMV José Maria Vieira, SA for kindly provide the sample for the study.

P4.51- UNVEILING COVID-19 EFFECTS: SARS-COV-2 SIGNIFICANTLY ALTERS THE OROPHARYNGEAL MICROBIOTA OF ASYMPTOMATIC ADULTS

Ana Cristina Paulo [1(*)], ***Sónia T. Almeida*** [1], ***Sara Handem*** [1], ***Alexandra S. Simões*** [1], ***Raquel Sá-Leão*** [1(**)]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa Oeiras, Portugal*

(*) *e-mail: acpaulo@itqb.unl.pt*; (**) *e-mail: rsaleao@itqb.unl.pt*

Keywords: COVID-19, Oropharynx, Nasophaynx, Microbiota, Asymptomatic adults.

ABSTRACT

The global COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been associated with disruptions in the microbiota homeostasis of patients who exhibit respiratory or gastrointestinal symptoms. However, a notable aspect of this pandemic has been the presence of many asymptomatic individuals. Among these, the impact of COVID-19 on the upper respiratory tract microbiota is a topic of ongoing study, yielding conflicting results. Our research aimed to understand how SARS-CoV-2 impacts on the upper respiratory tract microbiota of asymptomatic adults.

Nasopharyngeal and oropharyngeal samples of 118 participants aged 25-64 years old, non-smokers, asymptomatic for COVID-19, with no recent antibiotic use and no reported COVID-19 infection in the previous month, were retrospectively studied. Half of the participants were SARS-CoV-2 positive and half were negative. Sociodemographic information, recent history of respiratory symptoms, and adherence to COVID-19 prevention measures were collected through questionnaires. Microbiota composition was analyzed using 16S rRNA metagenomics, with PERMANOVA employed to assess differences between groups. Models were used to determine taxa differentially abundant between conditions.

In the oropharynx, individuals with and without COVID-19 exhibited distinct microbiota profiles (P=0.018). No differences were observed in the nasopharynx (P=0.282). The oropharyngeal microbiota of SARS-CoV-2 positive individuals showed an increase in *Porphyromonas*, *Aggregatibacter*, and *Moraxella*, and a decrease in *Prevotella* and *Selenomonas* (P<0.05). Additionally, lower *Prevotella* levels were associated with past COVID-19 infection (P=0.018) and shorter mask use (P=0.021). In the nasopharynx, higher *Neisseria* levels were found among those with past infections (P=0.009), while lower *Corynebacterium* (P=0.036) and higher *Fusobacterium* (P=0.051) levels were linked to shorter mask use.

Significant disparities in the oropharyngeal microbiota of individuals with and without SARS-CoV-2 infection were observed. Furthermore, COVID-19 past infection and daily mask duration were associated with variations in both oropharyngeal and nasopharyngeal microbiota.

P4.52 - GUT BACTERIAL METATAXONOMIC ANALYSIS OF ELDERLY FROM ALGARVE

José Matos [1,2], ***Nádia Silva*** [2], ***Marlene Trindade*** [2], ***Ana Teresa Rajado*** [2], ***Pedro Castelo-Branco*** [1,2,3], ***Leonor Faleiro*** [2,3,4(*)]

[1] *Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve Campus Gambelas, Faro, Portugal*

[2] *Algarve Biomedical Center—Research Institute, Faro, Portugal*

[3] *Champalimaud Research Program, Champalimaud Centre for the Unknown, Lisbon, Portugal;*

[4] *Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, Faro, Portugal*

(*) *e-mail: mfaleiro@ualg.pt*

Keywords: gut; bacteriome; elderly; *16S rRNA gene.*

ABSTRACT

Ageing is characterized by a progressive loss of homeostasis, impaired function, and susceptibility to death. The microbiome of elderly suffers variation, and geography is one of the factors that contributes to this variation. In this work the intestinal bacterial metataxonomic analysis of elderly from Algarve was performed by sequencing the *16S rRNA* gene using the Oxford Nanopore MinION system. Our results evidence that individuals from rural and urban locations from the Algarve region share the four most abundant phyla, namely *Bacillota, Pseudomonodota*, *Bacteroidota* and *Mycoplasmatota* but diverge on the fifth more abundant phylum, namely for rural individuals the phylum *Verrucomicrobiota* is the most abundant, in contrast to the urban individuals for which the phylum *Actinomycetota* is the fifth more abundant. The two groups of individuals are also distinguished among the ten most bacterial species, namely rural individuals show to be enriched with unique species, such as *Ruminococcus champanellensis*, *Blautia obeum*, *Gehongia tenuis* and *Phocaeicola vulgatus*, whereas the urban individuals are enriched in the species *Prevotella copri*, *Dialister succinatiphilus*, *Romboutsia timonensis* and *P. dorei*. The male individuals from rural and urban locations show similar abundances of *Anaerostipes h*adrus, in contrast with urban females that show lower abundance of this bacterial specie in comparison with rural females. A similar pattern is also observed for *Blautia luti*. This study expands the knowledge on the intestinal bacterial community between rural and urban individuals from Algarve that can impact their health status.

Acknowledgements:
The authors are grateful to the financial support through the Project Algarve Regional Operational Programme (CRESC – Algarve), Reference: ALG-01-0145-FEDER-072586. J. Matos acknowledges the support of Fundação para a Ciência e Tecnologia through the fellowship 2022.13925.BD.

P4.53 - QUANTIFICATION OF VAGINAL LACTOBACILLI USING AN OPTIMIZED FLUORESCENCE MICROSCOPY METHOD

Ângela Lima [1], Christina A. Muzny [2], Nuno Cerca [1,3(*)]

[1] CEB – UM. Centre of Biological Engineering, University of Minho, Braga, Portugal
2 Division of Infectious Diseases, University of Alabama, Alabama, United States of America
3 LABBELS – Associate laboratory, Braga, Guimarães, University of Minho, Portugal

(*) e-mail: nunocerca@ceb.uminho.pt

Keywords: vaginal lactobacilli*;* bacterial quantification; fluorescence microscopy; flow cytometer; colony-forming unit.

ABSTRACT

The vaginal microbiota of healthy women is generally dominated by *Lactobacillus* species (spp.). These microrganisms are fastidious, requiring rich media to growth, being also facultative anaerobes, which can explain the difficulty to cultivate them. As such, accurate bacterial quantification, which is essential to perform experiments, presents some challenges using standard culture-based methods. Alternatively, researchers can use flow cytometry (FC) or fluorescence microscopy (FM). FC is a robust and fast method, however, not all science laboratories can afford it. On the other hand, FM is more affordable but more time consuming and requires the use of ocular grids installed in the equipment or the use of a Neubauer chamber. The last is not appropriated for bacteria due to their small size, since bacteria can be located at different depths. The aim of this study was to determine the relation and initial concentration, using an easily cultivable bacteria, with the purpose of quantifying vaginal lactobacilli suspensions using FM.

Escherichia coli, a well characterized and easily culturable species, was used to calibrate bacterial concentrations using quantified by colony-forming units (CFU), FC and FM. However, FM was not possible to perform using a standard Newbauer chamber, and a further calibration was needed to transform a volume measurement in a surface area measurement. Following all calibrations, vaginal lactobacilli, namely, *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri* and *Lactobacillus iners* were quantified.

Contrary to *E. coli*, no linear relationship was found between CFU counting and FM counts for the 4 lactobacilli species. However, when using the calibrators optimized for *E. coli*, we were able to accurately quantify the 4 species of Lactobacilli.

The developed calibrations performed herein can be used to accurately quantify vaginal lactobacilli suspensions or other fastidious bacteria.

Acknowledgements:
This work was supported by the Portuguese Foundationfor Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit. Ângela Lima acknowledges FCT individual PhD fellowship 2022.13112.BD.

P4.54 - STAPHYLOCOCCUS AUREUS FROM ATOPIC DERMATITIS HAVE SPECIFIC GENOMIC SIGNATURES

Mariana Araújo [1(*)], Nuno A. Faria [1], Margarida Gonçalo [2], Joanna Nakonieczna [3], Carmen Torres [4], Floriana Campanile [5], Kátia Netto dos Santos [6], Rita Sobral [7], Maria Miragaia [1]

[1] Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB-NOVA, Portugal
[2] Department of Dermatology, Faculty of Medicine, University of Coimbra, Portugal
[3] Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Poland
[4] Area of Biochemistry and Molecular Biology, University of La Rioja, Spain
[5] Department of Biomedical and Biotechnological Sciences, University of Catania, Italy
[6] Department of Medical Microbiology, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Brazil
[7] Laboratory of Molecular Microbiology of Bacterial Pathogens, FCT, Portugal

[(*)] e-mail: mariana.araujo@itqb.unl.pt

Keywords: Atopic Dermatitis; Antimicrobial Resistance.

ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disorder caused by skin barrier defects, environmental factors and type 2 immune responses. Skin colonization by *Staphylococcus aureus* has been associated with AD severity. However, it is still unclear if specific *S. aureus* strains are more frequently associated to AD.

Our main goal is to establish a link between specific *S. aureus* genetic backgrounds and AD.

Whole-genome sequencing data of 349 AD-related isolates and 64 non-AD-related, from AD studies published in the European Nucleotide Archive (ENA), were analysed. Antimicrobial resistance and virulence genes were screened with ABRicate v1.0, using Resfinder and VFDB database; clonal types were identified by mlst v2.23.

We identified 83 different sequence types (ST) and 21 clonal complexes. The most prevalent STs among AD-related isolates were ST8 (16.9%) and ST1 (12.9%) (p=0.002), and among the non-AD-related isolates were ST45 (15.6%) and ST30 (14.0%).

Overall, 41 resistance genes were identified. AD-related-isolates carried genes encoding resistance to β-lactams (44.1%), tetracycline (41.5%), macrolides (21.8%), fosfomycin (18.9%), aminoglycosides (15.5%), mupirocin (7.7%), erythromycin (7.1%), fusidic-acid (3.7%), bleomycin (<1%), trimethoprim (<1%), lincosamide (<1%), and streptogramin A (<1%). Among the AD-related isolates 8.9% (n=31) were MRSA. Non-AD-related isolates carried less antibiotic resistance genes than those from AD lesions (n=18/39 genes). Among the non-related isolates only one was MRSA.

Furthermore, 97 virulence genes were identified, some of which were more prevalent in AD isolates than in non-AD isolates (p<0.0003). These included several proteins involved in evasion of host immune system and toxins typically transported within pathogenicity islands.

S. aureus from AD patients belonged to specific genetic backgrounds. Moreover, AD-related *S. aureus* harboured a higher number of antimicrobial resistance genes and were enriched in specific virulence factors associated to host immune evasion and host cells invasion.

P4.55 - RELIEF OF INFANT COLIC BY FENNEL SEED INFUSION: EFFECTS ON BREAST MILK AND THE BABY'S INTESTINAL MICROBIOTA

Célia C.G. Silva [1,2(*)], ***Beatriz F.M.P. Henriques*** [1], ***Susana C. Ribeiro*** [1], ***Henrique J.D. Rosa*** [1,2]
[1] *Instituto de Investigação e Tecnologias Agrárias e do Ambiente (IITAA), Universidade dos Açores, Angra do Heroísmo, Portugal*
[2] *Faculdade de Ciências Agrárias e do Ambiente (FCAA). Universidade dos Açores, Angra do Heroísmo, Portugal*

(*) *e-mail: celia.cg.silva@uac.pt*

Keywords: Infantile colic; milk microbiota; intestinal microbiota; fennel.

ABSTRACT

Infantile colic is defined as recurrent and persistent episodes of crying in infants that occur without apparent cause. Several clinical studies have found evidence for the use of fennel preparations in the treatment of this condition, although the mechanism underlying this effect is not well understood. The present study aims to elucidate the mechanisms by which fennel infusion drunk by the mother exerts a colic-reducing effect on the infant via breast milk. The study involved 12 mother-baby pairs recruited from local health centres on the island of Terceira (Azores). The donors were healthy, breastfeeding women and their infants under 5 months of age who were exclusively fed breast milk and had a symptomatology of persistent crying episodes. The mothers were asked to drink 1 L fennel infusion (20 g seeds) daily for one week. Samples of the mother's milk and the baby's stool were taken at the beginning (day 0) and at the end of the treatment (day 7). Identification of the bacterial community of the milk and stool samples was performed using Illumina sequencing and the database SILVA. Results showed that the bacterial diversity and richness of the neonatal faecal microbiota increased (p<0.05) after intervention. The ASVs assigned to the phylum *Actinobacteriota* and the genus *Bifidobacterium* increased in breast milk after ingestion of the fennel infusion (p<0.05). Most mothers also reported a reduction in the duration of their baby's crying after the intervention. In these responding infants, the ASV of *Firmicutes* and *Bifidobacterium* in the stool increased after the intervention (p<0.05). These results suggest that the fennel infusion exerts a prebiotic effect and modulates the microbiota of breast milk and the gastrointestinal tract of infants.

Acknowledgements:
This work was financially supported by Direção Regional da Ciência e Transição Digital, Governo dos Açores — Project M1.1.C/C.S./050/2021/01.

P4.56 - POLYMER TYPE AND ENVIRONMENTAL CONDITIONS DETERMINE THE PLASTISPHERE AND ASSOCIATED RESISTOME

Inês Alçada [1], Isabel Silva [1,2], Marta Tacão [2], Isabel Henriques [1(*)]
[1] Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal
[2] CESAM (Centre for Environmental and Marine Studies), Department of Biology, University of Aveiro, Aveiro, Portugal

[(*)] e-mail: isabel.henriques@uc.pt

Keywords: Antibiotic resistance; Plastisphere; Polypropylene; Polyethylene; Antuã River.

ABSTRACT

The worrisome impact of antibiotic resistance on public health, highlights the importance of assessing potential dissemination vectors. Microplastics have been suggested as carriers that facilitate the spread of pathogenic and antibiotic-resistant bacteria in rivers. Nevertheless, significant uncertainties persist concerning the role of these contaminants. The aim of this study was to investigate how location (river site) and type of polymer [polypropylene (PP) and polyethylene (PE)] impacts the community of microorganisms associated to the microplastics (plastisphere) and its resistome.

Thus, microplastics of PP and PE were exposed for 21 days, alongside sand particles, at two locations (L1 and L2) in the Antuã River. The structure of the plastisphere, sand and surrounding water communities was determined by 16S rRNA gene Illumina sequencing. The resistome, mobilome and potential pathogens were quantified by SmartChip Real-Time PCR.

The communities' diversity and richness varied according to location, sample and polymer type. For instance, at L1 a higher species diversity was identified in PE comparing to PP. The plastisphere communities proved to be distinct from the ones identified in water and sand, characterized by higher abundances of bacteria belonging to the Pseudomonadota and Bacteroidota phyla. Nineteen genera associated to potential pathogens were identified in microplastics (e.g. *Acinetobacter*, *Flavobacterium* and *Mycobacterium*), in lower relative abundance than in water samples. Out of 72 targeted genes for quantification, 31 were detected in water, 20 in microplastics and 13 in sand samples, varying between locations regarding the relative abundance and number of detected genes. Noteworthy, microplastics at both locations were significantly enriched in *bla*CTX-M and IS*CR1* but also *bla*VIM at L2, in comparison to water.

These results reveal that the plastisphere's bacterial communities, along with their resistome, differ based on polymer types and study locations. Additionally, there is evidence to suggest that microplastics could enhance the dissemination of antibiotic resistance.

Acknowledgements:
This work was supported by LabEx DRIIHM-Dispositif de Recherche Interdisciplinaire sur les Interactions Hommes-Milieux and OHMI-Observatoire Hommes-Millieux International Estarreja under the MICRANT Project: Microplastics in the rivers of Estarreja and their role in the spread of pathogens and antibiotic resistance and by Fundação para a Ciência e a Tecnologia (FCT) through individual grants to for Isabel Silva (SFRH/BD/144902/2019) and Marta Tacão (CEECIND/00977/2020).

P4.57 - IDENTIFICATION OF MYCOPLASMA GENITALIUM AND CHARACTERIZATION OF MACROLIDE AND FLUOROQUINOLONE RESISTANCE-ASSOCIATED MUTATIONS IN A POPULATION FROM THE LISBON AREA, PORTUGAL

Alexandra Rusu [1], Filomena Martins Pereira [1], Liliana Rodrigues [1(*)]
[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal

[(*)] e-mail: lrodrigues@ihmt.unl.pt

Keywords: sexually transmitted infections; molecular diagnostics; antimicrobial resistance.

ABSTRACT

Mycoplasma genitalium (MG) is an emerging agent of sexually transmitted infections (STI). The estimated global prevalence of 1-3.3% increases considerably in populations with high-risk sexual behaviour. An association with the Human Immunodeficiency Virus (HIV) infection has also been reported. Effective therapeutic options for the treatment of MG infections are limited, with antimicrobial resistance increasing in recent years. In this work, we determined the infection rate by MG in a population from the Lisbon area in Portugal. We also evaluated the presence of macrolide and fluoroquinolone resistance-associated mutations in MG positive samples.

Five hundred and forty three DNA isolates, mainly from vaginal swabs and urine samples collected between 2018 and 2020 from 501 individuals, were analyzed. Identification of MG was performed by qPCR targeting the MgPa adhesin gene. Positive samples were screened for the potential presence of macrolide resistance-associated mutations by a qPCR assay designed to detect the wild- type sequence of the 23S rRNA gene. The presence of macrolide and fluoroquinolone resistance-associated mutations was confirmed by sequencing analysis of 23S rRNA gene, *gyrA* and *parC*.

The MG infection rate in the studied population was 4.2%. A coinfection rate of 38% with other STI agents was observed, *Chlamydia trachomatis* was the most frequent (24%). Three types of mutations in the 23S rRNA gene (A2058G, A2058T and A2059G) were detected in 19% of MG positive samples. Mutation S83N was detected in *parC* in one sample, but no clinical significance has been reported.

This study detected a significant macrolide resistance rate in the MG positive samples of the studied population. Although no relevant fluoroquinolone resistance-associated mutations were detected, the worldwide increasing trend of resistance to these antibiotics should be considered in future management of these infections.

Acknowledgements:
Fundação para a Ciência e Tecnologia (FCT) for funds to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020).

P4.58 - EVALUATION OF TOXICITY AND *IN VITRO* ACTIVITY OF CANDIDATE DRUGS FOR REPURPOSING AGAINST *NEISSERIA GONORRHOEAE*

Beatriz Santos [1], ***Ana Armada*** [1], ***Liliana Rodrigues*** [1(*)]
[1] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisboa, Portugal*

(*) *e-mail: lrodrigues@ihmt.unl.pt*

Keywords: *Neisseria gonorrhoeae;* efflux pumps; antimicrobial resistance; drug repurposing.

ABSTRACT

Neisseria gonorrhoeae (NG) is the etiological agent of gonorrhea, a common sexually transmitted infection, with 82.4 million estimated cases in 2020. NG isolates resistant to the available therapeutic options have emerged, highlighting the threat of untreatable gonorrhea. Thus, it is crucial to develop new therapeutic alternatives. Efflux inhibitors potentially increase the intracellular concentration of currently used antimicrobials, thus restoring their activity in resistant strains. In this work, we studied the activity of repurposed candidate drugs, predicted to target membrane transporters or energy metabolism, in NG.

Minimum inhibitory concentrations (MICs) of acetazolamide, amlodipine, atovaquone, clomipramine and dequalinium were determined by microdilution against NG ATCC 49226. Each drug was tested (at ¼ their MIC) for the ability to reduce the MICs of antimicrobials such as azithromycin, kanamycin, and gentamicin. Efflux inhibitory activity was evaluated for each candidate drug by ethidium bromide (EtBr) accumulation fluorometric assays. The effect of each drug on the membrane potential of NG was assessed by fluorometry and fluorescence microscopy using the dye DiOC2(3). Drug cytotoxicity of drugs at 1×, 0.1×, 0.01× and 0.001× of their MIC was evaluated against Vero and HepG2 cell lines.

Dequalinium showed the highest antimicrobial activity (MIC of 3.79 µM). Most drugs caused a slight reduction of the MIC of the tested antimicrobials. Amlodipine promoted accumulation of EtBr, suggesting an efflux inhibitory activity. Atovaquone decreased membrane potential in NG. Cell viability above 90% was observed for most drugs up to 0.1× and 0.01× of their MICs for HepG2 and Vero cell lines, respectively.

This study identified promising candidate drugs that could be further explored for the development of new therapeutic approaches for NG infections, in particular efflux inhibitors that may help prevent the development of antimicrobial resistance.

Acknowledgements:
Fundação para a Ciência e Tecnologia (FCT) for funds to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020).

P4.59 - *IN VITRO* SELECTION OF *ALIARCOBACTER BUTZLERI* WITH ERYTHROMYCIN PRODUCE GENETIC CHANGES AND AFFECTS FITNESS AND VIRULENCE

Francisca Couto [1], Inês Martins [1(*)], Filipa Vale [2,3], Fernanda Domingues [1], Mónica Oleastro [4], Susana Ferreira [1]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

[2] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal

[3] BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Portugal.

[4] National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

[(*)] e-mail: ines.margarida.martins@ubi.pt

Keywords: *Aliarcobacter butzleri*; macrolide resistance mechanisms; efflux pumps; fitness.

ABSTRACT

Widely distributed, *Aliarcobacter butzleri* is pointed out as a potential public health concern due to its potential transmission to humans and animals. In humans, it is associated with gastroenteritis, bacteremia, and septicemia, with persistent diarrheal episodes, abdominal pain, nausea, vomiting, and fever as the most common symptoms. Although most infections are self-limiting, severe or persistent cases may require antibiotic therapy, with macrolides being recommended as a therapeutic option. In addition, this enteropathogen has demonstrated a multidrug resistance profile, with high variation in reported macrolide resistance rates. Despite this, the macrolide resistance mechanisms remain mostly unknown. Aiming to gain insights on this topic and unravel the phenotypic and genotypic changes associated with resistance, *A. butzleri* resistant strains from the *in vitro* adaptive laboratory evolution (ALE), in the presence of increasing concentrations of erythromycin, were studied. Several steps were performed: determination of the cross-resistance and collateral sensitivity profiles of ALE strains; analysis of the genetic causes of phenotypic antibiotic resistance by whole-genome sequencing and bioinformatic approach; functional correlation by accumulation of ethidium bromide assays. As acquisition of erythromycin resistance may alter fitness and virulence potential of ALE strains, growth profile in the presence and absence of erythromycin, motility and biofilm formation abilities were assessed. The results point to the contribution of mutations in ribosomal proteins L4 and L22 and to the role of the AreABC efflux pump in the acquisition of resistance to erythromycin in *A. butzleri*. Furthermore, the acquisition of resistance has been associated with an increased fitness cost and even impacted virulence factors, such as motility and biofilm formation, supporting the low worldwide prevalence of high-level resistance strains. Overall, this study reveal the mechanisms associated with macrolide resistance development in *A. butzleri,* and suggest that the acquisition of resistance to this antibiotic impairs bacterial fitness and virulence.

Acknowledgements:
Inês Martins is recipient of a doctoral incentive fellowship under the scope of the protocol established between UBI and Santander Totta Bank. This work was developed within the scope of the CICS-UBI projects

UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

P4.60 - DETERMINATION OF FOSFOMYCIN SUSCEPTIBILITY OF ESBL-PRODUCING *E. COLI* AND CARBAPENEM-RESISTANT *K. PNEUMONIAE* ISOLATES FROM HOSPITALIZED PATIENTS

Ricardo Goulart [1(*)], ***Sónia Ferreira*** [2], ***Sónia Mendo*** [1], ***António Maio*** [3], ***Tânia Caetano*** [1]
[1] *CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal*
2 Centro Hospitalar do Baixo Vouga - EPE, Clinical Pathology, Aveiro, Portugal
3Centro Hospitalar do Baixo Vouga – EPE, Infectiology, Aveiro, Portugal

(*) *e-mail: ricardo.mgoulart@ua.pt*

Keywords: Fosfomycin; Agar dilution; Extended-spectrum beta-lactamases; KPC; Beta-lactams.

ABSTRACT

Antibiotic resistance is a global public health threat. Enterobacterales, specifically *Escherichia coli* and *Klebsiella pneumoniae*, are the main pathogens associated with urinary tract infections (UTIs). The increasing of infections caused by multidrug-resistant (MDR) bacteria, frequently associated with beta- lactamases production, such as cabapenemases and extended-spectrum beta-lactamases (ESBLs), raises concern. In Portugal, carbapenem resistance among *K. pneumoniae* increased from 7.1% (2017) to 11.7% (2021), as reported by European Centre for Disease Prevention and Control (ECDC). The lack of new antibiotics against MDR bacteria has led to the urgency of new treatment strategies, including the reevaluation of old antibiotics. As a result, fosfomycin emerges as a promising alternative due to its unique mechanism of action and chemical structure, reducing the likelihood of cross-resistance. *In vitro* data demonstrated its effectiveness against beta-lactamases-producing Enterobacterales.

This study assessed fosfomycin susceptibility in 52 ESBL-producing *E. coli* and 32 carbapenem-resistant *K. pneumoniae* strains (n=84) isolated from hospitalized patients between 2016 and 2023, in the center of Portugal. ESBL genes were screened by PCR in *E. coli* isolates and *bla*CTX-M was the most prevalent (85%), followed by *bla*TEM (47%) and *bla*SHV (6%). Among *K. pneumoniae*, 6.25% were NDM-1 producers, while the remaining (94%) were KPC producers, all confirmed by immunochromatography. Minimum inhibitory concentrations were determined using agar dilution reference method, and results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Fosfomycin susceptibility was observed in 92% of *E. coli* and 78% of *K. pneumoniae* isolates. The genome of fosfomycin-resistant strains was sequenced and analyzed.

The high susceptibility rates found among the isolates highlight the potential of fosfomycin as an effective treatment for UTIs caused by ESBL-producing *E. coli* or carbapenem-resistant *K. pneumoniae*. Thus, management of fosfomycin in combination with other active agents can increase the effectiveness of antibiotic therapy and minimize the risk of resistance.

Acknowledgements: Tânia Caetano was funded by national funds (OE) through FCT in the scope of the framework contract (CEECIND/01463/2017). Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), through national funds.

P4.61 - EXPLORING THE VIRULENCE POTENTIAL OF CC152 AND CC121 *S. AUREUS* STRAINS RELATED TO PEDIATRIC COMMUNITY-ACQUIRED BACTERAEMIA IN THE MANHIÇA DISTRICT HOSPITAL, MOZAMBIQUE

Marcelino Garrine [1,2(*)], ***Mariana Andrade*** [2], ***Inácio Mandomando*** [1,3], ***Isabel Couto*** [2], ***Sofia Santos Costa*** [2]

[1] *Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique*
[2] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal*
[3] *Instituto Nacional de Saúde (INS), Ministério da Saúde, Maputo, Mozambique*

(*) *e-mail: marcelino.garrine@manhica.net*

Keywords: *Staphylococcus aureus*; pediatric bacteraemia; PVL; biofilm; *Galleria mellonella.*

ABSTRACT

Staphylococcus aureus is one of the most frequent causes of bacteraemia in humans. This bacterium displays a variety of virulence traits that allow the establishment and maintenance of infection, such as the Panton-Valentine leucocidin (PVL), encoded by the *lukSF-PV* genes, and the ability to form biofilms. This study aimed to describe the virulence profile of *S. aureus* causing bacteraemia (SAB) in children in Mozambique.

We analyzed 336 *S. aureus* isolated from blood cultures of children (<5 years) admitted to the Manhiça District Hospital between 2001 and 2019, previously characterized for antibiotic susceptibility and clonality. The presence of *lukSF-PV* genes was screened by PCR. Biofilm formation was evaluated by the crystal violet adhesion method. The virulence potential of strains representing relevant clonal lineages was assessed in the *Galleria mellonella* infection model.

Overall, carriage of PVL-encoding genes was frequent (43.7%, 147/336) amongst the SAB-related *S. aureus* with an increasing frequency (~70-100%) of PVL-positive strains during the last six years of the surveillance period, which could be linked to the emergence of the MLST clonal complex CC152 lineage in our setting. Nearly 80% (52/65) of the CC121 and CC152 strains produced biofilms, although this capacity was strongly enhanced in CC152 strains. These lineages showed higher virulence potential in the infection assays of *G. mellonella* compared to the *S. aureus* reference strain ATCC25923, but similar to the one displayed by strains from CC8, a clonal lineage with a decreasing frequency trend throughout the study period.

Our results highlight the importance of monitoring the emergent CC152-MSSA-PVL$^+$ and other lineages as they display important virulence traits that may impact negatively the management of SAB pediatric patients in our setting.

Acknowledgements:
Fundação para a Ciência e a Tecnologia (FCT, Portugal) through funds to GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020), grant UI/BD/154472/2022; Fundação Calouste Gulbenkian grant number 145278.

P4.62 - EVOLUTION OF *ALIARCOBACTER BUTZLERI* UNDER LOW CIPROFLOXACIN CONCENTRATIONS ASSOCIATED WITH THE SELECTION OF MULTIDRUG- RESISTANT MUTANTS

Inês Martins [1(*)], ***Alexandra Nunes*** [2], ***Mónica Oleastro*** [3], ***Susana Ferreira*** [1]
[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[2] *Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.*
[3] *National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.*

[(*)] *e-mail: ines.margarida.martins@ubi.pt*

Keywords: *Aliarcobacter butzleri;* antibiotic resistance; experimental evolution; low ciprofloxacin concentrations.

ABSTRACT

The threat that repeated exposure to sub-minimum inhibitory concentrations of antibiotics poses to public health is often overlooked but significant, and the risk to select multidrug-resistant mutants has recently begun to gain attention. The gastrointestinal pathogen *Aliarcobacter butzleri* is a ubiquitous bacterium for which high rates of multidrug resistance have been reported, and the role of these low concentrations in this phenotype remains unknown. In fact, considering the environmental distribution of this enteropathogen, resistance selection under low antibiotic concentrations is highly likely, with the risk of spreading MDR pathogens to human. The main goal of this work was to unveil the effect of low ciprofloxacin concentrations on the resistance potential of *A. butzleri*, with a focus on a One Health perspective. Ciprofloxacin is indicated for the treatment of infections caused by this microorganism and classified by the WHO as having the highest priority among the critically important antimicrobials and a member of the Watch category. To achieve the aim, three strains isolated from humans, food products, and the environment were phenotypically characterized regarding resistance to ciprofloxacin. Subsequently, short-term evolution experiments allowed to define the concentrations that select antibiotic resistance in *A.butzleri*. Following experimental evolution, the susceptibility of the evolved populations to ciprofloxacin was determined, as was their cross-resistance profile to several antibiotics, biocides, heavy metals, and ethidium bromide. The findings of this work showed that the *A. butzleri* adaptation in the presence of ciprofloxacin resulted in changes in the susceptibility of the evolved strains to other antibiotics classes, as well as to acriflavine and ethidium bromide. This suggests that resistance mechanisms likely involve the activity of efflux pumps. In sum, this work constitutes a further contribution to understand the harmful effect that antibiotic pollution represents in clinical and non-clinical ecosystems, particularly for pathogens such as *A. butzleri* with environmental niches.

Acknowledgements:
Inês Martins is recipient of a doctoral incentive fellowship under the scope of the protocol established between UBI and Santander Totta Bank. This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

P4.63 - BACTERIAL VAGINOSIS: ASSOCIATION BETWEEN ANTIMICROBIAL TOLERANCE AND *IN VITRO* BIOFILM FORMATION MODEL

Sofia A. Pereira [1], ***Lúcia G. V. Sousa*** [1], ***Nuno Cerca*** [1,2(*)]
[1] *Centre of Biological Engineering (CEB), Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal*
[2] *LABBELS – Associate Laboratory, Braga, Portugal*

(*) *e-mail: nunocerca@ceb.uminho.pt*

Keywords: bacterial vaginosis; antimicrobial tolerance; triple-species biofilm; microbial interactions.

ABSTRACT

Bacterial vaginosis (BV), the worldwide leading vaginal bacterial infection, is characterized by the development of a polymicrobial biofilm on the vaginal epithelium, primarily formed by *Gardnerella* spp. that coexists alongside other anaerobic species. Bacterial interactions in multi-species biofilms might confer increased virulence and enhanced antimicrobial tolerance, leading to treatment failure and high BV recurrence rates. Therefore, functional studies addressing these phenomena are a major challenge.

To evaluate the impact of polymicrobial interactions in triple-species biofilms (composed by *Gardnerella vaginalis*, *Fannyhessea vaginae* and *Peptostreptococcus anaerobius*) on antimicrobial tolerance, and its association to *in vitro* biofilm formation models.

Two *in vitro* models of biofilm formation were considered: the pre-conditioned (wherein *G. vaginalis* formed the early biofilm) and the competitive (wherein all three bacteria were co- incubated simultaneously) models. Total biofilm biomass and culturable cells were determined, prior to and after treatment with metronidazole or clindamycin.

Neither metronidazole nor clindamycin were able to eradicate biomass or culturable cells in triple-species biofilms, despite being effective against selective single-species biofilms. Furthermore, the enhancement of antimicrobial tolerance in polymicrobial biofilms was independent of the biofilm formation model. Since both methods yielded biofilms with different compositions and different structures, these results suggest that increased antimicrobial tolerance is likely the result of polymicrobial interactions.

Acknowledgements: This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit.

P4.64 - COLISTIN SUBINHIBITORY CONCENTRATIONS EFFECT ON GROWTH AND NATURAL TRANSFORMATION IN *ACINETOBACTER BAUMANNII*

Tiago Lima [1,2,3]***, Victoria Stubberud*** [1,4]***, Monica Doan*** [1,4]***, Gabriela Jorge da Silva*** [1,2]***, Sara Domingues*** [1,2(*)]

[1] *Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

[2] *Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal*

[3] *CIVG – Vasco da Gama Research Center / EUVG – Vasco da Gama University School, Coimbra, Portugal*

[4] *Oslo Metropolitan University, Oslo, Norway*

[(*)] *e-mail: saradomingues@ff.uc.pt*

Keywords: colistin; subinhibitory concentrations; growth rate; natural transformation.

ABSTRACT

Horizontal gene transfer (HGT) is a major contributor to the emergence and dissemination of multidrug resistance among bacterial pathogens. Natural transformation, one of the main HGT mechanisms, in *A. baumannii* is believed to be the principal contributor to the multidrug resistance of this species. Colistin destabilizes the cell wall of bacterial cells and has been suggested to facilitate the uptake of DNA. The aim of this work was to evaluate the effect of subinhibitory concentrations of colistin in the growth and transformability of naturally competent *Acinetobacter baumannii* strains.

The minimum inhibitory concentration (MIC) of colistin was determined by broth microdilution for *A. baumannii* 319 and A118 clinical isolates. Growth curves were determined in the absence and in the presence of colistin ([Col] = 0, 1/2, 1/4, 1/6 and 1/8 of the MIC). Natural transformation of A118 with homologous DNA from *A. baumannii* 121-1 was assessed in the presence of 1/4 colistin MIC during surface-motility.

A. baumannii 319 and A118 showed a colistin MIC of 8 and 4 mg/L, respectively. The growth rate of the clinical strains was highly affected by the presence of colistin even in sub-MICs, with cells remaining in the lag phase for several hours. Transformation of A118 in the presence of colistin was slightly higher than in the absence of colistin, with frequencies of 1.2×10^{-5} and 4.3×10^{-6}, respectively, but the increase in transformability was not statistically significant ($p = 0.492$).

Colistin impairs the growth rate of bacterial strains that present low resistance to colistin even at sub-MICs. However, natural transformation in *A. baumannii* A118 is not inhibited nor stimulated by the presence of sub-inhibitory concentrations of colistin.

P4.65 - INDUCED RESISTANCE TO VANCOMYCIN IMPAIRS BIOFILM FORMATION BY *S. EPIDERMIDIS*

Inês Santos [1], ***Ângela França*** [1,2(*)]
[1] *CEB – UM. Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2] *LABBELS – Associate laboratory, Braga, Guimarães, University of Minho, Portugal*

[(*)] *e-mail: afranca@ceb.uminho.pt*

Keywords: *Staphylococcus epidermidis;* biofilms; antibiotic resistance; vancomycin.

ABSTRACT

Coagulase-negative staphylococci (CoNS), particularly *Staphylococcus epidermidis*, are one of the leading causes of sepsis in preterm neonates mostly due to their capacity to form biofilms on medical devices. A major concern regarding CoNS infections is associated with the increased antibiotic resistance that has been observed among this bacterial group. Hence, the treatment of these infections is becoming progressively challenging, which may lead to increased morbidity and mortality in preterm neonates. In that sense, we aim to explore the consequences of antibiotic resistance in biofilm formation by *S. epidermidis*.

First, the susceptibility profile of *S. epidermidis* strains collected from Portuguese newborns with bloodstream infections was characterized. To induce resistance to vancomycin, the susceptible strains were exposed to increasing concentrations of the antibiotic and, after 36 passages, the MIC was re-evaluated and the capacity of vancomycin-induced resistance strains to form biofilms was assessed (Colony forming units (CFUs) and OD_{620nm} readings).

Overall, the strains with induced resistance to vancomycin formed biofilms with less biomass and CFUs than the isogenic strains.

The acquisition of resistance to vancomycin in clinical strains of *S. epidermidis* seems to cause a decrease in the capacity of these strains to form biofilms. As such, in *S. epidermidis,* the resistance to vancomycin does not seem to be related to a stronger biofilm formation capacity.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the project EXPL/BIA-MIC/0032/2021.

P4.66 - ST8475: A NEW SEQUENCE TYPE IDENTIFIED IN METHICILLIN-RESISTANT STAPHYLOCOCCI (MRS) IN PORTUGAL

Ricardo Oliveira [1,2,3(*)], ***Daniela Araújo*** [1], ***Beatriz Loureiro*** [1], ***Joana Castro*** [1,4], ***Sónia Silva*** [1,4,5], ***Carina Almeida*** [1,2,3]

[1] *INIAV, I.P - National Institute for Agrarian and Veterinarian Research, Rua dos Lagidos, Lugar da Madalena, Portugal*

[2] *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal*

[3] *ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*

[4] *Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga, Portugal*

[5] *LABBELS –Associate Laboratory, Braga/Guimarães*

(*) *e-mail: ricardo.oliveira@iniav.pt*

Keywords: *Staphylococcus aureus*; MRSA; Portugal; farm animals; companion animals.

ABSTRACT

Methicillin-resistant staphylococci (MRS) pose a challenge to public health, particularly in healthcare-associated infections, where they seriously threaten the effectiveness of antimicrobial therapy. MRS strains have also been implicated in community-associated infections, such as in companion animals, wild animals, fresh foods, and ready-to-eat foods, and livestock-associated infections, such as in poultry and cattle.

In this study, the antimicrobial susceptibility profile of one hundred *Staphylococcus* isolates from canine and feline infections and sixty-two *Staphylococcus* isolates from raw bovine milk was determined. Isolates with an MRS phenotype were tested for the presence of the methicillin-resistance genes, *mecA* and *mecC*. MRSA isolates were further characterized by *spa* typing and whole genome sequenced (WGS) for more in-depth characterization, including the determination of sequence types (ST)/clonal complexes (CC).

Six MRS isolates were identified from companion animals, including four *S. pseudintermedius*, one *S. hominis,* and one *S. haemolyticus*, while five *S. aureus* isolates from raw bovine milk presented an MRS phenotype. The *mecA* gene was detected as being responsible for the phenotype observed in all of them. The *spa* types identified were t011 and t2383, which are types normally associated with MRSA isolates in Europe. In turn, all MRSA isolates revealed a new ST, now designated as ST8475.

This study reveals the spread of MRS isolates not only in farm animals but also in companion animals, which could pose a risk to public health. Furthermore, not only *S. aureus* but also *S. pseudintermedius*, the species most commonly detected in companion animal, appears to be associated with the MRS phenomenon. More importantly, a new ST associated with MRSA isolates has been identified and submitted to the MLST database (ST8475). This could imply the emergence of a new genetic lineage in Portugal, and cattle as a possible reservoir of resistance.

Acknowledgements:
This work was supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC); Project PTDC/CVT-CVT/4620/2021, financed by FEDER funds through COMPETE2020 – Competitiveness and Internationalization Operational Program (POCI) and by national funds (PIDDAC) through FCT/MCTES. The authors are also grateful to FCT for the PhD Grant SFRH/BD/138883/2018 and for the CEEC Individual (2022.06886.CEECIND).

P4.67 - DIARRHEA IN UNDER FIVE CHILDREN IN BENGO AND LUANDA: ANTIBIOTIC SUSCEPTIBILITY AND *ROTAVIRUS*

Elsa Fortes-Gabriel [1,2], ***Maria de Fátima da Silva*** [1], ***Jocelyne Vasconcelos*** [1], ***Miguel Brito*** [1,3]
[1] *Centro de Investigação em Saúde de Angola (CISA), Bengo, Angola*
[2] *ISTM Instituto Superior Técnico Militar (ISTM), Estado Maior General - Forças Armadas Angolanas. Luanda, Angola*
[3] *Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa (IPL). Lisboa, Portugal*

(*) *e-mail: fortes.elsa@gmail.com*

Keywords: Diarrhea; Gram-negative bacteria; Rotavirus; antibiotic-resistance.

ABSTRACT

Diarrheal disease occur commonly among children and it is the leading cause of death in under five years in low- income countries and affect an estimated 370,000 childhood per year. Furthermore, the increase of antibiotic resistance could be disrupting of gut microbiome balance among this group. This study aims to monitor the pathogenic enterobacteria, assess antibiotic susceptibility and conduct *Rotavirus* genotyping among children in the main public Hospitals in Bengo and Luanda, respectively. The goal is to understand the fluctuations in prevalent strains and their associated genes.

About N=435 stools samples from children aged 1-59 months with diarrhoea symptoms were collected at the admission in the Hospitals of Luanda (n=106) and Bengo (n=330) and immediately screened with rapid test (SD bioline®) for *Rotavirus* infection. From those, n=145 samples were subjected to identity and antibiotic susceptibility tests for Gram-negative bacteria, according to the protocol of Vitek2 Compact® system. A molecular approach for Rotavirus genotyping by Nested-PCR using NZytech® protocol for RNA extraction and cDNA transcriptase followed by amplification with 20 combined primers was also carried out.

The results are ongoing processing. However preliminarily, in n=161 we observe the infection by pathogenic *Escherichia coli* spp n=67 (41.6%), *Klebsiella* spp n=53 (32.9%) and *Salmonella* spp n=3 (1.9%). The corresponding result for antibiotic susceptibility showed average 35% of resistance among the different classes of antibiotics. While selected n=245 samples showed 59% positive for *Rotavirus*, and genotypes in pairs of genes G9[P6] 21.6%, G10[P6] 18.6% were the most frequent.

The response rate from caregivers, reenforce the poverty, the low education, non-treated water as the factor for the occurrence of diarrhea in rural areas. In conclusion, our children could be infected with multidrug resistant strains of *Escherichia coli* and *Klebsiella pneumoniae* bacteria and non-vaccinal *Rotavirus* strains.

P4.68 - PREDICTION OF NOVEL SMALL NON-CODING RNAs FROM *PSEUDOMONAS AERUGINOSA* INVOLVED IN ANTIBIOTIC RESISTANCE AND BIOFILM FORMATION

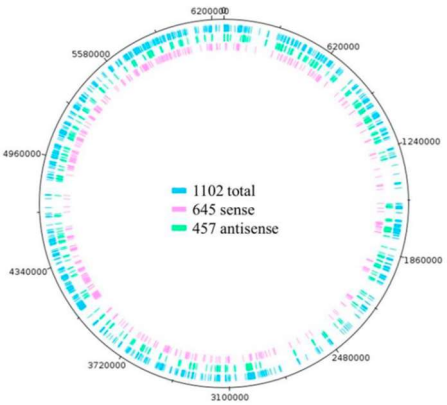
Joana Silva [1], Cecília Maria Arraiano [1], Vânia Pobre [1(*)]
[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

(*) e-mail: vaniapobre@itqb.unl.pt

Keywords: Small non-coding RNA, Antibiotic resistance, Biofilms, RNA-Seq, Bioinformatics

ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen that infects immunocompromised patients, particularly cystic fibrosis patients. When untreated, the infections result in lung failure and ultimately death. *P. aeruginosa* is very difficult to eradicate due to its strong resistance to several classes of antibiotics, as well as its capability to develop into a multi-drug resistant (MDR) bacterium and form biofilms. Consequently, it is crucial to develop alternative strategies to combat antibiotic resistance, such as small non-coding RNA (sRNA)-based therapies. These molecules play a crucial role in gene expression and have been shown to modulate antibiotic resistance and sensitivity in *P. aeruginosa*. Nonetheless, there is limited knowledge regarding sRNAs in this Gram-negative bacterium. In this work we predicted novel sRNAs using RNA-seq data from wild type PAO1 planktonic cells and biofilms grown with sub-lethal concentrations of four commonly used antibiotic classes: aminoglycosides (kanamycin), beta-lactams (ceftazidime), quinolones (nalidixic acid), and polymyxins (polymyxin B). As a control we grown PAO1 without any antibiotics. Using two bioinformatic tools (Artemis and Rockhopper), we successfully identified regions exhibiting significant expression of potential sRNAs, resulting in the prediction of 1102 novel sRNAs in at least one condition. Of these 809 were classified as potential cis-encoded sRNA, 268 were not clearly defined and only 25 were easily classified as trans-encoded. Moreover, we found that there is a significant difference in expression of majority of these sRNAs when comparing planktonic with biofilm growth conditions. However, the differences in expression are less pronounced when comparing the different antibiotics used in this study. Furthermore, we were able to experimentally validate some of these novel sRNAs through northern blots. Overall, this work considerably expanded our knowledge of *P. aeruginosa* sRNAs especially in the context of antibiotic resistance mechanisms and biofilm formation.



P4.69 - DISCLOSING AZOLE RESISTANCE MECHANISMS IN *CANDIDA GLABRATA* CLINICAL STRAINS THROUGH OMICS ANALYSES: BEYOND CGPDR1

Maria Joana Pinheiro [1,2], ***Sara B. Salazar*** [1,2], ***Carolina Soares*** [1,2], ***Vitória Rodrigues*** [3], ***Fábio Fernandes*** [1,2], ***Nuno P. Mira*** [1,2(*)]

[1] *iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico—Department of Bioengineering, Universidade de Lisboa, Lisboa, Portugal*

[2] *Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*

[3] *Seção de Microbiologia, Laboratório SYNLAB—Lisboa, Grupo SYNLAB Portugal, Lisboa, Portugal*

[(*)] *e-mail: nuno.mira@tecnico.ulisboa.pt*

Keywords: CgPdr1; CgPdr1-dependent and independent azole-resistance; azole resistance; *Candida glabrata.*

ABSTRACT

The pathogenic yeast *Candida glabrata* has the ability to rapidly acquire resistance to azoles, a trait that reduces the efficacy of antifungal treatments and, consequently, increases mortality rates in infected patients. A lot of knowledge has been gathered on the mechanisms by which *C. glabrata* acquires resistance to azoles *in vitro* but much less is known the extent at which these mechanisms underlie the resistance phenotype of resistant clinical strains. To tackle this issue our laboratory has been investigating clinical *C. glabrata* strains resistant to antifungals, in particular, to fluconazole and voriconazole. The vast majority of these resistant strains were found to encode hyperactive variants of the CgPdr1 transcriptional regulator, a central mediator of azole resistance in *C. glabrata*. Notably, we identified for the first time the I392M, E555K, G558C, I803T, K274Q, L332F and I616F substitutions as new gain-of-function CgPdr1 variants, enlarging the already big set of modifications described to result in hyperactivation of this protein. We have also performed comparative genomic analyses of these azole-resistant strains encoding different CgPdr1 variants and found that they encode varying alleles of several other genes implicated in azole resistance. Here, we will discuss the influence of these other azole-resistance genes in the resistance phenotype of the strains. Among our cohort of azole-resistant *C. glabrata* strains we identified three that encoded a wild-type *CgPDR1* allele, suggesting that CgPdr1-independent pathways mediated their acquisition of resistance. Results from comparative genomic and transcriptomic analyses suggest an involvement of genes mediating transport of sterol intermediates, adhesion and structuring of the cell wall, in that resistance phenotype. On the overall the results presented in this work pave the way for the understanding of the acquisition of resistance to azoles *in vivo* while also fostering the development of new diagnostic tools to rapidly detect strains encoding CgPdr1 hyper-active alleles.

Acknowledgements:
This study was supported by funds received from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project supporting the Research Unit Institute for Bioengineering and Biosciences (UIDB/04565/2020) and the project supporting Institute for Health and Bioeconomy, i4HB (LA/P/0140/2020). FCT is also acknowledged for funding a PhD grant (UI/BD/151180/2021) to Maria Joana Pinheiro.

P4.70 - PRELIMINARY STUDY OF PERMEABILITY IN ENTEROCOCCI OBTAINED FROM A VETERINARY BIOLOGICAL ISOLATION AND CONTAINMENT UNIT

Catarina Geraldes [1,2(*)], ***Sophie Burchael*** [3], ***Luís Tavares*** [1,2], ***Solange Gil*** [1,2,4], ***Marta Martins*** [3], ***Manuela Oliveira*** [1,2,5]

[1] *CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal*

[2] *Associate Laboratory for Animal and Veterinary Science (AL4AnimalS)*

[3] *Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Dublin, Ireland*

[4] *Biological Isolation and Containment Unit (BICU), Veterinary Hospital, Faculty of Veterinary Medicine, University of Lisbon, Portugal*

[5] *cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Portugal*

[(*)] *e-mail: cgeraldes@fmv.ulisboa.pt*

Keywords: *Enterococcus*, Efflux Pumps, Veterinary, Veterinary Biological and Isolation Units.

ABSTRACT

Enterococci are known for their resistance to many antimicrobials being associated with Hospital-Acquired Infections (HAIs). One of the several mechanisms responsible for antimicrobial resistance is lower permeability or increased efflux activity. Therefore, assessment of permeability and/or efflux in these bacteria is of utmost importance, especially in the hospital environment.

In this study, 33 *Enterococcus spp.* isolates (26 *E. faecium*, 4 *E. hirae* and 3 *E. faecalis,* identified through PCR), collected from a veterinary biological and isolation unit, were assessed for their permeability and potential efflux activity using an ethidium-bromide (EtBr) cartwheel assay[1]. Bacteria were inoculated onto Lysogeny Broth (LB) for 18 hours, diluted with PBS to achieve an optical density (600 nm) of 0.08-0.1, and streaked onto three different LB-agar plates with a cotton swab: one with no EtBr supplementation (negative control), one with a final concentration of EtBr of 0.5 µg/mL and another with a final concentration of EtBr of 2.5 µg/mL. Plates were then incubated for 18 hours at 37ºC, and then observed for fluorescence (ImageQuant™Las4000 imager).

It was possible to observe that 19 enterococci exhibited fluorescence and were consequently considered positive in 0.5 µg/mL plates while 22 isolates were positive in 2.5 µg/mL plates. The three bacteria that did not exhibit fluorescence at 0.5 µg/mL EtBr but did present it at 2.5 µg/mL may have a lower permeability or greater efflux capacity. On the latter, this can mean that bacteria are able to extrude the intracellular EtBr, when it is present in lower concentrations. Further studies are still needed to fully characterize efflux in these bacteria.

Assessment of permeability and/or efflux in bacteria collected from the hospital environment is important, considering that a higher efflux capacity cloud be associated with a potential decrease in susceptibility to many antimicrobials, leading to a higher number of complicated HAIs.

References:

1. Martins et al. Identification of Efflux Pump-mediated Multidrug-resistant Bacteria by the Ethidium Bromide-

agar Cartwheel Method. In Vivo, March 2011, 25 (2) 171-178.

Acknowledgements:
This research was funded by CIISA—Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, Project UIDB/00276/2020; and by Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), LA/P/0059/2020–AL4AnimalS (funded by FCT). Research in the Martins lab is supported by the Welcome Trust Strategic Fund, Trinity College Dublin, and the Irish Research Council (IRC), Dublin, Ireland.

P4.71 - DRUG REPURPOSING FOR IDENTIFICATION OF NEW EFFLUX INHIBITORS AND/OR ANTIBIOFILM AGENTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*

Joana Neves [1], Carolina Antunes [1], Mariana Andrade [1], Maria Bento [1], Isabel Couto [1], Liliana Rodrigues [1], Sofia Santos Costa [1(*)]

[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal

(*) e-mail: scosta@ihmt.unl.pt

Keywords: antimicrobial resistance; *Staphylococcus*; drug repurposing.

ABSTRACT

Staphylococcus aureus (SA) and *Staphylococcus epidermidis* (SE) are frequent agents of nosocomial infections. Staphylococcal efflux-mediated resistance and biofilm formation may render such infections resilient to antibiotherapy.

In this work, we used an *in silico* drug repurposing strategy to identify drugs targeting efflux and/or biofilm formation to be assessed, *in vitro*, for their efflux inhibitory and/or antibiofilm activities.

A list of potential targets, comprising all SA and SE membrane transporters and biofilm-associated proteins, was used to interrogate the DrugBank database and compile a list of drugs targeting homologues of these proteins. A subset of candidate drugs was tested in reference and isogenic strains differing in the expression of *norA*, encoding the NorA efflux pump. Candidate drugs (at ¼ their MIC) were assessed for their ability to inhibit efflux, through reduction of MICs of effluxable antimicrobials and ethidium bromide accumulation fluorometry. Drugs showing significant effect [≥ four-fold MIC reduction and/or a Relative Final Fluorescence value (RFF) ≥ 1] were tested for their potential to inhibit biofilm formation using the crystal violet adhesion method.

We identified over 200 drugs that potentially target SA and/or SE membrane transporters or biofilm-associated proteins. From these, we screened 56 drugs, which showed high MICs (>64 mg/L) against the evaluated strains. Nearly 25% of the candidate drugs significantly decreased the MICs of NorA substrates in *norA*-overexpressing strains and showed an RFF ≥ 1, indicative of efflux inhibitory activity. Additionally, eight drugs, including amlodipine and desipramine, diminished biofilm formation in SA and/or SE.

This study reveals approved drugs with dual targets that, in the future, may be included in the fight against antimicrobial-resistant SA and SE infections.

Acknowledgements:
Fundação para a Ciência e a Tecnologia (FCT, Portugal) through funds to Project DREBI, Ref.2022.07931.PTDC, GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020), BI grant 2022.07931.PTDC (JN) and grant UI/BD/154472/2022 (MA).

P4.72 - ATOPIC DERMATITIS MICROENVIRONMENT FAVOURS *STAPHYLOCOCCUS AUREUS* GROWTH AND PROMOTES ANTIBIOTIC RESISTANCE

Diana Caieiro [1], Nuno Faria [1], Mariana Araújo [1], Joanna Nakonieczna [2], Rita Sobral [3], Maria Miragaia [1(*)]

[1] Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB, Portugal

[2] Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Laboratory of Photobiology & Molecular Diagnostics, Gdansk, Poland

[3] Laboratory of Molecular Microbiology of Bacterial Pathogens, FCT, Portugal

[(*)] e-mail: miragaia@itqb.unl.pt

Keywords: Staphylococcus aureus; atopic dermatitis microenvironment; growth rate; biofilm production ability; antibiotic resistance.

ABSTRACT

Staphylococcus aureus is commonly isolated from severe lesions of atopic dermatitis (AD). Since the molecular basis of this link is still unknown, we aimed to understand the effect of AD host-related factors on *S. aureus* growth, pathogenicity, and antibiotic resistance.

S. aureus isolates from AD patients (nasal colonization, n=5; AD lesion, n=6) in Poland between 2014-2015 were studied. Nose/AD lesion pairs from the same patient were compared for their growth rate and biofilm production in conditions mimicking normal skin and AD lesion. We tested the impact of biotic (interleukin-4, progesterone, and beta-estradiol) and abiotic (pH and salinity) factors at concentrations typical of normal skin and AD lesions. Antimicrobial susceptibility was tested by disk diffusion in standard and AD-specific pH conditions.

The impact of biotic factors on growth rate and biofilm production was dependent on the *S. aureus* isolate and independent of its source (nose vs AD lesion). The high pH and low salinity promoted an increase in *S. aureus* growth (pH: 0.07-0.69 times NaCl: 9-192 times) and biofilm production (pH: 0.16-2 times, NaCl: 17-739 times). When analysing the influence of pH, NaCl and IL-4 simultaneously, we found that all AD isolates exhibited between 2-8 times higher growth rate than their nose counterpart under AD conditions, when compared to healthy skin-conditions. In standard conditions, most AD isolates exhibited an inhibition halo up to 20% smaller than the corresponding nose isolate. At AD lesion pH (8.4), we observed a decrease in susceptibility to 10 antibiotics, including fusidic acid.

AD microenvironment favoured *S. aureus* growth and promoted resistance to antibiotics, commonly used in AD treatment. *S. aureus* isolates from nasal colonization and AD lesions from the same patient had distinct phenotypic and metabolic features, suggesting adaptation to the AD lesions microenvironment.

Acknowledgements:
Funding by MOSTMICRO Research Unit.

P4.73 - CROSS-SPECIES GENE EXPRESSION PROFILING OF THE RESISTANCE TO INFECTION BY *MYCOBACTERIUM ULCERANS*

João Fevereiro [1,2]***, Nuno Osório*** [1,2]***, Maria Peixoto*** [1,2]***, Ana Mendes*** [1,2]***, Laurent Marsollier*** [3]***, Estelle Marion*** [3]***, Alexandra Fraga*** [1,2]***, Jorge Pedrosa*** [1,2]

[1] *Instituto de Investigação em Ciências da Vida e Saúde (ICVS), Escola de Medicina, Universidade do Minho, Braga, Portugal*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[3] *Centre de Recherche en Cancérologie et Immunologie Intégrée Nantes Angers (CRCINA), Institut National de la Santé et de la Recherche Médicale (INSERM), Université Angers, Angers, France*

[(*)] *e-mail: d8529@med.uminho.pt*

ABSTRACT

Mycobacterium ulcerans, the causative agent of the skin necrotizing disease Buruli Ulcer, presents a unique challenge to the host amongst human-pathogenic mycobacteria. First, *M. ulcerans* has undergone the loss of many immunogenic proteins throughout its evolution, some of which might account for the complex host-pathogen interactions fostered by its congeners. Secondly, it acquired a potent cytotoxic lipidic toxin – mycolactone – that blocks the export of proteins from the ER and ultimately kills the host cells. Yet, some individuals never develop BU despite being exposed to the pathogen throughout their lives. Moreover, a small percentage of BU patients can actually recover from BU lesions without any intervention. To better understand these findings, our groups have previously characterized two models of resistant phenotypes to *M. ulcerans* infection in Hartley guinea pigs and FVB/N mice. Now, in a follow-up transcriptomics approach, we show that guinea pigs significantly modulate lipid-related pathways in the early-stage infection, while mice hinge on humoral-mediated mechanisms during recovery from *M. ulcerans* lesions. Furthermore, intersection of these results with those from a recent Genome-Wide Association Study with BU patients revealed an undisclosed importance of a cytochrome for the outcome of infection. The current study thus provides an original resource to allow more accurate pinpointing of novel targets and pathways relevant for the host resistance against BU.

P4.74 - TLR2-DEPENDENT ACTIVATION OF MACROPHAGES DURING *MYCOBACTERIUM ULCERANS* INFECTION

Juan Dominguez [1,2&], ***Teresa Martins*** [1,2&], ***Bruna Ribeiro*** [1,2], ***Gabriela Trigo*** [1,2], ***José Gama*** [1,2], ***António Castro*** [1,2], ***Jorge Pedrosa*** [1,2], ***Alexandra Fraga*** [1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail: d8529@med.uminho.pt*

ABSTRACT

Mycobacterium ulcerans is the causative agent of Buruli ulcer (BU), a necrotizing disease of the skin, subcutaneous tissue and bone. *M. ulcerans* produces mycolactone, a macrolide exotoxin with cytotoxic and immunosuppressive properties, that can modulate macrophage antimicrobial activity. It has been shown in other mycobacterial infections that Toll-like receptor (TLR) recognition of pathogen components triggers the first mechanisms of macrophage activation.

Herein, we characterized the TLR2-dependent activation of macrophages during *M. ulcerans* infection. Infection of wild-type (WT) bone-marrow derived macrophages (BMDM) with mycolactone-producing or mycolactone-deficient strains of *M. ulcerans* resulted in an increase in the expression of TLR2, which was accompanied by an increase in the expression and protein levels of specific pro-inflammatory and anti-inflammatory cytokines. In the absence of TLR2, this inflammatory response was severely hampered, suggesting that TLR2 is highly involved in the recognition *M. ulcerans*, regardless the production of mycolactone. Importantly, the addition of purified mycolactone to mycolactone-negative infected-macrophages did not significantly impair TLR recognition of *M. ulcerans*. The contribution of TLR2 in *M. ulcerans* recognition was further corroborated through the activation of mitogen-activated protein kinases (MAPKs), particularly the extracellular signal-regulated protein kinase (ERK) and the p38 MAP kinase, in WT, but not TLR2KO, BMDM infected with *M. ulcerans*. Moreover, subcutaneous infection of the footpad of TLR2KO mice resulted in a worse outcome, with increased macroscopic pathology and bacterial load, when compared to WT mice.

Collectively, these results suggest that TLR2 is critical for the development of an innate inflammatory response against *M. ulcerans* and TLR2-*M. ulcerans* recognition ultimately impacts susceptibility/resistance to BU.

P4.75 - CHLORHEXIDINE AND BENZALKONIUM CHLORIDE ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM SKIN AND SOFT TISSUE INFECTIONS IN COMPANION ANIMALS

Carolina Ferreira [1(*)&], Catarina Morais [1(**)&], Paula Zacharias [1], Patrícia Abrantes [1], Constança Pomba [2,3], Sofia Santos Costa [1], Isabel Couto [1]

[1] *Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal*

[2] *Laboratory of Antibiotic Resistance, CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*

[3] *GeneVet, Laboratório de Diagnóstico Molecular Veterinário, Lisbon, Portugal*

[&] *These authors contributed equally to this work*

[(*)] *e-mail: carolinaf@ihmt.unl.pt;* [(**)] *e-mail: a21000758@ihmt.unl.pt*

ABSTRACT

Staphylococcus pseudintermedius and *Staphylococcus aureus* are important agents of skin and soft tissue infections (SSTIs) in companion animals. Antiseptics chlorhexidine (CHX) and benzalkonium chloride (BAC) are commonly used for the treatment of these infections.

This study analyzed the effectiveness of CHX and BAC against *S. pseudintermedius* and *S. aureus* causing SSTIs in companion animals.

CHX time-kill assays were performed according to the European Standard norm EN 1040[1] for reference strains *S. aureus* ATCC25923, *S. pseudintermedius* DSM21284 and for methicillin-resistant, multidrug-resistant clinical strains of *S. aureus* and *S. pseudintermedius*, corresponding to relevant clonal lineages previously identified in our studies; ST22/ST105 (*S. aureus*), ST71/ST118 (*S. pseudintermedius*). BAC time-kill assays were performed for *S. aureus* only. Assays were performed at 38□C (dog skin temperature). Antiseptics were tested in concentrations ranging from ½ MIC to the in- use concentration at different exposure times (1 min to 24h), including the recommended exposure times (5/10 min).

All biocides exhibited bactericidal activity at their in-use concentration. However, at lower concentrations, bacterial growth was still observed at the recommended exposure time (5/10 min). For some *S. aureus* and *S. pseudintermedius* clinical strains, no significant bactericidal effect was detected after 1h of exposure and/or bacterial growth was still observed at lethal concentrations (MIC) after 24h of exposure to CHX.

These results suggest that inappropriate use of antiseptics (e.g., insufficient rinsing) could potentially select for strains with reduced susceptibility towards these antiseptics, particularly CHX, as well as to antibiotics that share the same resistance mechanisms, promoting AMR dissemination in these relevant bacterial pathogens.

References:

1. Association Française de Normalisation. NF EN 1040 – Essai quantitative de suspension pour l'évaluation de l'activité bactericidé des antiseptiques et des désinfectants chimiques. 2006. AFNOR, La Plaine, France.

Acknowledgements:
Project BIOSAFE funded by FEDER through COMPETE and Fundação para a Ciência e a Tecnologia (FCT, Portugal), Grant LISBOA-01-0145-FEDER-030713, PTDC/CAL- EST/30713/2017. Further support by FCT to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020) and through grants 2021.05063.BD and UI/BD/151061/2021 awarded to CF and CM, respectively.

P4.76 - BIOFILM AND MULTIDRUG-RESISTANT BACTERIA: A STUDY IN BLACK-AND-WHITE RUFFED LEMUR

Carolina Silva [1,2(*)], ***João F. Requicha*** [1,2], ***José J. Martins*** [2,3,4], ***Isabel R. Dias*** [1,2,5], ***Carlos A. Viegas*** [1,2,5], ***Aida Duarte*** [6,7], ***Maria J. Saavedra*** [1,2,4,5]

[1] *Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal*

[2] *Animal and Veterinary Research Center (CECAV) - AL4AnimalS, UTAD, Vila Real, Portugal*

[3] *Department of Animal Science, UTAD, Vila Real, Portugal*

[4] *Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Matosinhos, Portugal*

[5] *Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB) - Inov4Agro, UTAD, Vila Real, Portugal*

[6] *Faculty of Pharmacy, Universidade de Lisboa, 1640-042 Lisboa, Portugal*

[7] *Egas Moniz Center for Interdisciplinary Research (CiiEM); Egas Moniz School of Health & Science, Caparica, Almada, Portugal*

[(*)] *e-mail: carolinasilva95@hotmail.com*

Keywords: oral biofilm; multidrug-resistant bacteria; lemurs; antibiotics.

ABSTRACT

Biofilms are obiquitous and have gained prominence in medicine. Characterised by being a dynamic biological system of microbial cells embedded in an organic polymeric matrix of microbial origin, biofilms deserve special attention when it comes to the problem of antibiotic resistance. In addition, there is increasing evidence that wild animals are carriers of multi-resistant bacteria. The aim of this study was to evaluate the biofilm formation and antibiotic susceptibility profile of Gram-negative bacterial isolates from the oral cavity of captive lemurs in a zoo in Portugal.

Eight black and white ruffed lemurs (*Varecia variegata*) were studied. Sterile oral swabs were taken from each animal and spread on Chromocult Coliform and MacConkey agars. Twenty-two isolates were selected to susceptibility tests for twenty-five antibiotics belonging to seven different classes: Beta-lactams, Aminoglycosides, Quinolones, Macrolides, Chloramphenicol, Fosfomycin, Sulfamethoxazole/trimethoprim. Biofilm formation assay was performed by Stepanović method, and the biomass was quantified by crystal violet, according to Simões and collaborators.

All tested isolates showed resistance to, at least, one antibiotic and 50% (n=11/22) were resistant to all antibiotic classes. Of the 11 isolates from different lemurs, six were able to form biofilms, although with different kinetics. Two isolates (RL3 and RL8) produced highest biomass at both sampling times, 24 and 48 hours.

Biofilms may physically protect bacteria from antimicrobial exposure when compared to planktonic forms, which generate biofilm-forming bacteria more resistant to antibiotics. Although the antibiotic susceptibility of biofilm-forming bacteria was not investigated, these results highlight the importance of these multi-resistant bacteria's ability to form biofilm.

Most often human infections occur through by contact with environmental reservoirs. The ability to acquire antibiotic resistance and to form biofilms allows bacteria to persist in the environment for long periods and to be transmitted to multiple hosts.

Acknowledgements:
This work was funded by national funds through the Portuguese Foundation for Science and Technology (FCT) under the projects UIDB/AGR/04033/2020, UIDB/CVT/00772/2020, AL4AnimalS and Inov4Agro; and under the Scientific Employment Stimulus—Institutional Call—CEECINS/00127/2018 (J.F. Requicha).

Ruffed Lemurs	24 h	48 h
RL1 (1/6)	+	++
RL2 (1/2)	+	++
RL3 (1/2)	++	+++
RL5 (1/7)	+	++
RL7 (1/4)	+	+
RL8 (1/1)	++	+++

P4.77 - *STREPTOCOCCUS PNEUMONIAE* CARRIAGE AND SEROTYPE DISTRIBUTION IN SALIVA SAMPLES FROM PRE-SCHOOL CHILDREN

Sónia T. Almeida [1(*)], ***Alexandra S. Simões*** [1], ***Sara Handem*** [1], ***António Brito-Avô*** [2], ***Hermínia de Lencastre*** [3,4], ***Raquel Sá-Leão*** [1]

[1] *Laboratory of Molecular Microbiology of Human Pathogens (LMMHP). Instituto de Tecnologia Química e Biológica António Xavier. Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal*
[2] *Clinical Associate, MostMicro Unit. ITQB NOVA, Oeiras, Portugal*
[3] *Laboratory of Molecular Genetics. ITQB NOVA, Oeiras, Portugal*
[4] *Laboratory of Microbiology and Infectious Diseases. The Rockfeller University, New York, USA*

(*) *e-mail: soniaalmeida@itqb.unl.pt*

Keywords: *Streptococcus pneumoniae*; carriage; saliva; children; qPCR.

ABSTRACT

Streptococcus pneumoniae is a leading cause of infectious disease worldwide, and a common colonizer of the human nasopharynx. Young children are often asymptomatically colonized, contributing significantly to the transmission in the community. In 2015, a 13-valent pneumococcal conjugate vaccine (PCV13, targeting 13 of 103 serotypes) was introduced in the Portuguese National Immunization Plan. We aimed to evaluate pneumococcal carriage and serotype distribution among young children.

A cross-sectional study was conducted in November-December 2022 among children 2-6 years old attending day-care centers in Oeiras. Saliva samples, vaccination data, and demographic/clinical data were obtained. Pneumococcal carriage was determined by qPCR targeting *lytA* and *piaB* genes; molecular serotyping targeting 41 serotypes/serogroups was also done by qPCR. Various criteria to screen for false positive serotyping results were used following current best-practise recommendations.

Pneumococci were detected in the saliva of 34.9% of 584 participants. Among the 204 pneumococcal positive samples, the most frequent serotypes/serogroups detected were 23A (10.8%), 15B/C (10.3%), 23B (9.8%), 11A/D (9.3%), 10A/B (8.8%), and 3 (7.8%). PCV13 serotypes were detected in 17.6% of all samples, of which serotype 3 was the most frequent (7.8%), followed by 19F (5.4%). Potential coverage of PCV15 and PCV20 were 22.5% and 48.5%, respectively. Children aged 4-6 years old were more likely to carry PCV13 serotypes than children 2-4 years old (9.6% vs 1.5%, $p<0.001$).

Saliva can be used to detect pneumococcal carriage among young children. Carriage rates, however, were lower than those typically obtained with nasopharyngeal samples. The combined use of saliva and molecular methods allows the detection of multiple serotype carriage. Vaccines with more valencies have the potential to target a more significant fraction of serotypes carried by children.

P4.78 - CHARACTERIZATION OF MOLECULAR FEATURES AND VIRULENCE PROFILE OF *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA OXYTOCA* ISOLATES FROM COMPANION ANIMALS IN PORTUGAL

Daniela Araújo [1(*)], ***Joana Castro*** [1,2], ***Ricardo Oliveira*** [1,3,4], ***Hugo Oliveira*** [2,5], ***Liliana Fernandes*** [2,5], ***Isabel Carvalho*** [2,5], ***Gyu-Sung Cho*** [6], ***Charles Franz*** [6], ***Carina Almeida*** [1,2,3,4], ***Sónia Silva*** [1,2,5]

[1] *INIAV, IP - National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Lugar da Madalena, 4485-655 Vairão, Portugal*
[2] *Centre of Biological Engineering, University of Minho, Braga, Portugal*
[3] *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal*
[4] *ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*
[5] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*
[6] *Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany*

[(*)] *e-mail: daniela.araujo@iniav.pt*

Keywords: *Klebsiella spp.*; companion animals; K-locus; genome characterization; virulence; *Galleria mellonella*

ABSTRACT

Klebsiella spp. are important pathogens that affect both humans and animals and can cause serious life-threatening diseases. The increasing incidence of *Klebsiella* infections in companion animals (*e.g.,* cats and dogs) can result in the death of animals and become a serious public health concern. The study of strains isolated from animal infections can be a means of assessing the risk of transmission to humans, including zoonotic potential.

The aim of this study was to characterize the genetic and phenotypic features of *Klebsiella pneumoniae* and *Klebsiella oxytoca* previously isolated from ill companion animals by whole genome sequencing, followed by *in vitro* evaluation of biofilm formation. The *Galleria mellonella* model was also used to evaluate the *in vivo* pathogenicity of *Klebsiella* isolates.

K. pneumoniae isolates tested exhibited two LPS O-types (O3B and O1/O2v2) and only one LPS O-types was detected for *K. oxytoca* isolates (OL104). Among the STs, ST11 and ST266 were the most frequently found. In turn, *K. pneumoniae* showed a high diversity of K-locus types (KL102; KL105; KL31, and KL13). Among *K. pneumoniae*, a specific pattern (*i.e.,* KL105-ST11-O1/O2v2) raises concern due to its high resistance and virulence towards human hosts. Furthermore, this pattern was associated with a high inflammatory response observed in *G. mellonella* larvae, with approximately 80% of the larvae dead at 72 h post-infection, which is not directly related to the ability of *Klebsiella spp.* to form a biofilm.

The present study highlights a noteworthy level of pathogenicity associated with *Klebsiella* spp. isolated from companion animals. Consequently, it underscores the potential for dogs and cats to serve as reservoirs of resistant *Klebsiella* spp. that could pose a risk of transmission to humans.

Acknowledgements:
This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC). In addition, this study was also funded by FCT (Portuguese Foundation for Science and Technology), under the scope of the strategic funding of UIDB/04469/2020 unit, LABELS—Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems—LA/P/0029/2020 (CEB). J.C. also thanks FCT for the CEEC Individual (2022.06886.CEECIND).

P4.79 - GENOMIC AND PHENOTYPIC PROFILING OF MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* AND *KLEBSIELLA VARIICOLA* ISOLATED FROM NORTHERN PORTUGUESE HOSPITALS

Joana Castro [1,2(*)], ***Daniela Araújo*** [1], ***Hugo Oliveira*** [2,3], ***Liliana Fernandes*** [2,3], ***Ricardo Oliveira*** [1,4,5], ***Gyu-Sung Cho*** [6], ***Charles Franz*** [6], ***Maria José Saavedra*** [7,8,9], ***Sónia Silva*** [1,2,3], ***Carina Almeida*** [1,2,4,5]

[1] *INIAV, IP - National Institute for Agrarian and Veterinary Research, Vairao, Portugal*
[2] *Centre of Biological Engineering, University of Minho, Braga, Portugal*
[3] *LABBELS—Associate Laboratory, Braga, Portugal*
[4] *LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal*
[5] *ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*
[6] *Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany*
[7] *Department of Veterinary Sciences, Laboratory Medical Microbiology, Antimicrobials, Biocides and Biofilms Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[8] *CITAB—Center for the Research and Technology Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[9] *Inov4Agro—Associate Laboratory for Innovation, Capacity Building and Sustainability in Agri-Food Production, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[(*)] *e-mail: joana.castro@iniav.pt*

Keywords: *Klebsiella variicola*; *K. pneumoniae*; virulence; biofilm; antimicrobial resistance.

ABSTRACT

With the alarming increase in antibiotic resistance and the global spread of resistance mechanisms, the emergence of antibiotic-resistant *Klebsiella* spp. has become a significant challenge in clinical settings. It is therefore important to monitor the characteristics of *Klebsiella* isolates in circulation to develop mitigation solutions.

The aim of this study was to characterize the genetic and phenotypic characteristics of 21 Multidrug-Resistant (MDR) *Klebsiella* isolates, including 19 *K. pneumoniae*, and 2 *K. variicola* isolated from patients admitted to two central hospitals in northern Portugal using whole-genome sequencing (WGS) and subsequent bioinformatic analysis of several virulence factors, including capsule types (K-types), LPS O antigen serotype and sequence types (ST). Furthermore, we assessed the *in vitro* biofilm-forming capacity and the hypermucoviscosity of *Klebsiella* isolates, as well as the ability of *Klebsiella* spp. to infect *Galleria mellonella*, a larval *in vivo* model.

Genetic results showed a high prevalence of O1/O2 serotypes (14/21; 67%) among the isolates tested, which is consistent with previous reports from Portugal. In contrast, a wide variety of K locus types was found, in our study, where ST15-KL19 (4/21; 19%) associated to serotype O1/O2v2 was the dominant. Within the O1/O2v2 serotype, a ST15-KL23 *K. variicola*, was the one harbouring a high number of virulence genes. We also found statistical differences in the ability to produce biofilm biomass within the strains, with a ST280-KL23 *K. pneumoniae* outcompeting with nine other strains.

According to our results, the most prominent serotype able to cause the death of *G. mellonella* was the KL105-O1/O2v2.

In conclusion, our results emphasize the importance of conducting continuous molecular surveillance in order to identify the key molecular features to be considered in the development of novel strategies for the treatment of *Klebsiella* spp.-associated infections.

Acknowledgements:
This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC). In addition, this study was also funded by FCT (Portuguese Foundation for Science and Technology), under the scope of the strategic funding of UIDB/04469/2020 unit, LABELS—Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems—LA/P/0029/2020 (CEB), Inov4Agro-Associate Laboratory for Inovation, Building and Sustainability in Agri-Food Production—LA/P/0126/2020, and projects UIDB/04033/2020 and UIDP/04033/2020 (CITAB). J.C. also thanks FCT for the CEEC Individual (2022.06886.CEECIND).

P4.80 - LOW-COST SALIVA TESTS FOR SARS-COV-2 INFECTION CONTROL

Catarina Amaral [1(*)], ***Sofia M. da Silva*** [1], ***Diana Grilo*** [1], ***Wilson Antunes*** [2], ***Elin Moe*** [1], ***Américo Duarte*** [1], ***Luís M. P. Lima*** [1], ***Cláudia Luís*** [1], ***Inês Morais*** [1], ***Gonçalo Afonso*** [1], ***Nuno Faria*** [1], ***Inês Gomes*** [2], ***Ana Margarida Henriques*** [3], ***Miguel Fevereiro*** [3], ***Raquel Sá-Leão*** [1], ***Maria Miragaia*** [1], ***Mónica Serrano*** [1], ***Catarina Pimentel*** [1]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

[2] *Centro de Investigação da Academia Militar (CINAMIL), Unidade Militar Laboratorial de Defesa Biológica e Química (UMLDBQ), Lisboa, Portugal*

[3] *Instituto Nacional de Investigação Agrária e Veterinária,I.P., Laboratório de Virologia, Oeiras, Portugal*

[(*)] *e-mail: caamaral@itqb.unl.pt*

Keywords: COVID-19, molecular diagnostics, Mass-screening, Infection control, LAMP, RT-PCR.

ABSTRACT

The gold standard of COVID-19 testing is real-time RT-PCR, which detects the genetic material of SARS-CoV-2 in nasopharyngeal (NP) samples with high specificity and sensitivity. However, RT-PCR diagnostics are complex and expensive, which hamper their application in mass screening scenarios. To enable large-scale implementation of COVID-19 testing, even in resource-limited settings, we have developed and implemented two low-cost saliva tests.

We initially set up an RT-LAMP colorimetric test for direct detection of SARS-CoV-2 in saliva samples from infected individuals. However, this colorimetric format presented certain disadvantages. Firstly, the acidity of the saliva samples could affect the test results. Secondly, identifying positive samples in a multi-well array using colorimetry proved to be challenging. To address these limitations, we developed a new pH-independent method for colorimetric identification of infected saliva samples. We also implemented a fluorescent assay, allowing for rapid and real-time analysis of a large number of samples.

While RNA extraction from saliva samples significantly enhanced the sensitivity of our RT- LAMP test, it also introduces additional costs and time, thereby partially restricting its utility in mass screening scenarios. This prompted us to assess whether an inexpensive RT-PCR test (based on the intercalating probe SYBR Green) could reliably be used to detect SARS-CoV-2 in saliva samples, without the need for prior RNA extraction.

The RT-PCR SYBR Green test demonstrated excellent analytical sensitivity and specificity. To assess its suitability for mass screening, we made it available to students from public schools in Oeiras. In a partnership between ITQB NOVA, the Municipality of Oeiras and local schools, we conducted testing on 4445 students within just one month. Among them, we identified 80 asymptomatic children whose saliva samples tested positive for SARS-CoV-2 infection. Subsequent gold standard testing with NP sampling confirmed the infection in all students who had received a positive saliva test.

P4.81 - USE OF ANTISENSE OLIGOMERS FOR THE CONTROL OF TRANSCRIPTION FACTORS INVOLVED IN BIOFILM FORMATION OF *CANDIDA GLABRATA*

Daniela Araújo [1(*)], ***Tânia Ribeiro*** [2], ***Joana Castro*** [1,2], ***Mariana Henriques*** [2,3], ***Sónia Silva*** [1,2,3]
[1] *INIAV, IP - National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Lugar da Madalena, 4485-655 Vairão, Portugal*
[2] *Centre of Biological Engineering, University of Minho, Braga, Portugal*
[3] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail: daniela.araujo@iniav.pt*

Keywords: Candidiasis; nucleic acid mimics; therapeutic oligomers; adhesion; biofilm formation.

ABSTRACT

Candida glabrata infections are currently a major public health problem, with high rates of morbidity and mortality due to its strong ability to form biofilms and increasing levels of resistance to traditional antifungal therapies [1]. Therefore, new alternative therapies with novel mechanisms of action are urgently needed to reach the market, and antisense therapy (AST) may be the answer to this problem. AST uses synthetic oligomers to bind to mRNA and block the expression of the target gene [2]. This method has been used to treat non-infectious diseases, but AST-based applications to control *Candida* infections are scarce [3, 4]. Therefore, the aim of this work was to use antisense oligonucleotides (ASOs) to control the expression of three transcription factors (*EPA3*, *EPA6,* and *PDR1)* involved in the first step of *C. glabrata* biofilm formation, namely the adhesion phenomenon. The synthesized ASOs were chemically modified by LNA modification. The ASOs' ability to control the gene expression was assessed *in vitro* by RT-qPCR, and the individual and combined effect on *C. glabrata* adhesion reduction was assessed by quantification of the number of cultivable cells. The effect of ASOs was also analyzed using the *in vivo G. mellonella* model.

In vitro, all ASOs were able to reduce gene expression of their respective target, however, only anti-*EPA3* and anti-*EPA6,* at a concentration of 100 nM, were able to significantly reduce *C. glabrata* cell adhesion at pH 4. ASOs showed no *in vivo* toxicity in the *G. mellonella* model. Furthermore, and contrary to observe *in vitro* the anti-*PDR1* oligomer revealed to be the most promising ASO, increasing the survival of *G. mellonella* larvae infected with a sub-lethal dose of *C. glabrata* cells by 20%.

In summary, this work validates the applicability of antisense oligomers for the control of *C. glabrata* virulence determinants.

References:

[1] Lamoth, F.; Lockhart, S.R; Berkow, E.L.; Calandra, T. (2012) Changes in the epidemiological landscape of invasive candidiasis. *J Antimicrob Chemother.* 73, i4-i13
[2] Sully, E.K. and Geller, B.L. (2016) Antisense antimicrobial therapeutics. *Curr Opin Microbiol* 33, 47–55
[3] Araújo, D., Azevedo, N. M., Barbosa, A., Almeida, C., Rodrigues, M. E., Henriques, M., Silva, S. (2019) Application of 2'-OMethylRNA' antisense oligomer to control *Candida albicans EFG1* virulence determinant. *Mol Ther Nucleic Acids*, 18, 508-517
[4] Araújo, D.; Mil-Homens, D.; Henriques, M.; Silva, S. (2022) Anti-EFG1 2'-OMethylRNA oligomer inhibits Candida albicans filamentation and attenuates the candidiasis in Galleria mellonella. Molecular Therapy – Nucleic Acids, 27, 8, 517-523.

Acknowledgements: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechnaical Systems, LA/P/0029/2020.

P4.82 - DETECTING ENTEROTOXIGENIC *ESCHERICHIA COLI* IN ANIMAL PRODUCTION: METHOD DEVELOPMENT AND VALIDATION

Maria Margarida Barros [1,2], Ricardo Oliveira [1,3,4(*)], Joana Castro [1,5], Ana Campos [1], Gonçalo Nieto Almeida [1], Daniela Araújo [1], Sónia Silva [1,5,6], Divanildo Outor-Monteiro [2], Carina Almeida [1,3,4]

[1] INIAV, I.P - National Institute for Agrarian and Veterinarian Research, Portugal

[2] Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

[3] LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

[4] ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

[5] Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga, Portugal

[6] LABBELS –Associate Laboratory, Braga/Guimarães

[(*)] e-mail: ricardo.oliveira@iniav.pt

Keywords: ETEC; colibacillosis; toxins; fimbriae; PCR.

ABSTRACT

Swine enteric colibacillosis is a disease characterized by an intestinal infection caused by enterotoxigenic *Escherichia coli* (ETEC). This infection mostly causes illness or death in neonatal and weaned pigs making it responsible for significant economic losses. Bacterial fimbriae (F4/F5/F6/F18/F41) are responsible for the adhesion to epithelial cells, and when both the immunological systems and the gut microbiota are poorly developed, ETEC colonizes and produces one or more enterotoxins (LT/STa/STb/Stx2e) that can have local and systemic effects. Therefore, it is of prime importance to monitor and characterize ETEC in the swine industry to develop mitigation strategies.

In this study, our aim was to develop a methodology to detect ETEC and its major virulence factors (*i.e.,* toxins/fimbriae) from swine. Firstly, we optimized a qPCR methodology using ETEC control strains for the screening of ST/LT/stx2 toxins. Thus, the efficiency of primers, sensitivity, and specificity were determined. Also, the limit of detection was performed in artificially contaminated samples. This methodology is intended to be applied to the initial screening of enriched liquid cultures. Secondly, to better characterize the virulence features of ETEC isolates, we developed a multiplex PCR to specifically determine both the fimbriae (F4/F5/F6/F18/F41), and toxins (LT/Sta/STb/Stx2e). To validate our method, we collected rectal swabs from pigs. Our results showed that the qPCR approach can detect between 20-400 DNA copies/µL depending on the toxin tested and is also highly specific and sensitive. Furthermore, we also optimized the multiplex PCR technique using the ETEC control strains. Regarding its validation, our preliminary data with porcine rectal samples showed a high prevalence of the above-mentioned toxins (namely ST) as well as the F4 and F18 fimbriae.

In sum, this methodology has the potential to be adopted as a routine technique for the rapid detection of ETEC strains in livestock, since the method exhibit a robust performance.

Acknowledgements: This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). The authors are also grateful to FCT for the PhD Grant SFRH/BD/2023.04664.BDANA and for the CEEC Individual (2022.06886.CEECIND).

P4.83 - ASSESSING THE ABILITY OF *LACTOBACILLUS* STRAINS TO COUNTERACT ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) INFECTION BY USING A *GALLERIA MELLONELLA IN VIVO* MODEL

Daniela Araújo [1], Ana Rita Silva [1], Ana Oliveira [2,3], Nuno Pereira Mira [4,5], Sónia Silva [1,2,3], Carina Almeida [1,2,6,7], Joana Castro [1,2(*)]

[1] INIAV, IP - National Institute for Agrarian and Veterinary Research, Vairão, Portugal
[2] Centre of Biological Engineering, University of Minho, Braga, Portugal
[3] LABBELS—Associate Laboratory, Braga, Portugal
[4] Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Department of Bioengineering, University of Lisbon, Portugal
[5] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Portugal
[6] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal
[7] ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

(*) e-mail: joana.castro@iniav.pt

Keywords: Enterotoxigenic Escherichia coli (ETEC); probiotics; colibacilosis; *Galleria mellonella; infection.*

ABSTRACT

Enteric colibacillosis is a common disease in weanling pigs, with postweaning diarrhea (PWD) as the main symptom in piglets. It is caused by the colonization of the small intestine by enterotoxigenic strains of *Escherichia coli* (ETEC). Of the control strategies, antibiotics and zinc oxide have been the most effective in reducing the economic losses caused by PWD. However, concerns about antibiotic resistance have led to restrictions on the use of critically important antimicrobials in food-producing animals, and in June 2021 zinc oxide was banned in the European Union due to the environmental risks it poses. As a result, efforts are underway to develop more environmentally friendly alternatives to combat ETEC infections, such as probiotics.

In this study, we evaluated the ability of three potential probiotics (*Lactobacillus gasseri, L. acidophilus* and *L. reuteri*) to reduce the ETEC infection by using a *Galleria mellonella in vivo* model in 2 different perspectives: co-infection (i.e. *Lactobacillus* + ETEC); and prophylactic strategy (i.e. prior infection with *Lactobacillus* for 4 h followed by ETEC infection). Survival rate and health index scores of *G. mellonella* were assessed at 24, 48, and 72 h post-infection. In addition, real-time PCR was also performed to determine the transcript levels of genes encoding the *G. mellonella* antimicrobial peptides to infer the immune response to ETEC infection.

Our results suggest that a co-infection strategy was not effective in controlling ETEC infection. On the other hand, when a prophylactic strategy was used, we observed significant differences between the treated larvae and the control. Overall, we observed that *L. acidophilus* was able to reduce ETEC strain SP11 infection. Differences in the expression of antimicrobial peptides were also found when comparing treated and control conditions. In conclusion, specific *Lactobacillus* species seem to have the potential to protect against ETEC infection.

Acknowledgements:
This work was financially supported by the Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. It was also supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC); and under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). J.C. also thanks FCT for the CEEC Individual (2022.06886.CEECIND).

P4.84 - MARINE MICROALGAE MICROBIOME: A SOURCE OF POTENT ANTIFUNGAL COMPOUNDS

Teresa Pissarro [1(*)]***, Francisco Xavier Nascimento*** [2] ***and Catarina Pimentel*** [1]
[1] *Instituto de Tecnologia Química e Biológica António Xavier - ITQB NOVA, Oeiras, Portugal*
[2] *Instituto da Bilogia Experimental e Tecnológica - iBET, Oeiras, Portugal*

(*) *email: t.pissarro@itqb.unl.pt*

Keywords: Yeast; *Candida*; antifungals; marine bacteria.

ABSTRACT

Invasive fungal infections caused by the opportunistic yeast *Candida* spp. are generally associated with high rates of mortality and morbidity among hospitalized patients, imposing significant socioeconomic burdens. Advances in life-saving medical treatments, the limited repertoire of effective drugs, and the growing resistance to available antifungals have worsened this scenario, creating a substantial demand for new treatments.

The marine environment remains an untapped source of valuable medicines. In particular, the microbiome of marine organisms has triggered great interest because, if cultivable, it represents a sustainable and low-cost approach to developing effective drugs.

In this study, we evaluated the antifungal potential of a collection of bacteria isolated from the marine microalgae *Phaeodactylum tricornutum* and *Nannochloropsis oceanica*. Through agar diffusion assays, we were able detect antifungal activity of some bacteria against *Candida albicans*, the species responsible for more than 50% of all cases on invasive candidiasis. For one bacterium, we found that while conditioned medium - i.e., growth medium from overnight cultures after removal of bacteria by centrifugation and filtration - was not active against *C. albicans*, when the bacterium was co-cultured with *C. albicans*, the respective conditioned medium led to a significant reduction in *C. albicans* growth. This effect suggests the presence of a secreted compound and is unlikely to be attributed to nutrient exhaustion. In fact, conditioned medium from co-cultures of *C. albicans* with other marine bacteria that did not exhibit apparent activity against the yeast did not impact *C. albicans* growth.

We are currently investigating the nature of this compound.

P4.85 - EXPLORING THE ANTIMICROBIAL EFFECTS OF VARIOUS HONEY TYPES FOR THE MANAGEMENT OF DIABETIC FOOT INFECTIONS

Andréa Bezerra [1,2(*)]***, Maria José Alves*** [3,4]***, Maria José Saavedra*** [2]***, Hélder Fonseca*** [1]***, Francisca Rodrigues*** [5]***, Cristina Delerue-Matos*** [5]***, Irene Gouvinhas*** [2]***, Juliana Garcia*** [2,3]

[1] *CIAFEL, Faculdade de Desporto da Universidade do Porto, Rua Dr. Plácido Costa, Portugal.*

[2] *CITAB – Centre for the Research and Technology of Agro-Environment and Biological Sciences/ Inov4Agro - Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production, University of Trás-os-Montes e Alto Douro, Portugal.*

[3] *AquaValor – Centro de Valorização e Transferência de Tecnologia da Água – Associação, Portugal*

[4] *CIMO - Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Portugal.*

[5] *REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, Porto, Portugal.*

**e-mail: andreab@utad.pt*

Keywords: Multidrug-resistant bacteria; Diabetic foot ulcer; diabetic wounds; honey; biomaterial.

ABSTRACT

Diabetic foot ulcers (DFUs) pose a significant global healthcare challenge, exacerbated by the emergence of multidrug-resistant microorganisms (MRMs) [1]. Honey dressings, known for their antimicrobial properties, have demonstrated effectiveness in combatting these microorganisms and expediting wound healing [2]. However, a consensus has yet to be reached regarding the optimal honey characteristics for combating resistant bacteria. This study aims to investigate the efficacy of various types of honey against MRM biofilms. Multiple honey samples were sourced from the Trás-os-Montes region in Portugal, and their antimicrobial activity was evaluated through Minimum Inhibitory Concentration (MIC) assays. These honey samples were tested at three concentrations against biofilms formed by *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Candida albicans*. Results revealed that select honey samples exhibited substantial efficacy against bacteria, with a MIC of 1.68%, particularly effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Furthermore, certain honey samples significantly reduced biomass, eliminating nearly 78% in *Candida albicans* and approximately 60% in *Staphylococcus aureus* and *Escherichia coli* biofilms. Metabolic inactivation exceeding 85% was observed in biofilms of *Candida albicans, Klebsiella pneumoniae*, and *Staphylococcus aureus* at higher honey concentrations (10xMIC). Fluorescent microscopy using LiveDead staining assays indicated a prevalence of red-stained cells in honey-treated biofilms, suggesting a higher density of deceased microorganisms and reduced biofilm viability. Honey seems to be effective against MRMs and might be a promisor biomaterial for DFUs management.

References:

1. Al-Waili, N., et al., *Synergistic Effects of Honey and Propolis toward Drug Multi- Resistant Staphylococcus Aureus, Escherichia Coli and Candida Albicans Isolates in Single and Polymicrobial Cultures.* Int. J. Med. Sci., 2012. **9**(9): p. 793-800.

2. Moghazy, A.M., et al., *The clinical and cost effectiveness of bee honey dressing in the treatment of diabetic foot ulcers.* Diabetes Res. Clin. Pract., 2010. **89**(3): p. 276-281.

Acknowledgements:
Andréa Bezerra thanks to FCT (Fundação para a Ciência e Tecnologia) for funding through Honey+ project (MTS/SAS/0077/2020). Francisca Rodrigues thanks to FCT (Fundação para a Ciência e Tecnologia) for funding through the Scientific Employment Stimulus - Individual Call (CEECIND/01886/2020). Irene
Gouvinhas thanks to FCT (Fundação para a Ciência e Tecnologia) for funding through the Scientific Employment Stimulus - Individual Call (2022.00498.CEECIND). Juliana Garcia is grateful to the FCT for the projects titled "AquaValor—Centro de Valorização e Transferência de Tecnologia da Água" (NORTE-01- 0246-FEDER-000053) and "Emprego altamente qualificado nas empresas ou em COLABS—Contratação de Recursos Humanos Altamente Qualificados (PME ou CoLAB)" (NORTE-06-3559-FSE-000095), supported by the Norte Portugal Regional Operational Programme (NORTE 2020) under the PORTUGAL 2020 Partnership Agreement through the European Regional Development Fund (ERDF). Projects UIDB/AGR/04033/2020 (CITAB) and LA/00037/2022 (Inov4Agro) funded by the Portuguese Foundation for Science and Technology (FCT).

P4.86 - *STREPTOMYCES LUNALINHARESII* A54A, AN ENDOPHYTIC ACTINOBACTERIA FROM SÃO PAULO COAST-BRAZIL AS A POTENTIAL BIOCONTROL AGENT OF PHYTOPATHOGENIC BACTERIA

Jairo I. Quintana-Bulla [1], Gabriel Luiz Padoan Gonçalves [1], Roberto G. S. Berlinck [2], Simone Possedente de Lira [1(*)]

[1] Departamento de Ciências Exatas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil

[2] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, São Paulo, Brazil

[(*)] email: splira@usp.br

Keywords: secondary metabolites; biological control; phytopathogenic bacteria.

ABSTRACT

Phytopathogenic fungi and bacteria affect crop yield and quality, leading to annual crop losses. Additionally, increasingly strict regulations for synthetic chemical pesticides, demand the urgent discovery of new crop protection agents. Biological control with microorganisms and their natural products has become an efficient alternative for crop disease management. In this study, we report the evaluation of the endophytic actinobacteria *Streptomyces lunalinharesii* A54A as a biocontrol agent against the phytopathogenic bacteria *Xanthomonas citri* IBSBF 1421, *Xanthomonas citri* (copper resistant strain), *Xanthomonas campestris* pv. *campestris* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. The antibacterial activity of the crude extract was evaluated, showing inhibition rates ranging from 15.4-67.3% at 256 µg mL^{-1}. Reversed phase solid phase extraction (RP-SPE) fractionation of crude extract, revealed the active fractions even with increased antibacterial activity against some phytopathogenic bacteria tested. Furthermore, preliminary liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS) molecular networking and dereplication analysis, showed no previously reported compounds for the masses detected. These results suggest *S. lunalinharesii* A54A as a producer of new secondary metabolites that could be used as antibacterial agents, indicating its potential as a biocontrol agent against crop disease-causing bacteria.

Acknowledgements:
The authors thank the São Paulo Research Foundation (FAPESP) for financial support (grants nº 2019/17721-9 and 13/50228-8); J.I.Q.B thanks the Foundation for Agrarian Studies Luiz de Queiroz (FEALQ) for the postdoctoral scholarship (104517); the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES Print program) – Finance Code 001".

MICROBIOTEC 23

Poster Session

Topic 5

P5.1 - BIOCHEMICAL PORTRAIT OF THE INNER MEMBRANE CYTOCHROME CBCA: INSIGHTS INTO ITS ROLE IN EXTRACELLULAR ELECTRON TRANSFER

Jorge M. A. Antunes *, Carlos A. Salgueiro, Leonor Morgado

Associate Laboratory i4HB - Institute for Health and Bioeconomy
UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal

(*) e-mail: jma.antunes@campus.fct.unl.pt

Keywords: Geobacter, extracellular electron transfer, membrane associated cytochrome, protein-protein interactions, NMR

ABSTRACT

Extracellular electron transfer (EET) is a respiratory mechanism that allows electrogenic bacteria to sustain their growth by using exterior electron acceptors, including electrode surfaces. This process that involves the transfer of electrons through consecutive redox partners connecting the inner membrane to the cell exterior is a key metabolic feature that can be used in several biotechnological areas, namely bioremediation, bioenergy production and microbial electrosynthesis [1]. CbcBA from *Geobacter sulfurreducens* is an inner-membrane quinone oxidoreductase complex essential for electron transfer to extracellular electron acceptors with a low redox potential, as Fe(III) minerals or electrodes poised between –210 mV and –280 mV [2]. The complex is formed by CbcA, a cytochrome containing 7 *c*-type heme groups anchored to the membrane by a C-terminal α-helix, and CbcB, an integral membrane di-heme *b*-type cytochrome.

The CbcA periplasmic domain (37 kDa) was heterologously produced in *E. coli* and characterized by different spectroscopic techniques at structural and functional level. Circular Dichroism was used to determine its secondary structural elements and thermal stability. The reduction potential of the protein was measured by UV-visible potentiometric redox titrations. Finally, Nuclear Magnetic Resonance was used to determine the spin state of the heme groups and to probe biomolecular interactions with putative periplasmic redox partners. The results obtained contribute to the understanding of the complex networks for EET in *Geobacter*.

1. Kumar, R.; Singh, L.; Wahid, Z. A.; Din, M. F. M. Exoelectrogens in microbial fuel cells toward bioelectricity generation: a review. *Int. J. Energy Res.* **2015**, *39* (8), 1048–1067.

2. Joshi, K.; Chan, C. H.; Bond, D. R. *Geobacter sulfurreducens* inner membrane cytochrome CbcBA controls electron transfer and growth yield near the energetic limit of respiration. *Mol. Microbiol.* **2021**, 1–16.

Acknowledgements:
This work was supported by Fundação para a Ciência e a Tecnologia through grants 2022.11900.BD (JMAA), PTDC/BIA-BQM/4967/2020 (CAS), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB).

P5.2 - THE FLAVODIIRON PROTEIN OF *SHARPEA AZABUENSIS* HAS O_2 AND H_2O_2 REDUCTASE ACTIVITY

Diogo Cunha *, Susana F. Fernandes , Miguel Teixeira , Filipe Folgosa

ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

(*) e-mail: diogocunha801@gmail.com

Keywords: Oxygen reductase, Oxidative stress, Nitrosative stress

ABSTRACT

Flavodiiron Proteins (FDPs) are cytoplasmatic enzymes that contain a minimal core composed by a metallo-β-lactamase, harboring the catalytic diiron center, and a flavodoxin-like domain, with a flavin mononucleotide (FMN). The FDPs characterized so far can reduce either oxygen, nitric oxide or both, which confer many pathogens the ability to protect themselves against oxidative and nitrosative stress. [1], [2]

Here we present the biochemical and spectroscopic characterization of a class G flavodiiron protein from *Sharpea azabuensis*, which has, in addition to the core domains, a short-chain rubredoxin and a flavin reductase-like domain. [2]

The protein was successfully produced in *E. coli* and purified to homogeneity. The pure protein has a 68 kDa monomer and behaves as tetramer in solution. The UV-Visible spectral deconvolution is consistent with the presence of all protein's domains.

The O_2 reductase activity was determined amperometrically, obtaining a turnover rate of 26±2.9 s^{-1} and 13±2.5 s^{-1} using NADH and NADPH as electron donors, respectively. This indicates that NADH is the preferred electron donor for this flavodiiron protein. Having not detected an increase in oxygen concentration after catalase addition in the reaction chamber, the peroxidase activity was also determined. Using a concentration of

100 µM of H_2O_2, we have obtained turnover rates of 16±5.4 s^{-1} and 5±1.4 s^{-1}, respectively, using NADH and NADPH as electron donors.

References:

[1] M. C. Martins *et al*, Free Radic. Biol. Med., vol. 140, 36-40, 2019.

[2] F. Folgosa *et al*, FEMS Microbiol. Lett., vol. 365, 1-8, 2018.

P5.3 - *CANDIDA ALBICANS* 5'-3'-ECTONUCLEOTIDASE ACTIVITY: A COMPARATIVE STUDY OF ISOLATES FROM DIFFERENT HUMAN INFECTION NICHES

Mariana Afonso [1,2*], ***Bernardo Bandeira*** [1,2], ***Rodrigo A. Cunha*** [1,2], ***José Roberto Meyer-Fernandes*** [3], ***Teresa Gonçalves*** [1,2+], ***Lisa Rodrigues*** [1,2+]

[1] *CNC - Center for Neurosciences and Cell Biology, Coimbra, Portugal*
[2] *FMUC- Faculty of Medicine, University of Coimbra, Coimbra, Portugal*
[3] *Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[+] *These authors share senior authorship*

[*] *e-mail: marianaladeiro@gmail.com*

Keywords: *Candida albicans,* Human infection niches, Ectonucleotidases, Nucleotide metabolism

ABSTRACT

Fungi, such as *Candida albicans* (*Ca*), are commensals in human microbiota, but also important agents of opportunistic infections. The purinergic system, namely adenosine and ATP, operates important roles in immunity and inflammation homeostasis. Through ectophosphatases and ectonucleotidases activities, converting ATP into adenosine, this system is known to influence the infectious potential and survival strategies of several microbes. Previous work from our group showed that *Ca* is capable of hydrolyzing AMP into adenosine (a stop signal of inflammation), using this ectonucleotidase activity. Thus, this study aimed to characterize 5' and 3'-nucleotidase/nuclease (5'&3'NT/NU) enzymatic activity in clinical isolates of *Ca* obtained from different human infection niches. For that, 50 clinical isolates of *Ca* were selected from oral, bloodstream, vaginal, medical devices, and gut-associated infections. The 5'&3'NT/NU activities were quantified from Pi released at different pH values (4-8), using a well-established Fiske-Subbarow spectrophotometric method. *Ca* SC5314 was used as control. Regardless of isolation place, all the *Ca* isolates were endowed with 5'&3'NT/NU activity. The highest enzymatic activity was found at pH 4 for all niches, with three isolate clusters identified. Isolates from bloodstream, vaginal and medical devices niches showed similar 5'&3'NT/NU activities (mean±SEM: 5'AMP 383.124±38.718 nmol Pi $h^{-1}10^{-9}$ cells; 3'AMP 530.195±41.681 nmol Pi $h^{-1}10^{-9}$ cells). Variances were mostly seen in isolates from gut- (5'AMP 743.200±319.403 nmol Pi $h^{-1}10^{-9}$ cells; 3'AMP 720.633±333.579 nmol Pi $h^{-1}10^{-9}$ cells) and oral-associated infections (5'AMP 257.431±43.639 nmol Pi $h^{-1}10^{-9}$ cells; 3'AMP 321.905±46.148 nmol Pi $h^{-1}10^{-9}$ cells), corresponding, respectively, to the highest and the lowest enzymatic activities at pH 4.
In conclusion, although the nature and specificity of yeasts ectonucleotidases are not completely established, we highlight these enzymes importance in infection context, most probably helping yeasts to adapt to host defenses in different infection niches.

P5.4 - TWO NEW FLAVODIIRON PROTEINS TO FIGTH OXIDATIVE STRESS IN AN ANAEROBIC BACTERIUM?

Maria C. Martins [1]*, Filipe Folgosa [1] and Miguel Teixeira [1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

(*) e-mail: mcmartins@itqb.unl.pt

Keywords: oxidative stress, nitrosative stress, oxygen reductase, hydrogen peroxide reductase, [3Fe-4S] cluster

ABSTRACT

Flavodiiron proteins (FDPs) constitute a widespread family of metalloenzymes with a crucial role in O_2/ROS and/or NO detoxification, through the reduction of these species to H_2O or N_2O, respectively. All the FDPs is composed by a minimal catalytic unit with two domains: a metallo-β-lactamase-like domain, harboring the catalytic diiron site and a flavodoxin-like domain.[1,3] The majority of FDPs, already characterized, have only this core, but more complex arrangements were found.[1,2] Recently, we identified nine classes of FDPs, based on their domain's architecture.[1,2]
Two of the more complex classes (FDP_E and FDP_H) are encoded in the genome of the anaerobe *Syntrophomonas wolfei subsp*. *wolfei* str. Goettingen G311. Besides the core domains, FDP_E posses a third domain with an FeS cluster and FDP_H contains two short-chain rubredoxins and a NAD(P)H:rubredoxin oxidoreductase-like domain.[3]
This enzymes were produced and characterized and the presence of the predicted cofactors was investigated by a set of biochemical and spectroscopic methodologies.
The study of FDP_E allows the characterization of a new C-terminal domain in FDPs field, a FeS cluster with a $[3Fe4S]^{1+/0}$ center with structural and spectroscopic features similar to [3Fe4S] ferrodoxins. These results can contribute to the establishment of a new type of electron transfer chain in this family of enzymes.
The kinetic characterization of FDP_H shows a remarkable O_2 reduction activity with a k_{cat}= 52.0 ± 1.2 s^{-1} and a negligible NO reduction activity (~100 times lower than with O_2), with NADH as electron donor, i.e., it is an oxygen selective FDP. In addition, this enzyme showed the highest turnover value for H_2O_2 reduction (k_{cat}= 19.1 ± 2.2 s^{-1}) ever observed among FDPs, as well as of other diiron containing peroxidases.[4]

1. Martins MC,*et al*, (2019), FRBM, *140*, 36-60.
2. Folgosa F,*et al,* (2018), FEMS Micro Lett, *365*(3).
3. Sieber JR,*et al*,(2010), *Env Micro*,*12*(8), 2289–2301.
4. Martins MC, *et al*. 2023, submitted.

P5.5 - UNCOVERING THE SECRETS OF GEOBACTER URANIIREDUCENS: A STUDY OF KEY PLAYERS IN URANIUM BIOREMEDIATION PATHWAY

Alexandre Almeida *, Marta A. Silva and Carlos A. Salgueiro

Associate Lab i4HB – Institute for Health and Bioeconomy
UCIBIO – Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal

(*) e-mail: asa.almeida@campus.fct.unl.pt

Keywords: Geobacter, Bioremediation, Extracellular Electron Transfer, Multiheme Cytochromes

ABSTRACT

The remarkable respiratory versatility displayed by *Geobacter* bacteria has garnered significant interest. These microorganisms can couple the oxidation of a wide range of organic compounds with the reduction of diverse electron acceptors located outside their cells. This is accomplished through a mechanism known as extracellular electron transfer (EET). Studies on EET mechanisms in *Geobacter* species have primarily focused on *Geobacter sulfurreducens (Gs)* [1]. However, the hypothesis that other species might adopt different EET strategies is a subject of ongoing research. One environmentally relevant *Geobacter* strain, *Geobacter uraniireducens*, is part of these efforts. *Geobacter uraniireducens* shares numerous metabolic similarities with *Gs* and was originally isolated from subsurface sediments contaminated with uranium [2]. A distinctive hallmark of *Geobacter* bacteria is a conserved family formed by 4 to 6 triheme cytochromes. These families have been studied in *Gs* and *G. metallireducens* (*Gm*) and their thermodynamic characterization has revealed notable differences in the functional mechanisms of the cytochromes. In this work, we present the results obtained for the family of triheme cytochromes from *G. uraniireducens*. The data show that the *G. uraniireducens*' triheme cytochromes have unique properties compared to their homologs in *Gs* and *Gm* and start to reveal the secrets of the uranium reduction pathways, shedding light how strategies to bioremediate toxic contaminants in the environment can be explored.

[1] D. R. Lovley *et al.*, *Geobacter. The Microbe Electric's Physiology, Ecology, and Practical Applications*, 1st ed., vol. 59. Elsevier Ltd., 2011.

[2] E. S. Shelobolina, H. A. Vrionis, R. H. Findlay, and D. R. Lovley, "Geobacter uraniireducens sp. nov., isolated from subsurface sediment undergoing uranium bioremediation," *Int. J. Syst. Evol. Microbiol.*, vol. 58, no. 5, pp. 1075–1078, 2008.

Acknowledgements:
Supported by Fundação para a Ciência e Tecnologia through the grants PTDC/BIABQM/4967/2020, EXPL/BIA-BQM/0770/2021 and by FCT projects UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020 (i4HB).

P5.6 - ARSENATE RESPONSE IN YEAST: EXPLORING NEW PATHWAYS

***Teresa Pissarro*, Américo G. Duarte, Catarina Amaral, Catarina Pimentel, Sofia M. da Silva**
Instituto de Tecnologia Química e Biológica António Xavier - ITQB NOVA, Oeiras, Portugal

(*) *e-mail: t.pissarro@itqb.unl.pt*

ABSTRACT

The development of sustainable and environmentally friendly processes able to mitigate arsenic contamination, a major public health concern, relies on the comprehensive knowledge of arsenic detoxification pathways. In *Saccharomyces cerevisiae*, a powerful eukaryotic model organism, the arsenate (As^{V}) reductase Acr2, the arsenite (As^{III}) permease Acr3 and the aquaglyceroporin Fps1 are key players in arsenic detoxification, and their mode of action is well documented. However, our recent data indicate that arsenic detoxification is far more complex than generally assumed. We found that the deletion of both *ACR2* and *ACR3* genes increased *S. cerevisiae* sensitivity to As^{V} and led to a massive cellular accumulation of arsenic, which does not occur in *Δacr2* single mutant. These results challenge the accepted model for As^{V} detoxification in yeasts, and suggest the direct involvement of the Acr3 transporter in As^{V} detoxification. On the other hand, although the deletion of *FPS1* from *Δacr2* background also renders the cells more sensitive to As^{V}, it does not lead to arsenic accumulation. Instead, it triggers the accumulation of large amounts of glycerol in the presence of As^{V}, suggesting that osmolyte export is required to avoid As^{V} toxicity.

In order to determine if As^{V} is indeed a substrate of the Acr3 permease we are currently expressing a recombinant form of the protein on yeast *Δacr2Δacr3* cells. The ability to transport As^{V} by the recombinant Acr3 will be evaluated using membrane vesicles.

P5.7 - ELUCIDATING THE PHYSIOLOGICAL ROLE OF THE DSRJ CYTOCHROME IN DISSIMILATORY SULFATE METABOLISM

Raquel Mendes Bernardino *, Ana Catarina Barbosa, Inês Cardoso Pereira

ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

(*) e-mail: rm.bernardino@itqb.unl.pt

Keywords: Sulfate-reducing organisms; Dissimilatory sulfite reduction; Respiratory membrane complexes; DsrMKJOP; Phenotypic characterization.

ABSTRACT

The overgrowth of sulfidogenic bacteria in the human gut, due to dysbiosis, has been associated with chronic inflammatory disorders and cancer. These bacteria produce high levels of sulfide, which can break the mucus barrier, allowing microbial access to the gut epithelium and thus inducing inflammation [1]. Sulfide is mainly produced by these bacteria via the dissimilatory sulfite reduction (Dsr) pathway. In the Dsr pathway, sulfite is reduced by the dissimilatory sulfite reductase DsrAB with the involvement of DsrC, yielding a DsrC-trisulfide. It is believed that the DsrC-trisulfide is then reduced by the conserved DsrMKJOP membrane complex to produce the final product, sulfide, recycling DsrC [2]. This last step likely provides a link to chemiosmotic energy conservation through a still unknown mechanism. One of the most puzzling subunits of the DsrMKJOP complex is its periplasmic subunit, DsrJ. DsrJ is a triheme cytochrome *c* with a very unusual His/Cys coordination for one of the hemes, which may be involved in sulfur chemistry and electron transfer [3].

To disclose the physiological role of DsrJ, we performed growth studies with variants of the gut bacterium *Desulfovibrio vulgaris*, lacking DsrJ, and with variants of the key Cys residue that is a heme ligand in this protein, produced by site-directed mutagenesis.

Our results show that DsrJ is crucial for sulfate reduction, as its absence compromised the functional role of the DsrMKJOP complex. Moreover, the conserved Cys residue from the unique His/Cys heme coordination did not prove to be essential, as the growth of its variants was not affected. The results of this work provide new insights concerning the role of DsrJ in the dissimilatory sulfite reduction pathway in sulfidogenic organisms.

References:

[1] N. Ijssennagger, R. Van Der Meer, and S. W. C. Van Mil, "Sulfide as a Mucus Barrier-Breaker in Inflammatory Bowel Disease?," Trends Mol Med, vol. 22, no. 3, pp. 190–199, 2016, doi: 10.1016/j.molmed.2016.01.002.

[2] A. A. Santos, S. S. Venceslau, F. Grein, W. D. Leavitt, C. Dahl, D. T. Johnston, I. A. C. Pereira, "A protein trisulfide couples dissimilatory sulfate reduction to energy conservation," Science, vol. 350, no. 6267, pp. 1541–1545, Dec. 2015, doi: 10.1126/science.aad3558.

[3] F. Grein, S. S. Venceslau, L. Schneider, P. Hildebrandt, S. Todorovic, I. A. C. Pereira, C. Dahl, "DsrJ, an essential part of the DsrMKJOP transmembrane complex in the purple sulfur bacterium *Allochromatium vinosum*, is an unusual triheme cytochrome *c*," Biochemistry, vol. 49, no. 38, pp. 8290–8299, Sep. 2010, doi: 10.1021/bi1007673.

P5.8 - OPTIMIZING BACTERIAL CELLULOSE PRODUCTION IN *KOMAGATAEIBACTER SPP.* THROUGH METABOLIC ENGINEERING

Pedro Montenegro-Silva [1], ***Tom Ellis*** [2,3], ***Fernando Dourado*** [1,4], ***Miguel Gama*** [1,4], ***Lucília Domingues*** [1,4*]

[1] *CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2] *Centre for Synthetic Biology, Imperial College London, London*
[3] *Department of Bioengineering, Imperial College London, London*
[4] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail: luciliad@deb.uminho.pt*

Keywords: Bacterial cellulose (BC), *Komagataeibacter,* PQQ-dependent membrane dehydrogenases, Gluconate, Ethanol Supplementation, Metabolic Engineering

ABSTRACT

Bacterial cellulose (BC), primarily synthesized by acetic acid bacteria (AAB), holds promise across diverse industries from biomedicine to electronics. However, cost-effective production remains elusive. *Komagataeibacter* perform periplasmic oxidation of sugars and alcohols into weak acids (e.g., glucose to gluconate, ethanol to acetate) by PQQ-dependent membrane dehydrogenases (PQQ-mDH). The dramatic pH drop that occurs due to glucose oxidation to gluconate is reported to impact BC yields negatively, while ethanol supplementation is well known to boost BC production. This study evaluates the effects of PQQ- mDH knockouts (KO) on BC production and its modulation by ethanol supplementation.

The genome of *K. sucrofermentans* ATCC 700178 (KS001) codes four PQQ-mDHs. Mutant strains, KS002 to KS005, were developed by knocking out these genes. The KO effects were evaluated by performing agitated and static cultures, HPLC, and pH monitoring. BC yield was analyzed by gravimetry.

All mutants, barring KS003, quickly consumed glucose, with KS003 avoiding gluconate production. All strains except KS004 oxidize ethanol to acetate in the periplasm, while KS004 seems to be metabolizing the alcohol in the cytosol. Evaluating BC production, KS003 emerged as a front-runner, showing a 5.77-fold BC production increase in glucose compared to KS001.

The mutant strains KS003 and KS004 behave differently from KS001 when the culture medium is supplemented with ethanol. KS001 and KS003 showed maximum productivity with 1.0% and 2.0% (v/v) ethanol, respectively. KS003 shows a 2-fold yield increase when comparing the optimal condition for each strain. In contrast, ethanol supplementation does not have a positive effect on BC production by KS004.

Metabolic engineering, particularly targeting PQQ-dependent dehydrogenases offers a promising avenue to optimize BC production in *Komagataeibacter*. The study underscores the importance of understanding the requirement of PQQ-mDHs for substrate utilization, their role on energy generation, and their implication on BC synthesis.

Acknowledgements:
Portuguese Foundation for Science and Technology (FCT) - strategic funding of UIDB/ 04469/2020 Pedro Montenegro PhD scholarship (2021.07153.BD)

P5.9 - THE ELECTROGENIC QRCABCD COMPLEX FROM SULFATE-REDUCING PROKARYOTES PLAYS AN IMPORTANT ROLE ON SULFATE RESPIRATION

Gonçalo Manteigas *, Américo G. Duarte, Inês A. C. Pereira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

(*) e-mail: goncalo.manteigas@itqb.unl.pt

Keywords: Sulfate-reducing prokaryotes, Sulfate reduction, Sulfite reduction, Redox-loop

ABSTRACT

In anaerobic sulfate rich environments, sulfate-reducing prokaryotes are commonly present and play an important role in the carbon and sulfur cycles. Through highly conserved respiratory membrane complexes these microorganisms couple the oxidation of hydrogen or carbon compounds with the dissimilatory reduction of sulfate or sulfite. In *Desulfobacterota*, such as *Desulfovibrio vulgaris* Hildenborough (*Dv*H), the Quinone-reductase complex (QrcABCD) is an electrogenic respiratory membrane complex that links the periplasmic hydrogen and formate oxidation to the reduction of the membrane quinone pool. The quinol pool can be used for sulfate or sulfite reduction through the QmoABC and DsrMKJOP complexes, respectively.

In this project, we genetically engineered *Dv*H to knock-out the *qrcABCD* operon. A homologous overexpression system for QrcABCD was constructed using this strain. Growth studies were performed with strains expressing the QrcABCD complex and variants, in several growth conditions. The recombinant Qrc complex and different variants were purified and reconstituted in menaquinone containing liposomes. The electron transfer from reduced TpIc*3* (Qrc physiological partner) to the quinones was evaluated through stopped-flow experiments, using the Qrc proteoliposomes.

The growth studies show that QrcABCD is essential to couple hydrogen and formate oxidation to sulfate reduction, but it is not essential for sulfite respiration. Electron transfer experiments with Qrc variants reconstituted in liposomes allowed the identification of key aminoacids that are essential for quinone reduction.

This work elucidates the molecular mechanism for energy conservation in QrcABCD and will enable further understanding of the energy metabolism of sulfate reducers, as well as other relevant bacteria, since several homologues of Qrc are present in bacteria with different bioenergetics contexts.

Acknowledgements:
Funding by Fundação para a Ciência e Tecnologia (Portugal) through the grant FCT SFRH/BD/04641/2021, Research Unit MOSTMICRO (Portugal) – UIDB/04612/2020 and UIDP/04612/2020, and Associate Laboratory LS4FUTURE (Portugal) – LA/P/0087/2020.

P5.10 - THE ROLE OF PNPASE IN THE BIOFILM FORMATION OF *ESCHERICHIA COLI*

Manuel Condinho [1], Sandra Cristina Pinto [2,3], Cecília Maria Arraiano [1], Vânia Pobre [1(*)]
[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.
[2] iBB-Institute for Bioengineering and Biosciences (IBB), Instituto Superior Técnico, Portugal.
[3] i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Portugal

(*) email: vaniapobre@itqb.unl.pt

Keywords: Biofilms; Ribonucleases; Microscopy; c-di-GMP; Adhesion

ABSTRACT

Bacterial communities constantly deal with stress situations, having to quickly regulate their gene expression to ensure survival. Biofilm formation is one of these response mechanisms, as it gives bacterial aggregates protection against harsh environments. Switch from planktonic to a sessile lifestyle is a highly controlled process in bacteria and is dependent on RNA regulators. Ribonucleases (RNases) are the enzymes that regulate all RNA levels in the cell, being responsible for their degradation, maturation, and processing. RNases regulate various processes in bacteria, including biofilm formation, aggregation, adhesion, among others. In our work we described the role of an exoribonuclease, PNPase, in the biofilm formation of *Escherichia coli*. The crystal violet quantification showed that Δ*pnp* forms less biofilm than the wild type. We observed by confocal microscopy that the Δ*pnp* has clear difficulties in biofilm formation and in the adhesion to a surface. Compared to the wild type the PNPase mutant has an elongated shape, what suggests that this RNase can be important for cell division. We found that the PNPase mutant has its aggregation compromised, which is a fundamental process at an initial stage of biofilm formation. To comprehend the molecular role of PNPase in this process we quantified by mass spectrometry the intracellular levels of c-di-GMP, which is a signaling molecule that regulates biofilm growth in several bacteria. We observed that the Δ*pnp* has decreased levels of c-di-GMP compared to wild type, which agrees with the observed biofilm phenotype. We hypothesized that PNPase is involved in the expression of the proteins that synthetize and degrade c-di-GMP, and by them affect the levels of this metabolite. To test this hypothesis, we evaluated the expression of several transcripts for these proteins using qPCR. In summary, with our work we established a link between PNPase and biofilm formation in *E. coli*.

P5.11 – ROLE OF ENOLASE IN THE FORMATION OF *CLOSTRIDIOIDES DIFFICILE* BIOFILM

Rute Canhão-Pereira [1]***, Isabel Roseiro*** [1]***, Mónica Oleastro*** [2]***, Adriano O. Henriques*** [1] ***and Mónica Serrano*** [1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, NOVA University, Lisbon, Portugal
[2] National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

(*) *e-mail: rute.pereira@itqb.unl.pt*

Keywords: Matrix, Moonlighting protein, Recurrence

ABSTRACT

Bacterial biofilms are a form of multicellular organization that confers protection against harmful conditions and creates nutrient-rich niches. Biofilms were also shown to comprise an important aspect of microbial persistence in the human gut. The endosporeformer *Clostridioides difficile* is a major cause of nosocomial infections associated with antibiotic therapy. About 30% of *C. difficile* infections lead to disease recurrence. If this organism perseveres in the intestine as biofilms, spores or biofilm-associated spores is still unknown.

The extracellular matrix is an important feature of the biofilm and is composed of exopolysaccharides, extracellular DNA, and proteins. We recently identified several *C. difficile* biofilm-matrix proteins. Most of these proteins were found to be abundant cytoplasmic enzymes. Here, we focused on the role of enolase. Enolase is a moonlighting protein, performing different functions in addition to its role in glycolysis. We demonstrated that enolase is universally present in the *C. difficile* biofilm matrix. Enolase is encoded by the essential *eno* gene, and therefore to understand its role in *C. difficile* biofilms, we used a CRISPRi vector targeting *eno*. Depletion of enolase led to defects in growth and impaired biofilm production. Extracellular complementation with a catalytic inactive form of enolase restored biofilm formation. We also showed by Clear-Native gels that enolase forms an octamer and that the replacement of arginine at position 396 by alanine (R396A) impairs the formation of the octamer curtailing enolase activity. Single-cell analysis revealed that *eno* is homogeneously and constitutively expressed during biofilm formation.

Giving the importance of enolase in many pathways, including biofilm formation, an important factor for persistence of the organism, our work will provide new insights on strategies to fight infections by *C. difficile*.

P5.12 - PRELIMINARY CHARACTERIZATION OF A HYDROXILAMINE OXIDOREDUCTASE WITH AN NON-CANONICAL N-TERMINAL DOMAIN

Susana F. Fernandes *, Catarina M. Alves and Filipe Folgosa

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

(*) e-mail: scfernandes@itqb.unl.pt

Keywords: Anammox Bacteria, Hydroxylamine oxidoreductases, MultiCopper proteins

ABSTRACT

Anaerobic ammonium-oxidizing (anammox) bacteria are unique organisms that belong to the planctomycetes phylum and convert ammonium and nitrite into nitrogen, anaerobically, with the formation of nitric oxide as an intermediate1. The enzymes responsible for the NO production in anammox bacteria were not yet elucidated. In other bacteria, NO formation from nitrite is performed either by the heme-containing cd1 nitrite reductase or by a Cu-containing nitrite reductase2. However, in these organisms, the genes coding for canonical nitrite reductases are either inexistent, hardly expressed or not expressed at all3. Several hydroxylamine oxidoreductases (HAO)-like proteins are considered candidates to perform this reaction; one of them is a fusion protein, with a very fascinating combination of domains: an HAO-like domain and a multicopper oxidase (MCO)-like domain (N-terminal), denominated hereon as MH.

The aim of this work was to biochemically and spectroscopically characterize MH from the anammox bacterium Candidatus Brocadia pituitae and understand the role of MCO and HAO-like domains including the hypothesis of the simultaneous use of two different substrates.

The MCO domain was heterologously overexpressed in E. coli and purified to homogeneity and was characterized by UV-Visible and EPR spectroscopies. This domain showed laccase activity with ABTS as substrate at pH 4, but no nitrite reductase activity.

The production of the MH and HAO domain was performed in both E. coli and Shewanella oneidensis. This is still an ongoing work.

References:

1. Peeters SH, van Niftrik L. Trending topics and open questions in anaerobic ammonium oxidation. Curr Opin Chem Biol. 2019;49:45-52. doi: 10.1016/j.cbpa.2018.09.022
2. Bartossek R, Nicol GW, Lanzen A, Klenk HP, Schleper C. Homologues of nitrite reductases in ammonia-oxidizing archaea: Diversity and genomic context. Environ Microbiol. 2010;12(4):1075-1088. doi:10.1111/j.1462-2920.2010. 02153.x
3. Kartal B, Maalcke WJ, De Almeida NM, et al. Molecular mechanism of anaerobic ammonium oxidation. Nature. 2011;479(7371):127-130. doi:10.1038/nature10453

P5.13 - AN9181 MAY REGULATE *ASPERGILLUS NIDULANS* METABOLIC RESPONSE TO DIFFERENT CHEMICAL STRESSES

João Jorge [1]*, Celso Martins [1], Tiago Martins [1], Diego Hartmann [1], Gustavo H. Goldman [1,2], Cristina Silva Pereira [1,#]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Nova University of Lisbon, Oeiras, Portugal

[2] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

[#] corresponding author

[(*)] e-mail: joao.jorge@itqb.unl.pt

Keywords: transcriptomics; aromatic compounds; stress responses; regulators

ABSTRACT

The aspergilli comprise a diverse group of saprophytic filamentous fungus covering over 200 million years of evolution [1]. They have been extensively studied due to their biotechnological and ecological relevance and pathogenic potential. Earlier studies on the genome's structure and evolution between aspergilli revealed an impressive level of structural and functional conservation and synteny [1, 2]. These high levels of genomic similarity in aspergilli underline the likely existence of conserved genes responsive to variable chemical stresses (*e. g.*, regulators) [1, 3].

In this study, we used a computational strategy to test the far-reaching hypothesis that exposure to unusual organic compounds, triggers unknown conserved responses across aspergilli. We resorted to transcriptome-base data on aspergilli upon exposure to organic compounds having an aromatic building block in their structure (5 datasets) to identify genes showing a conserved response. Only one gene showed similar upregulation in the five datasets, *AN9181*. This gene encodes for a protein containing a *NmrA*-like domain. *Aspergillus nidulans* - a well-established model organism for genetics and cell biology studies - was chosen as the "receiver species" to construct deletion-mutants in order to functionally characterize AN9181.

Specifically, we compared the phenotypes of the deletion mutant and the wild-type strain, namely their germination fitness, metabolic profile in different nitrogen and carbon sources, radial growth rate, and susceptibility to antifungals under different conditions. The collected data are suggestive that deletion of *AN9181* lead to higher metabolic activities in different nitrogen sources.

This gene possibly acts as a metabolic repressor of *A. nidulans* response upon exposure to aromatic-based compounds, with unexpected links to antifungal response, specifically to cell wall damaging agents. This study is a step forward to better understand general stress responses in this model organism.

References:

1. Galagan, J.E., et al., Sequencing of Aspergillus nidulans and comparative analysis with A. fumigatus and A. oryzae. Nature, 2005. 438(7071): p. 1105-15.
2. Steenwyk, J.L., et al., Variation Among Biosynthetic Gene Clusters, Secondary Metabolite Profiles, and Cards of Virulence Across Aspergillus Species. Genetics, 2020. 216(2): p. 481-497.
3. Martins, T.M., C. Martins, and C. Silva Pereira, Multiple degrees of separation in the central pathways of the catabolism of aromatic compounds in fungi belonging to the Dikarya sub-Kingdom. Adv Microb Physiol, 2019. 75: p. 177-203.

P5.14 - A DNA-BINDING BACTERIOFERRITIN FROM THE ANAMMOX BACTERIUM *CANDIDATUS BROCADIA PITUITAE*

Catarina M. Alves *, Susana F. Fernandes and Filipe Folgosa

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República 2780-157, Oeiras, Portugal

(*) e-mail: catarina.alves@itqb.unl.pt

Keywords: Iron, Anammox bacteria, Bacterioferritin, DNA-Binding activity

ABSTRACT

Iron is a transition metal, essential for all living organisms, present mostly as cofactor in a plethora of enzymes/proteins, responsible for critical processes such as O_2 transport, DNA synthesis and gene regulation[1]. Anammox (Anaerobic Ammonium Oxidation) bacteria are one of the most iron-dependent microorganisms, relying on this metal for growth and metabolism[1]. These bacteria produce an abundant number of multiheme cytochromes, cofactors with Fe-S and iron nanoparticles, that contribute to the anaerobic oxidation of NH^{4+}, resulting in an efficient removal of this contaminant from residual waters[1,2]. However, iron-induced toxicity can occur, when this metal is present in high concentrations, leading to the accumulation of toxic intermediates and destruction of cellular components[1]. Thus, microorganisms developed strategies to allow iron sufficiency within the cell and simultaneously prevent iron-induced toxicity, namely by expressing iron storage proteins. A family of these proteins are bacterioferritins, known for forming a spherical hollow structure, capable of storing up to 4500 iron ions[3].

Here, expression, purification and biochemical characterization of a recently identified bacterioferritin in the Anammox organism *Candidatus Brocadia pituitae* will be presented, such as its iron loading capacity, oligomeric state and DNA-binding and DNA-protection activities. These two activities are not common in bacterioferritins and may imply a novel function of this family in gene regulation.

Understanding how these proteins function is essential to comprehend the Anammox process and consequently improve this efficient and ecological tool for wastewater treatment plants.

References:

1. Peng, M. W., et al. (2022). ACS Sustainable Chemistry and Engineering, 10(24), 7896–7906.
2. Kuypers, M. M. M., et al. (2018) Nat. Rev. Microbiol. 16, 263–276
3. Rivera, M. (2017). Accounts of Chemical Research, 50(2), 331–340.

Acknowledgements:

This work was financially supported by FCT through EXPL/BIA-BQM/0768/2021, MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020, UIDP/04612/2020), LS4FUTURE Associated Laboratory (LA/P/0087/2020).

P5.15 - MICROBIAL PRODUCTION OF THE BUILDING BLOCK P-COUMARIC ACID: UNLEASHING THE POTENTIAL OF *KLUYVEROMYCES MARXIANUS*

Marlene Baptista [1*] **, Carlos E. Costa** [1,2], **Lucília Domingues** [1,2]

[1] *CEB—Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal;*

[2] *LABBELS –Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail: marlene.baptista@ceb.uminho.pt*

Keywords: *Kluyveromyces marxianus,* p-coumaric acid, tyrosine ammonia lyase, precision fermentation

ABSTRACT

The interest in several plant secondary metabolites, such as naringenin or resveratrol, for their known biological functions, is growing at a fast pace. These properties encompass antioxidant, anti-inflammatory, and anti-microbial, among many other activities. The phenolic acid *p*-coumaric acid is naturally produced by plants, being a key precursor of many of these secondary metabolites. Nevertheless, *p*-coumaric acid is currently extracted from plants, which poses several drawbacks for its industrial production including low efficiency and dependence on plant availability. On the other hand, microbial production of *p*-coumaric acid has been emerging as a sustainable and economically viable alternative.

The unconventional yeast *Kluyveromyces marxianus* is garnering increasing interest as an alternative cell platform to produce ethanol and high-value compounds with a span of applications across industries [1]. This is due to its distinctive attributes, such as rapid growth rate, thermotolerance, and the capacity to metabolize different sugars [2]. Due to its Crabtree-negative metabolism, this yeast produces acetyl-coenzyme A in the presence of oxygen and high sugar concentration. As such, it could be an interesting chassis to produce aromatic compounds derived from *p*-coumaric since some of them require malonyl-CoA (derived from acetyl-CoA) as a precursor. For that, in yeast, the expression of heterologous enzymes involved in the conversion of aromatic amino acids into *p*-coumaric acid is required.

Building upon this knowledge, here, two *K. marxianus* strains were engineered and screened for their capacity for *p*-coumaric acid production. Initially, a heterologous enzyme, tyrosine ammonia lyase (TAL), which converts tyrosine to *p*-coumaric acid, was integrated into both strains. Further, the effects of different carbon sources and agitation conditions on *p*-coumaric acid production and yeast primary metabolism were evaluated. Overall, this work shows the potential of *K. marxianus* for *p*-coumaric acid production and its derivatives through an integrated process.

References:

[1] Baptista, M; Cunha, JT; Domingues, L. Establishment of Kluyveromyces marxianus as a microbial cell factory for lignocellulosic processes: production of high value furan derivatives. Journal of Fungi, 7(12), 1047, 2021.

[2] Baptista, M; Domingues, L. Kluyveromyces marxianus as a microbial cell factory for lignocellulosic biomass valorisation. Biotechnology Advances, 60(108027), 2022

Acknowledgements:

Portuguese Foundation for Science and Technology (FCT) - strategic funding of UIDB/ 04469/2020; Marlene Baptista PhD scholarship (2020.06888.BD).

P5.16 - DISENTANGLING TANGLED CYTOCHROMES: INSIGHTS ON THE FUNCTIONAL MECHANISMS OF A MICROBIAL HEME-TETHERED REDOX STRING

Tomás M. Fernandes [1,2]*, Marta A. Silva [1,2], Leonor Morgado [1,2], Carlos A. Salgueiro [1,2]

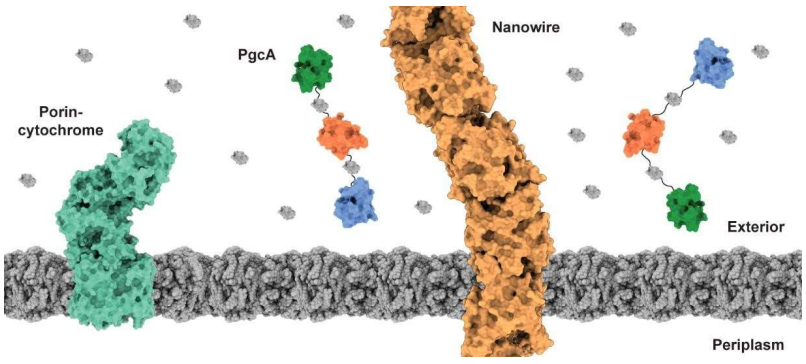
[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy.
[2] UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal.

(*) e-mail: tmo.fernandes@campus.fct.unl.pt

Keywords: Extracellular electron transfer, Geobacter, Multiheme cytochromes, NMR

ABSTRACT

Microbial extracellular reduction of insoluble compounds requires soluble electron shuttles that diffuse in the environment, freely diffusing cytochromes, or direct contact with cellular conductive appendages that release or harvest electrons to assure a continuous balance between cellular requirements and environmental conditions. PgcA is an extracellular triheme cytochrome that contributes to Fe(III) and Mn(IV) oxides reduction in *Geobacter sulfurreducens.* The AlphaFold model of PgcA shows that its mature form possesses a fuzzy global arrangement with three monoheme cytochrome domains linked by unstructured stretches. The three domains were heterologously expressed and studied using complementary biophysical techniques. The domains are structurally homologous, but their heme groups show variable axial coordination and reduction potential values. Electron transfer experiments monitored by NMR and visible spectroscopy show the variable extent to which the domains promiscuously exchange electrons while reducing different electron acceptors. The results suggest that PgcA is part of a new class of cytochromes - microbial heme-tethered redox strings - that use low-complexity protein stretches to bind metals while conferring elasticity and promoting intra- and intermolecular electron transfer events amongst its cytochrome domains and to electron acceptors at variable distances[1].



[1] Fernandes, T. M., Silva, M. A., Morgado L., Salgueiro, C. A. (2023) Hemes on a string: insights on the functional mechanisms of PgcA from *Geobacter sulfurreducens*. J. Biol. Chem., 105167.

Acknowledgements:
This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the following grants: SFRH/BD/145039/2019 and COVID/BD/153449/2023 (TMF), PTDC/BIA- BQM/4967/2020 (CAS), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB).

P5.17 - EXPLORING GEOBACTER QUINONE OXIDOREDUCTASE COMPLEXES: EXPRESSION AND PURIFICATION OF CBC3 AND CBC4 PROTEINS

Mafalda V. Fernandes *, Jorge M. A. Antunes, Carlos A. Salgueiro, Leonor Morgado

Associate Laboratory i4HB - Institute for Health and Bioeconomy
UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal

(*) e-mail: mv.fernandes@campus.fct.unl.pt

Keywords: Geobacter, quinone oxidoreductases, multiheme cytochromes, iron-sulfur proteins

ABSTRACT

Exoelectrogenic microorganisms such as *Geobacter* bacteria are in the spotlight since they emerged as remarkable microorganisms for applications in several fields including biotechnology, bioremediation and microbiology. *Geobacter* species are known for their unique metabolic capabilities, with special relevance to the usage of several extracellular compounds as terminal electron acceptors, such as insoluble metals, in extracellular electron transfer (EET). This has enabled their crucial role in biogeochemical cycling of metals and carbon, as well as in electron transfer processes [1]. *Geobacter sulfurreducens* is the best studied *Geobacter* species and gene-knockout and proteomics studies were used to identify essential proteins for the EET mechanisms. One crucial step in this process is the transfer of the electrons from the quinone pool to the inner membrane associated quinone oxidoreductases for subsequently transfer to their redox partners in the periplasm.

Quinone oxidoreductases are typically composed by a membrane associated *b*-type diheme domain that is fused or adjacent to a multiheme c-type cytochrome located in the periplasm, and in some complexes, to an iron-sulfur protein. *G. sulfurreducens* genome encodes for six inner membrane quinone oxidoreductase gene clusters, from which three of them, CbcL, ImcH and CbcBA, have already been implicated in specific metabolic pathways [2,3]. The goal of this work is to characterize the inner-membrane electron transfer complexes Cbc3 (CbcVWX) and Cbc4 (CbcSTU). Cbc3 is composed by a transmembrane domain that binds three *b*-type hemes (CbcW), a pentaheme *c*-type cytochrome (CbcX) and a Rieske iron-sulfur protein (CbcV). Cbc4 complex is composed by a transmembrane domain (CbcU), a tetraheme *c*-type cytochrome (CbcS) and a protein that binds four [4Fe-4S] centers (CbcT). The biochemical characterization of the multiheme cytochromes and the iron-sulfur proteins of these complexes will be presented.

[1] Lovley DR, Ueki T, Zhang T, *et al*. (2011) Adv. Microb. Physiol. 59, 1-100.
[2] Joshi K, Chan CH, Bond DR (2021) Mol. Microbiol. 116, 1124-1139
[3] Antunes JMA, Silva MA, Salgueiro CA, Morgado L (2022) Front. Microbiol. 13, 1-11.

Acknowledgements:
This work was supported by Fundação para a Ciência e a Tecnologia through grants 2022.11900.BD (JMAA), PTDC/BIA-BQM/4967/2020 (CAS), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB).

P5.18 – CELL DIVISION PROTEIN FTSK COORDINATES BACTERIAL CHROMOSOME SEGREGATION AND DAUGHTER CELL SEPARATION IN *STAPHYLOCOCCUS AUREUS*

Helena Veiga [1]*, Ambre Jousselin [1], Simon Schäper [1], Bruno M. Saraiva [1], Leonor B. Marques [1], Patricia Reed [1], Joana Wilton [1], Pedro M. Pereira [1], Sérgio R. Filipe [1,2], Mariana G. Pinho [1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

[2] UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

[(*)] e-mail: hveiga@itqb.unl.pt

Keywords: bacterial cell cycle; chromosome replication and segregation; FtsK; peptidoglycan hydrolases; *Staphylococcus aureus*

ABSTRACT

Unregulated cell cycle progression may have lethal consequences and therefore, bacteria have various mechanisms in place for the precise spatiotemporal control of cell cycle events. We have uncovered a new link between chromosome replication/segregation and splitting of the division septum.

We show that the DNA translocase domain-containing divisome protein FtsK regulates cellular levels of a peptidoglycan hydrolase Sle1, which is involved in cell separation in the bacterial pathogen *Staphylococcus aureus*. FtsK interacts with a chaperone (trigger factor, TF) and establishes a FtsK-dependent TF concentration gradient that is higher in the septal region.

Trigger factor binds Sle1 and promotes its preferential export at the septal region, while also preventing Sle1 degradation by the ClpXP proteolytic machinery. Upon conditions that lead to paused septum synthesis, such as DNA damage or impaired DNA replication/segregation, TF gradient is dissipated and Sle1 levels are reduced, thus halting premature septum splitting.

Acknowledgements:
- FCT– Fundação para a Ciência e a Tecnologia grant PTDC/BIA-MIC/6982/2020
- European Research Council grant ERC-2017-CoG-771709
- FCT– Fundação para a Ciência e a Tecnologia MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020, UIDP/04612/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020)

P5.19 - TRACKING STRESS ADAPTATION IN BACTERIA USING A "LIBRARY OF GLOBAL REGULATORS OF GENE EXPRESSION

Ines S. C. Baptista *, Suchintak Dash, Andre S. Ribeiro

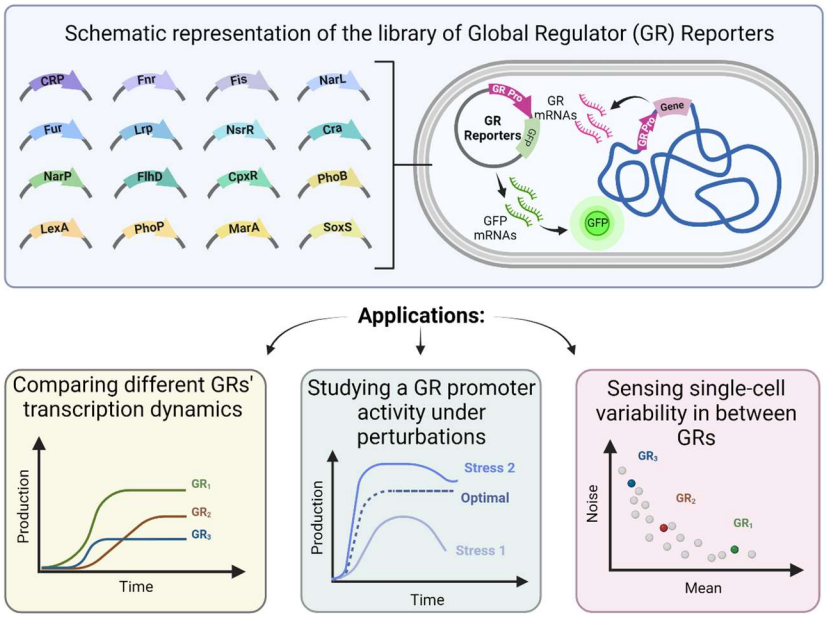
Laboratory of Biosystem Dynamics, Faculty of Medicine and Health Technology, Tampere University, Finland.

(*) e-mail: ines.baptista@tuni.fi

Keywords: Global Regulators of Gene Expression; Transcriptional Reporters; Strain Library.

ABSTRACT

E. coli has evolved many mechanisms to adapt to stresses. At the core of some of these mechanisms are several global regulators (GR) of gene expression, which are transcription factors that control tens to hundreds of genes. GRs control a broad range of biological tasks, from regulating how carbon is used by cells when in the absence of glucose (CRP), to controlling genes responsible for resistance to antibiotics and acidifying the cytoplasm (MarA). Tracking the numbers of the global regulators over time should provide new insight into their role in the activation of specific cellular functions during stress conditions. So far, there is no synthetic gene library that allows tracking and comparing the numbers of GRs of *E. coli* over time. Here, we present a library of 16 strains, each reporting the mRNA expression dynamics of a GR. We show that the reporter strains are robust to changes in the growth phase. Also, we show that the reporters have high sensitivity and specificity to different genome-wide perturbations. Finally, we show that these reporters can be used to study single-cell variability at the transcription level. This library can contribute to several studies, including those focusing on the global transcriptional stress-response programs of *E. coli*. In the long term, they may assist the development of synthetic bacteria that tune these programs to prioritize productivity, instead of cell growth, which would be of value to the bioindustry.



P5.20 - TIME SERIES EXOMETABOLOMICS DEPICT METABOLIC FEATURES OF *STAPHYLOCOCCUS EPIDERMIDIS* PATHOGENICITY

Elisabete Morais [1]***; Maria Miragaia*** [1]***; Luís G Gonçalves*** [1]***; Ana V Coelho*** [1(*)]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

(*) *e-mail: varela@itqb.unl.pt*

Keywords: *S. epidermidis*; pathogenicity; commensalism; exometabolomics; ^{1}H-NMR.

ABSTRACT

A common human skin colonizer, *Staphylococcus epidermidis* (SE), is often the cause of infections associated with medical devices. Strains that retain this pathogenic and commensal potential coexist in human skin, belonging to clonal complexes 2 (CC2) and non-CC2, respectively. Pathogenicity mechanisms are poorly understood in this organism. Recognizing how they deal with increased pH is required to design more effective prevention and treatment strategies against SE infections. To investigate the metabolic adaptation of CC2 and non-CC2 representative strains to an increase in pH, we mimicked the pH conditions of skin and bloodstream (5.5 and 7.4). Biomass formation, growth media pH and ^{1}H-NMR exometabolomic data were followed until stationary phase. The commensal strain reached higher biomass when grown at pH 7.4 than the pathogenic strain. For all experimental conditions, media pH significantly changed during growth, revealing different pH adaptation mechanisms. 48 exometabolites were quantified and their profiles were clustered in uptake, secretion or mixed. Commensal strain uptake and secretion clusters were further subdivided into early and late, suggesting a metabolic shift during growth. A strong negative correlation of media pH with acetate and 2,3-butanediol was observed during growth, while acetoin only correlates at skin pH. Some exometabolite profiles show strain-dependent differences. Most significative difference is the formate accumulation at blood pH in pathogenic cells, justified by the absence of formate dehydrogenase gene in this strain. We also reported for the first time in SE, the use of exported lactate as potential carbon source after mid-exponential phase in aerobiose. This work depicted metabolic traits associated to pathogenicity, which will be helpful to develop new preventive and treatment strategies against SE infections.

Acknowledgements:
MOSTMICRO (LISBOA-01-0145-FEDER-007660); Cermax (AAC/01/SAICT/2016) ITQB NOVA; PD/BD/150980/2021; SymbNET project (952537) EU Horizon 2020.

P5.21 - EFFECT OF *HTRA* GENE IN THE PATHOGENICITY OF *ALIARCOBACTER BUTZLERI*

Cristiana Mateus [1(*)], Fernanda Domingues [1], Roland Bücker [2], Mónica Oleastro [3], Susana Ferreira [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[2] *Clinical Physiology/Nutritional Medicine, Medical Department of Gastroenterology, Infectious Diseases, Rheumatology, Charité–Universitätsmedizin Berlin, Berlin, Germany*

[3] *National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal*

(*) *e-mail: cristiana.lopes.mateus@ubi.pt*

Keywords: *Aliarcobacter butzleri*; *htrA* gene; pathogenicity; biofilm.

ABSTRACT

Aliarcobacter butzleri is a motile Gram-negative bacterium that can be isolated from distinct environments and hosts. As an emergent pathogen, this bacterium has been most commonly associated with enteritis in humans, with symptoms such as diarrhea, abdominal cramps and nausea. From the vast virulome of this enteropathogen, several genes may be associated with pathogenicity and adaptation of *A. butzleri*, however, its role in pathogenicity is still to clarify. The serine proteinase High Temperature Requirement protein A (HtrA) proved to be important for bacterial survival and colonization under challenging environmental conditions or to conquer host defense mechanisms in several pathogens. Thus, the aim of this work is to fill the gap in knowledge about the role of *htrA* gene on pathogenicity of *A. butzleri*. To achieve this, firstly a mutant was constructed by insertional mutagenesis, interrupting the *htrA* gene by a kanamycin resistance cassette, using the natural transformation method. The effect of this deletion on growth curves, bacterial motility, biofilm formation, survival to acidic, osmotic and oxidative stress was evaluated. Regarding the results, no significant difference in the growth curves and bacterial motility was observed; however, the mutant strain presented an increase in the biofilm formation when compared to the parental strain. Concerning the survival to stress conditions, the mutant shows to be slightly more tolerant to oxidative stress and to osmotic stress conferred by magnesium chloride, with a change on growth profile when compared to the parental strain. In sum, HtrA may have a potential role on pathogenicity of *Aliarcobacter butzleri*, nonetheless more studies will be required for a clearer assessment.

Acknowledgements:
Susana Ferreira acknowledges UBI and FCT by the contract of Scientific Employment according to DL57/2016. Cristiana Mateus is recipient of a doctoral fellowship (UI/BD/151023/2021) under the scope of the CICS-UBI Programmatic Funding (UIDP/00709/2020). This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.

P5.22 - PHAGE HUNTING ACROSS *MYCOBACTERIUM BOVIS* GENOMES

Daniela Pinto [1,2], Inês Mendes [1], Mónica V. Cunha [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*

(*) e-mail: mscunha@ciencias.ulisboa.pt

Keywords: Bacteriophages; *Mycobacterium bovis;* Genomics.

ABSTRACT

Mycobacterium bovis is the primary causative agent of animal tuberculosis (TB), impacting both livestock and wildlife. Traditionally considered a clonal pathogen with slow evolution, limited recombination, no horizontal gene transfer, and host-associated lifestyle, recent research has revealed that, after shedding by infected hosts, *M. bovis* is indeed able to persist in the environment in a viable state. This novel finding raises the question on whether interactions of *M. bovis* bacteria with mycobacteriophages and gene transfer events might occur outside the host. In this study, we leveraged a comprehensive dataset of over 200 *M. bovis* genomes obtained during epidemiological surveillance efforts. Our analysis initially focused on the presence of phiRv1, the prophage associated with *M. bovis* lineage ancestors, revealing instances of prophage excision within certain branches. Subsequently, we employed PHASTER to explore the presence of additional prophages, with ongoing efforts to explore more recent databases. To investigate previous phage infections and potential CRISPR-mediated responses, we conducted a meticulous assessment of CRISPR arrays within these genomes using CRISPRfinder, with no evidence of recent infection events. We then explored sequencing data from *M. bovis* isolated from infected host tissues using Genome Detective but no mycobacteriophage sequences were detected. We broadened our search to environmental matrices collected from regions of endemic TB, known to contain viable *M. bovis*. Through PCR and Sanger sequencing, we searched for mycobacteriophage families reportedly capable of infecting *M. bovis*. Thus far, we have not confirmed the presence of these phages. Our future endeavors involve attempting the isolation of *M. bovis* phages from environmental samples and address the intriguing absence of evidence of *M. bovis* infection by mycobacteriophages, disclosing the biological reasons behind this refractory behavior.

Acknowledgements:
Fundação para a Ciência e a Tecnologia, IP [PTDC/CVT- CVT/29783/2017, UIDB/00329/2020 UIDB/04046/2020, LA/P/0121/2020], PORLisboa [LISBOA-01-0145-FEDER-029783], COMPETE [POCI-01-0145- FEDER-029783].

P5.23 - GENOME-WIDE SIGNATURES OF ADAPTATION TOWARDS THE SESSILE LIFESTYLE IN *STAPHYLOCOCCUS AUREUS*

Sara Teixeira [1], Beatriz Ramos [1,2], Mónica V. Cunha [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

(*) *e-mail: mscunha@ciencias.ulisboa.pt*

Keywords: *S. aureus*; Biofilms; Comparative Genomics; Adaptive evolution.

ABSTRACT

Staphylococcus aureus is a commensal pathobiont of humans and other mammals, driving nosocomial and community-acquired infections that exert a high burden upon healthcare systems. *S. aureus* ubiquity and ecological adaptability have been associated to a sessile lifestyle, involving a complex set of genes and regulatory networks. The main aim of this work was to expand knowledge of the genetic bases of biofilm formation in *S. aureus* by means of an integrated approach pairing comparative genomics and adaptive laboratory evolution (ALE). To achieve this goal, 115 *S. aureus* isolates were subjected to whole genome sequencing and comparative genomics, highlighting polymorphisms on selected genes, previously associated to the biofilm development process, that were then related with the biofilm phenotype exhibited *in vitro* by the isolates. In addition, ALE assays towards the selection of biofilm traits or tolerance to linezolid enabled biofilm phenotype-genotype correlations after whole genome resequencing of evolved isolates. Sequence-type ST6133 isolates, associated with the epidemiologically relevant clonal complex 398, exhibited significantly stronger biofilm phenotypes. The strong biofilm producers could be distinguished from weak biofilm producers by missense variants in *eno, clfA, fib, ebpS, agrC, fnbB, fnbA, clfB, icaB and icaC* genes. ALE assays towards biofilm formation led different parental strains to convergent evolution in specific genes related with nucleotide transport and metabolism, transcription, and signal transduction COG functional categories. ALE towards linezolid resistance led to convergent evolution of specific genes related with carbohydrate transport and metabolism and, also, transcription. Our findings highlight that *S. aureus* specialization towards the sessile mode of growth is accompanied by adhesin and extracellular matrix remodelling, along with mutations in effectors at different regulatory levels.

Acknowledgements:
This work was funded by Fundação para a Ciência e a Tecnologia, IP (project OCELOT: 2022.01539.PTDC, UIDB/00329/2020, LA/P/0121/2020, UIDB/04046/2020, DFA/BD/7777/2020).

P5.24 - CABLE BACTERIA'S GROUNDBREAKING PROTEIN: ON THE JOURNEY FOR IMPROVED BIOELECTRICITY GENERATION AND BIOELECTRONICS COMPONENTS

Marta A. Silva [1,2(*)], Benjamin S. Korsgaard [3], Pilar C. Portela [1,2], Krutika Bavishi [3,4], Thomas Boesen [3,4], Carlos A. Salgueiro [1,2]

[1] *Associate Lab i4HB – Institute for Health and Bioeconomy*

[2] *UCIBIO – Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Caparica, Portugal*

[3] *Center for Electromicrobiology, Aarhus University, Denmark*

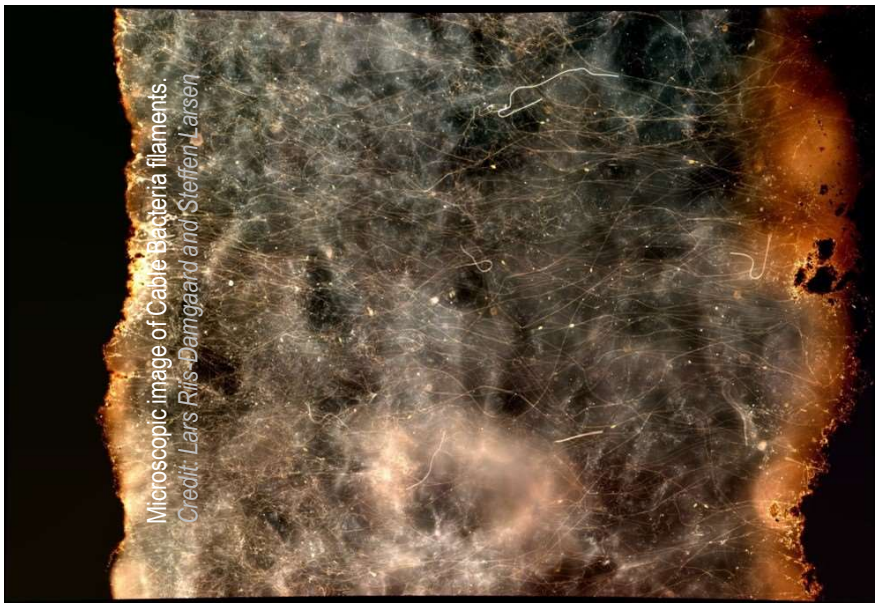
[4] *Interdisciplinary Nanoscience Center & Department of Molecular Biology and Genetics, Aarhus University, Denmark*

(*) *e-mail: mafs@fct.unl.pt*

Keywords: Cable bacteria; Bioenergy; Biogenic conductive nanomaterials; Extracellular electron transfer; Protein structure and function.

ABSTRACT

Exoelectrogenic bacteria (EXS) have the intriguing ability to link their oxidative metabolism with electron transfer (ET) to extracellular acceptors. In *Geobacter* bacteria, this process involves electrically conductive filaments formed by the assembly of polymeric multiheme *c*-type cytochromes, enabling ET to micrometer-scale distance[1]. Cable Bacteria (CB) are recent discovered EXS, which form electrically centimetre-long filaments that couple the oxidation of sulfide in deeper sediment layers with the reduction of oxygen near the sediment-water interface[2]. The discovery of this unique cellular organization shows that CB developed a mechanism to efficiently conduit ET over longer distances, extending the known length scale of biological electron transfer, and providing a source of inspiration for novel materials and technologies. The analysis of CB's genomes also revealed another remarkable hallmark, which is the absence of conventional terminal oxidases, despite the high oxygen reduction rates of these microorganism[3]. Instead, the genomes code for a distinctive group of multidomain redox proteins that appear to be the key to the understanding of CB's unique features. It consists of a *c*-type pentaheme cytochrome domain bound to one or two truncated hemoglobin (trHbs) domains. Each trHb domain contains a *b*-type heme and belongs to a family of small O_2-binding proteins[4]. Through a combination of redox measurements and complementary spectroscopic techniques, including NMR, CD, and UV-visible spectroscopy, the biochemical and biophysical properties of each individual domain were determined and will be presented. These findings establish a new research frontline for improving and developing innovative bioelectricity-based applications and bioelectronics components.



References:

1 Yalcin SE *et al.* (2020) Nat Chem. Biol 16, 1136-1142;

2 Bjerg JT *et al.* (2018) PNAS 115, 5786;

3 Scilipoti S *et al.* (2021) Sci Adv 7, eabe1870;

4 Kjeldsen KU *et al.* (2019) Proc Natl Acad Sci USA 116, 19116–19125.

Acknowledgements:

Work supported by Fundação para a Ciência e Tecnologia through: 2020.04717.BD (PCP), PTDC/BIABQM/4967/2020 (CAS), UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020 (i4HB).

P5.25 - INSIGHTS INTO THE GENOME AND TRANSPORTOME OF THE YEAST *CYBERLINDNERA JADINII*

Maria Sousa-Silva [1,2], Ricardo Franco-Duarte [1,2], Simão Rodrigues [3], Margarida Casal [1,2(*)], Pedro Soares [1,2], Isabel Soares-Silva [1,2]

[1] *IB-S – Institute of Science and Innovation for Bio-Sustainability, University of Minho, Portugal*

[2] *CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal*

[3] *School of Applied Sciences, University of Huddersfield, United Kingdom*

(*) *e-mail: mcasal@bio.uminho.pt*

Keywords: genomics; plasma membrane transporters; biotechnology.

ABSTRACT

The yeast *Cyberlindnera jadinii* is an appealing platform for industrial applications due to its intrinsic robust fermentation features and ability to utilize a wide range of substrates, including hexoses, pentoses, amino acids, and carboxylic acids[1]. However, *C. jadinii* is still lagging behind when compared to other non-*Saccharomyces* yeasts, mainly due to the inexistence of extensive knowledge on its metabolism, regulatory networks, and transport mechanisms. The genomic characterization of different *C. jadinii* strains is crucial to reveal the genetic features underlying the existing interspecies variability. Thus, we sequenced the genome of four *C. jadinii* strains by Illumina NGS: two wild strains isolated from agro-food by-products (TB104, TB115) and two from the Portuguese Yeast Culture Collection (PYCC2578, PYCC3092). The four strains presented a highly variable number of predicted proteins, from 4600 to 5600 The total genome length size was higher in wild isolates (~20 Mbp) than in PYCC strains (~18 Mbp). In addition, the publicly available genomes from the strains NBRC0988, Y-1542, Y1112B, and CBS1600 were also included in this analysis. In total, we explored the genomes of eight *C. jadinii* strains, including a ploidy analysis and intraspecies variability. A PCA analysis revealed the presence of three major groups that correlate with the strain origin. In addition, we analysed the predicted transportome of the *C. jadinii* NRRL Y-1542 strain, which corresponds to ~10 % of its proteome, revealing an enrichment in carboxylic acid transporters when compared to *Saccharomyces cerevisiae*. The exploration of the *C. jadinii* genome, including the prediction of metabolic pathways combined with the study of its predicted transportome, paves the way for better exploitation of this biotechnologically relevant yeast species.

References:
1 Sousa-Silva, M. et al. J. Fungi 2021, 7, 36.

Acknowledgements:
This work was supported by the Strategic Programme UID/BIA/ 04050/2020 and the project LA/P/0069/2020 granted to the Associate Laboratory ARNET, both funded by Portuguese funds through the FCT-IP.

P5.26 - STRUCTURE-FUNCTION STUDIES OF THE *SCHIZOSACCHAROMYCES POMBE* DICARBOXYLIC ACID TRANSPORTER MAE1

Tayane Fontes [1,2], João Alves [1,2], Filipe Kuhlmann [1,2], Pedro Soares [1,2], Sandra Paiva [1,2], Margarida Casal [1,2], Isabel Soares-Silva [1,2]

[1] *IB-S – Institute of Science and Innovation for Bio-Sustainability, University of Minho, Portugal*

[2] *CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal*

(*) *e-mail: id9021@alunos.uminho.pt*

Keywords: Carboxylic acids; Transporter Proteins; Site-directed mutagenesis.

ABSTRACT

The industrial production of organic acids by microbial cell factories is severely influenced by the ability to import carbon and energy sources, as well as to export the desired organic acid. Thus, the optimization of transport mechanisms plays a fundamental in the overall efficiency of this process. Nevertheless, transporter engineering is not straightforward as the limited knowledge of transport mechanisms hampers the functional and structural characterization of membrane proteins [1]. The dicarboxylate transporter Mae1 from *Schizosaccharomyces pombe* belongs to the Tellurite-resistance/Dicarboxylate Transporter (TDT) family and is responsible for the transport of malate and succinate through the plasma membrane. Mae1 was initially described as a dicarboxylate proton symporter, promoting the intracellular accumulation of dicarboxylates[2]. In addition, it is also able to promote the export of these substrates, as its expression in carboxylic acid-producing strains leads to increased titers of succinic, malic and fumaric acid[3]. Here, we used *in silico* tools for 3D structure prediction and molecular docking of Mae1, to unravel the amino acid residues involved in substrate binding sites and translocation through the protein pore. Seventeen amino acid residues were selected for site-directed mutagenesis, based on predicted interactions with substrates, conservation status within the TDT family, and localization in the constriction zones of the pore. The mutant alleles of Mae1p were expressed in the *Saccharomyces cerevisiae* and the ability of cells to grow on different carbon sources was evaluated. Altered growth phenotypes were obtained in cells expressing Mae1 mutant alleles, due to altered protein activity or subcellular localization. Further studies are ongoing to fully characterize these mutants, increasing our knowledge of the structure of the Mae1 transporter.

References:

[1] Soares-Silva et al. (2020) FEMS Microbiol Lett, 367(15):fnaa118

[2] Sousa M.J. *et al.* (1992) Yeast, 8(12), 1025-1031

[3] Darbani B. *et al.* (2019) PNAS, 116(39), 19415-19420

Acknowledgements:
This work was supported by the Strategic Programme UID/BIA/ 04050/2020 and the project LA/P/0069/2020 granted to the Associate Laboratory ARNET, both funded by Portuguese funds through the FCT-IP.

P5.27 - GLYCINE BETAINE ROLE IN *STAPHYLOCOCCUS EPIDERMIDIS* PH ADAPTATION AND PATHOGENICITY

Luís G. Gonçalves [1(*)]; Elisabete Morais [1]; Susana Santos [1]; Jean Armengaud [2]; Maria Miragaia [1]; Ana V. Coelho [1]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

[2] *Département Médicaments et Technologies pour la Santé (DMTS), SPI, Université Paris-Saclay, CEA, INRAE, 30200 Bagnols-sur-Cèze, France*

(*) *e-mail:* lgafeira@itqb.unl.pt

Keywords: *Staphylococcus epidermidis*; Metabolomics and Proteomics; Glycine betaine; pH adaptation; pathogenicity.

ABSTRACT

Glycine betaine (GB) is a positively charged quaternary ammonium linked to glycine. The most common role of GB is as osmolyte, however could be involved in other biologic processes. In Staphylococcus epidermidis (SE), an opportunistic pathogen skin bacteria colonizer, GB has been described as an osmolyte. In an integrative proteomic and metabolomic study to understand the metabolic adaptation to physiologic pH changes, mimicking the transition from the skin (pH 5.5) to blood (pH 7.4) of a commensal and a pathogenic SE strain. At blood pH, despite the lower levels of the enzymes involved in GB synthesis, choline dehydrogenase (CDH) and betaine aldehyde dehydrogenase (BADH), and the betaine transporters, BetL and OpuCA; the GB levels are 4 times higher in the commensal than in pathogenic strain. The GB extracellular profiles are similar for both strains, being exhausted from the media during growth. At skin pH, the commensal strain GB levels are equivalent to those at blood pH but 2.5 times higher than those of the pathogenic strain. In this condition, commensal strain CDH and BADH levels increase becoming like those of the pathogenic strain. GB extracellular levels for both strains decrease until mid-exponential phase, then increase, suggesting that this metabolite is exported. For both strains, the lowest GB levels were determined in a situation that mimics SE blood colonization (abrupt transfer from pH 5.5 to 7.4), the same occurs for the GB biosynthetic enzymes. A new role for GB on pH adaptation in SE was disclosed that, interestingly is different between SE commensal and pathogenic strains. New studies are being performed to better understand this novel GB function and its relationship with environmental pH and pathogenicity.

Acknowledgements:
CERMAX (AAC/01/SAICT/2016); MOSTMICRO (LISBOA-01-0145-FEDER-007660 and UID/Multi/04378/2019); FCT (Transnational Cooperation Portugal/France – Pessoa Program and contract according to DL57/2016 (SFRH/BPD/111100/2015))

P5.28 - HETEROLOGOUS EXPRESSION OF A LANM HOMOLOGOUS, FROM STRAIN *MESORHIZOBIUM QINGSHENGII* J19, INVOLVED IN YTTRIUM IMMOBILIZATION

Carina Coimbra [1], Rita Branco [1], Paula V. Morais [1(*)]

[1] *University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, Coimbra, Portugal.*

(*) *e-mail: pvmorais@ci.uc.pt*

Keywords: yttrium accumulation; rare earth elements; EF-hand motifs; protein expression.

ABSTRACT

Yttrium (Y) is a metallic element belonging to the group of rare earth elements (REE) that has high industrial and economic importance due to its unique properties (luminescence, magnetism, and resistance). Although Y is considered non-essential for living organisms, its role as an essential or toxic element is not well defined. Lanthanides (LnIII) are assumed to bind to external surfaces due to carboxylate and phosphate groups, as demonstrated for europium in *Bacillus thuringiensis*. Research has been done on biosorption as well as on the localization of some REE in bacterial cells, but knowledge regarding bacteria-Y interactions is currently scarce, thus becoming a topic of considerable interest. A periplasmic protein lanmodulin (LanM), which contains four carboxylate-rich EF-hand motifs, was reported to confer metal ion binding cooperativity. Its structural analysis confirmed the Y-LanM binding. Based on genome analysis of strain *Mesorhizobium qingshengii* J19 (a great Y bioaccumulator), it was identified a gene encoding a protein homologous to LanM named EF-hand domain-containing protein. To validate and study the functionality of this gene for metal binding, the *EF-hand* gene from strain J19 was exploited through overexpression in *Escherichia coli* BL21 cells. Protein expression in the presence of Y was confirmed by SDS-PAGE, exhibiting the expected molecular mass weight of approximately 18 kDa. Furthermore, these engineered cells were tested for Y accumulation, using R2Ab medium supplemented with increasing Y concentrations (0.01 to 0.4 mM), demonstrating the greatest Y accumulation at the highest tested concentration, up to 3-fold higher than control cells. The results demonstrated that recombinant cells overexpressing the EF-hand protein from strain J19 could be explored as an alternative strategy to improve the Y recovery from the environment.

Acknowledgements:
This work was supported by the projects Biorecover from the European Union's Horizon 2020 research and innovation program under grant agreement No 821096 and ERA-MIN 2 REVIVING ERA-MIN-2019_67 from the Fundação para a Ciência e Tecnologia (FCT) and sponsored by the FEDER funds through the program COMPETE – Programa Operacional Factores de Competividade – and by national funds through FCT, under projects UIDB/00285/2020, LA/P/0112/2020 and UIDB/04564/2020. Carina Coimbra was supported by POCh (cofounded by the European Social Fund and national funding by MCTES), through a PhD research grant (SFRH/BD/147292/2019) from FCT.

P5.29 - BIOCOMPATIBLE ENGINEERED POLYUREA (PURE) DENDRIMERS ARE A NEW HOPE AGAINST MULTIDRUG-RESISTANT (MDR) BACTERIA

Dalila Mil-Homens [1], Laura Pereira [2], Rita F. Pires [1], José M. Andrade [2], Marta M. Alves [3], Gabriel Serafim [1], Nuno Martinho [1], Manuel Melo [2], Arsénio M. Fialho [1,4], Vasco D.B. Bonifácio [1,4], Sandra N. Pinto [1(*)]

[1] iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Portugal

[2] Instituto de Tecnologia Química Biológica António Xavier, Universidade NOVA de Lisboa, Portugal

[3] Centro de Química Estrutural (CQE), Institute of Molecular Sciences, Instituto Superior Técnico (IST), Universidade de Lisboa, Lisboa, Portugal

[4] Bioegineering Department, Instituto Superior Técnico, Portugal

[(*)] e-mail: sandrapinto@ist.utl.pt

Keywords: Antimicrobial Dendrimers; Multidrug-resistant (MDR) bacteria; anti-biofilm activity; confocal microscopy.

ABSTRACT

Infectious diseases caused by bacteria are a disturbing societal burden that puts in risk millions of people worldwide. This is a problem of major concern and utmost priority especially due to the emergence of novel resistant pathogens and infectious diseases. In this sense, the development of new antimicrobial agents with novel mechanisms of action is of critical importance. In 2017, the World Health Organization (WHO) made an appeal to the scientific community, stating that R&D strategies should focus on the discovery and development of new antibiotics.

Considering this scenario, we developed engineered polyurea (PURE) dendrimers, densely positive-charged core-shell nanoparticles that mimic antimicrobial peptides, as a novel class of antimicrobial agents. Novel cationic core-shell PURE dendrimers were synthetized following a sustainable protocol. The antimicrobial capacity (MIC and MBC values and colony count kinetic assay) was tested against several multidrug-resistant (MDR) bacteria. In addition, we also preformed distinct assays (e.g. confocal microscopy) to study the effectiveness of PURE dendrimers against *L. monocytogenes* biofilms.

The results obtained point towards a high efficiency of dendrimers in the treatment of MDR infections without causing significant toxicity against *in vitro* healthy cells model and Galleria mellonella *in vivo* model. We also found that polycationic core-shell dendrimers have an impact on cell density and biofilm adhesion. Using electron microscopy and coarse-grained molecular dynamics simulations it was possible to observe that the dendrimers display a disruptive action at the membrane level.

In a scenario of increasing resistant pathogens and infectious diseases, cationic core-shell PURE dendrimers emerge has a step forward in the development of effective and reliable antibiotics.

Acknowledgements:
This study was supported by funds received from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects SentinelEVs (EXPL/BTM-MAT/0910/2021) and DENDRICARE (2022.03627.PTDC); the project supporting the Research Unit Institute for Bioengineering and Biosciences (UIDB/04565/2020) and the project supporting Institute for Health and Bioeconomy, i4HB (LA/P/0140/2020).

P5.30 - RNA-SEQUENCING ANALYSIS REVEALS KEY CHANGES IN *G. VAGINALIS* TRANSCRIPTOME AFTER ANTIMICROBIAL CHALLENGE IN POLYMICROBIAL BIOFILMS

Lúcia G. V. Sousa [1], ***Vânia Pinheiro*** [1], ***Nuno Cerca*** [1,2(*)]

[1] *Centre of Biological Engineering (CEB), Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal*
[2] *LABBELS –Associate Laboratory, Braga, Portugal*

[(*)] *e-mail: nunocerca@ceb.uminho.pt*

Keywords: Bacterial vaginosis; polymicrobial biofilms; recurrence.

ABSTRACT

Bacterial vaginosis (BV) affects about 30% of women worldwide and is characterized by an increase of anaerobic bacteria and a reduction of protective *Lactobacillus* in the vaginal microbiota. The presence of a polymicrobial biofilm in the vaginal epithelium is a well-known feature of BV, despite the poor knowledge about the etiology of the infection. *Gardnerella* species are the most common bacteria found in cases of infection and thus, its role cannot be neglected. In this work, we aimed to study how the transcriptome of *G. vaginalis* is affected after challenging with different antimicrobial agents, when growing in the presence of a polymicrobial community typical of BV.

Five-species biofilms were prepared and incubated at anaerobic conditions. At 48h, the biofilms were incubated with different antimicrobial agents, namely, metronidazole, clindamycin, and essential oil (EO) from *Thymbra capitata*, which we previously showed to have a strong antimicrobial potential against BV polymicrobial biofilms. After 72h, RNA was extracted from the biofilms and sent for sequencing using an Ilumina Novaseq platform.

Our data demonstrated that three agents have distinct effects on the transcriptional response of *G. vaginalis*. A Euclidian distance cluster analysis demonstrated that the EO was the antimicrobial that had the lower influence in the global gene expression, with both antibiotic challenges resulting in a more diversified gene expression adaptation. However, when looking at more than 5-fold up- or down-regulated genes, clindamycin challenge was the condition that resulted in a lower number of highlighted genes, followed by the EO challenge. Metronidazole, the drug of choice for treatment in Portugal, resulted in the most striking effect on *G. vaginalis* transcriptome.

Ongoing work is now focused on understanding how the most affected genes in *G. vaginalis* transcriptome can contribute to the virulence or possible role in recurrence of BV.

Acknowledgements:
This work was funded by the Portuguese Foundation for Science and Technology (FCT), under the scope of the exploratory project EXPL/SAU-INF/1000/2021. LS acknowledges FCT the financial support of individual Grant 2020.04912.BD.

P5.31 - EXPLORING THE STRATEGIES OF ADAPTATION OF *STAPHYLOCOCCUS EPIDERMIDIS* TO THE HOSPITAL ENVIRONMENT USING A PANGENOMIC APPROACH

Ana Botelho [1(*)], ***Henrik Westh*** [2], ***Joana Rolo*** [3], ***Maria Miragaia*** [1]
[1] *Laboratory of Bacterial Evolution and Molecular Epidemiology, MOSTMICRO, ITQB NOVA, Oeiras, Portugal.*
[2] *Hvidrove Hospital, Copenhagen, Denmark*
[3] *CICS-UBI — Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

[(*)] *e-mail: abotelho@itqb.unl.pt*

Keywords: *Staphylococcus epidermidis*; 1960s; recombination.

ABSTRACT

Staphylococcus epidermidis is a common skin commensal but has emerged as an opportunistic pathogen during the 1960s. Since the 1990s it has established as one of the main causes of medical device-associated infections in hospitals. But the mechanisms of adaptation to a pathogenic lifestyle are still elusive. We aim to contribute to the understanding of the strategies used by *S. epidermidis* to adapt to the clinical setting.

A collection of 35 *S. epidermidis* isolates from colonisation and infection, collected during the 1960's and the 1990's in Denmark, was sequenced by NextSeq (Illumina). Strains relatedness was evaluated by SNP analysis of the core genes and recombination was calculated using Gubbins. The presence/absence of accessory genes was assessed through pangenome construction using PROKKA/Roary. Gene presence frequency was determined for 1960s and 1990s isolate groups, percentiles were defined and difference in frequencies between groups calculated for 1st percentile genes.
1960s and 1990s isolates were grouped into two clusters and were frequently intermixed in the phylogenetic tree, suggesting core genetic background conservation overtime. However, the number of recombination blocks and the number of mutations inside recombination blocks were significantly higher in 1990s isolates when compared to the 1960s (p-value<0.05). The pangenome contained 4735 genes. Genes most frequent in the 1960s group were related to: insertion sequences (IS200 family), bacteriophages, plasmid replication, arsenic and mercury resistance, oxidative stress resistance, sugar transport iron uptake and host-microbe interactions. Genes most frequent in the 1990s group were related to: IS1595 and IS256 families; SCC*mec*; ACME; resistance to penicillin, antiseptics, mercury, copper and zinc; recombination; Restriction/Modification systems; and secretory antigens.

Our results suggest that adaptation of *S. epidermidis* to the clinical setting involved a genome remodelling towards an increased ability to recombine and acquire mobile genetic elements carrying antimicrobial resistance and immune evasion genes.

Acknowledgements:
This work was supported by FCT - Fundação para a Ciência e a Tecnologia, I.P., through MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020, UIDP/04612/2020), LS4FUTURE Associated Laboratory (LA/P/0087/2020) and PhD Studentship (2022.11966.BD)

P5.32 - TOWARD COST-EFFECTIVE PRODUCTION OF RECOMBINANT HUMAN SERUM ALBUMIN FOR USE IN MYCOTOXIN CAPTURE DEVICES

Diana G. Rodrigues [1], ***Heidar Adalsteinsson*** [1], ***Lavínia Pinto*** [1], ***Tatiana Q. Aguiar*** [1,2], ***Carla Oliveira*** [3], ***Luís Abrunhosa*** [1,2], ***Lucília Domingues*** [1,2(*)]

[1] *CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[3] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[(*)] *e-mail: luciliad@deb.uminho.pt*

Keywords: Human serum albumin; *Pichia pastoris*; Recombinant production; Mycotoxin binding.

ABSTRACT

Human serum albumin (HSA) is a monomeric multidomain macromolecule with wide clinical and biotechnological applications. Thus, there is a high demand for HSA, which is increasing due to new emerging applications. Among these, the extraordinary ligand binding capacity of HSA has been recently explored to capture mycotoxins from food and feed matrices. However, HSA is currently produced commercially primarily from human plasma, which conveys supply limitations. Recombinant production may be a solution to overcome this obstacle. Among the expression systems explored for HSA production, the yeast *Pichia pastoris* has drawn attention for its simplicity of genetic manipulation and high-cell density in low-priced medium, along with efficient secretory capacity and ability to form disulfide bonds, which are major advantages over bacterial systems.

Envisioning cost-effective production of recombinant HSA for its widespread use in mycotoxin capture devices, *P. pastoris* KM71H was transformed with the pPICZ9ssHSAH6 vector to produce recombinant HSA in fusion with the His6 tag at the C-terminal (rHSA). After selecting the best-performing clones, minimal and complex media were tested for rHSA production in shake-flask. The best production of stable extracellular rHSA was achieved in complex medium within 96 h of methanol induction at nearly 0.5 g/L culture. Following rHSA purification by immobilized metal ion affinity chromatography, fluorescence quenching studies were used to assess the suitability of the produced rHSA for the capture of different mycotoxins. The binding constants (Ksv, L/mol) obtained for rHSA and commercial HSA were: 8.29×10^5 and 6.22×10^5 for ochratoxin A, 6.02×10^4 and 2.97×10^4 for zearalenone, and 1.26×10^4 and 9.50×10^3 for patulin, respectively. rHSA thus displayed results that matched those of native HSA, forming strong complexes with these mycotoxins.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and project MycoProAffinity 2022.03438.PTDC (https://doi.org/10.54499/2022.03438.PTDC).

P5.33 - EXPLORING *HANSENIASPORA GUILLIERMONDII* AS A CELL FACTORY: INSIGHTS INTO ACETATE ESTER PRODUCTION AND ETHANOL TOLERANCE MECHANISMS

Isabel Seixas [1,2]***, Diogo Santos*** [3,4(*)]***, Nuno P. Mira*** [3,4]***, Ana Mendes-Ferreira*** [1,2]

[1] *WM&B - Laboratory of Wine Microbiology & Biotechnology, Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[2] *BioISI-Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa Campo Grande, Lisboa, Portugal*

[3] *iBB, Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.*

[4] *Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.*

[(*)] *e-mail: diogo.alexandre.santos@tecnico.ulisboa.pt*

Keywords: *Hanseniaspora guilliermondii;* non-*Saccharomyces* yeasts; alcohol acetyltransferases; acetate esters and higher alcohols; transcriptome; ethanol stress; wine yeasts..

ABSTRACT

Hanseniaspora guilliermondii is a non-conventional yeast with recognized potential to modulate wine aroma, mainly through the production of volatile acetate esters and higher alcohols associated with fruity and floral aromas. Recently, the first genomic sequence of a *H. guilliermondii* strain was disclosed by our laboratories, revealing what appears to be a novel family of alcohol acetyltransferases (AATs), enzymes involved in acetate ester production. Following functional analysis of these AAT genes, we found that *H. guilliermondii* overproduces acetate esters and higher alcohols at low carbon-to-assimilable nitrogen ratios, using amino acids as precursors, in striking contrast to *Saccharomyces cerevisiae*, which produces these volatiles at high C:N ratios and using carbohydrates of central carbon metabolism. The higher acetate ester production was accompanied by a higher expression of the HgAATs genes, confirming their involvement in this process.

On a complementary angle we also address, for the first time, the response of *H. guilliermondii* to ethanol-induced stress, considering that ethanol tolerance is a major bottleneck in the exploration of this species. Thus, the transcriptome profile of *H. guilliermondii* in response to ethanol (6% v/v during 1h) was analyzed using high-throughput RNA sequencing and leveraging the previously obtained genomic sequence. In these conditions, 392 genes were down-regulated and 305 up-regulated, 55 of which being believed to be specific to the *Hanseniaspora* genus. Besides general stress responses (such as upregulation of genes involved in energy generation or protein folding), ethanol exposure activated several genes involved in lipid metabolism, particularly in phospholipid and ergosterol biosynthesis, likely reflecting an adaptation of membrane composition to respond to the deleterious effects of ethanol. Altogether, these results contribute to elucidate fundamental aspects of *H. guilliermondii*'s biology and physiology, advancing its potential to be used in the wine industry as a bioflavorant and also as a cell factory for production of add- value volatiles.

Acknowledgements:
This study was supported by funds received from FCT—Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects SMARTWINE (PTDC/AGR-TEC/3315/2014); SMARTWINE 2.0 (2022.06777.PTDC) and for the
contracts supporting the Research Units for Biosystems and Integrative Sciences Institute (UIDB/04046/2020) and Institute for Bioengineering and Biosciences (UIDB/04565/2020) and for the project supporting the Associate Laboratory Institute for Health and Bioeconomy—i4HB (LA/P/0140/2020).

P5.34 - REDUCING POWER IN *GEOBACTER*'S PERIPLASM: NMR INTERACTION STUDIES BETWEEN TRIHEME CYTOCHROMES

Leonor Morgado [1(*)], ***Marisa R. Ferreira*** [1], ***Carlos A. Salgueiro*** [1]
[1] *Associate Laboratory i4HB - Institute for Health and Bioeconomy UCIBIO - Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal.*

[(*)] *e-mail: mlmorgado@fct.unl.pt*

Keywords: *Geobacter*; multiheme cytochromes; electron transfer; NMR.

ABSTRACT

The analysis of several *Geobacter* genome's revealed abundant cytochromes with multiple duplications for some families of proteins (1). From the different strains analyzed, *G. sulfurreducens* has been the model organism for the study of the complex electron transfer networks of exoelectrogens and the development of genetic systems for its manipulation has been the basis for several studies aiming to identify the key players in each metabolic pathway. In these studies, the apparent redundancy was shown to be necessary since the proteins were found to be involved in different electron transfer routes. For the family of triheme periplasmic cytochromes PpcA-E, knockout and complementation mutants revealed that in the absence of all other homologs, any of the proteins was able to support wild-type levels of Fe(III) reduction (2). However, in contrast, RNA transcriptomics showed that the five triheme cytochromes are differently expressed depending on the final acceptor (3). The combination of both studies suggests that despite the specificity towards terminal electron acceptors found for other cytochromes, the periplasmic cytochromes are promiscuous and able to interact with different redox partners.

By exploring the distinctive Nuclear Magnetic Resonance (NMR) spectroscopic signatures of PpcA-E (4), pairwise interactions and electron transfer reactions between the five cytochromes were performed. The results show that the five proteins can exchange electrons between each other while forming transient and unspecific redox complexes. This suggests that the constitutively expressed pool of cytochromes in *Geobacter* cells can synergistically establish and maintain a proper periplasmic redox potential providing reducing power to the cell to ensure electron transfer across the periplasm independently of the terminal acceptor or even a specific redox partner.

References

1. JE Butler *et al* (2010) BMC Genomics 11.
2. S Choi *et al* (2022) J Bacteriol 204, e0032222.
3. K Joshi *et al* (2021) Mol Microbiol 116, 1124.
4. JM Dantas *et al* (2011) Dalton Trans 40, 12713.

Acknowledgements:
This work was supported by Fundação para a Ciência e a Tecnologia through the following grants: SFRH/BD/132969/2017 (MRF), PTDC/BIA-BQM/4967/2020 (CAS), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO), and LA/P/0140/2020 (i4HB).

P5.35 - EXPLORING THE BIOSYNTHETIC CAPABILITY OF A CYANOBACTERIUM FROM LEGE-CC FOR THE PRODUCTION OF NOVEL COMPOUNDS

Dora Ferreira [1], Leonor Ferreira [1,2], Ralph Urbatzka [1], Joana Almeida [1], Mariana Reis [1(*)]

[1] CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

[2] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

[(*)] e-mail: mreis@ciimar.up.pt

Keywords: cyanobacteria; natural products; biosynthetic gene cluster; metabolism.

ABSTRACT

Cyanobacteria are photosynthetic microorganisms with undeniable biotechnological potential. Given their remarkable ecophysiological diversity and metabolic plasticity, they produce a wide array of unique secondary metabolites that have shown diverse bioactivities. On this basis, a cyanobacterium from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC, CIIMAR) was found to produce novel anticancer compounds in a previous bioactivity-guided project. Genome mining of this strain revealed the putative biosynthetic gene clusters (BGC) related to the cytotoxic compounds, as well as other BCGs whose products we were unable to identify by untargeted metabolomics analysis. Lack of natural environmental cues in the usual cultivation conditions has been linked to the absence or poor expression of BGCs in the lab setting. Successful strategies for awakening such BGCs in heterotrophic bacteria and fungi involve altering cultivation conditions. Its use in cyanobacteria is, however, understudied.

The main goal of this study was to investigate how changing cultivation parameters affected the production of known BGC products (anticancer compounds), and whether it also induced the production of silent or cryptic BGC products in a cyanobacterium from LEGE-CC. The strain was grown in photobioreactors, under different light conditions (white, blue, and red) and photoperiods (16/8h light/dark cycle and 24h light). Methanolic extracts of biomass and culture media were analyzed by HRLC-MS/MS to investigate the chemical profile in different culture conditions. The results of the metabolomics analysis will be presented, demonstrating whether the various growth conditions affected the metabolome and whether the silent/cryptic compound was discovered. Bioactivities for anticancer, anti-fouling, anti-obesity, and antimicrobial will also be presented and compared to metabolomics profiles.

Acknowledgements:
CIIMAR's strategic fund support(FCT: UIDB/04423/2020 and UIDP/04423/2020). Recovery and Resilience Plan co-funded by the Portuguese Republic and the European Union, ProjectNo. C644915664-00000026 (WP2 Vertical Bivalves and WP9-Portuguese Blue Biobank), application to NoticeNo. 02/C05-i01/2022. JA acknowledges FCT2022.03876.CEECIND. LF acknowledges FCT2022.11979.BD

P5.36 - ENHANCING LACTONE BIOSYNTHESIS IN *ASHBYA GOSSYPII*: TOWARDS SUSTAINABLE PRODUCTION FROM DIVERSE CARBON SOURCES

Carlos E. Costa [1,2(*)], ***Luís Freitas*** [1], ***Miguel Francisco*** [1], ***Tatiana Q. Aguiar*** [1,2], ***Lucília Domingues*** [1,2]

[1] *CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[(*)] *e-mail: carlos.costa@ceb.uminho.pt*

Keywords: *Ashbya gossypii,* lactones, metabolic engineering, biosynthesis, circular economy.

ABSTRACT

Lactones are volatile organic compounds sourced from lipid metabolism, with diverse applications, especially fragrances and flavours, being naturally present in fruits such as strawberries and peaches. Traditional lactone production is attained by biotransformation of hydroxylated fatty acids, which relies on limited precursor resources, such as vegetable or fish oils. However, the filamentous fungus *Ashbya gossypii* can naturally synthesize γ-lactones *de novo* from varied carbon sources [1]. This extended range of substrates can enable its production from several renewable resources. This study aims to enhance lactone biosynthesis by improving its biosynthetic pathway in *A. gossypii* strains and optimising production conditions. Different *A. gossypii* strains were genetically modified to intensify lactone biosynthesis, with emphasis on the enzymes like desaturases, involved in fatty acid synthesis. The fermentation conditions were optimized considering factors like C/N ratio, different carbon sources, nutritional supplementation, and oxygenation levels. These optimal conditions were then evaluated in a bioreactor configuration, which increased total lactone content over 2-fold. Finally, we successfully demonstrated the feasibility of using different wastes for lactone production. This study not only underscores the potential of recombinant *A. gossypii* strains in elevating lactone production but also introduces sustainable avenues for lactone synthesis using waste products. These findings provide promising insights for commercial lactone production and future applications.

References:

[1] Silva, R. et al. Metabolic engineering of *Ashbya gossypii* for deciphering the de novo biosynthesis of γ-lactones. *Microb Cell Fact* 18, 1–11 (2019).

Acknowledgements:

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, the ESSEntial Project (PTDC/BII-BTI/1858/2021), and PhD grant 2023.04144.BD to M.F.

P5.37 - PHYSIOLOGICAL DIFFERENCES IN EXPONENTIAL AND STATIONARY PHASE CELLS OF *DEBARYOMYCES HANSENII* IN RESPONSE TO NACL

Tomás Gonçalves [1], ***Catarina Prista*** [1,2*]
[1] *Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal*
[2] *LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Lisbon, Portugal*

(*) *e-mail: cprista@isa.ulisboa.pt*

Keywords: Microalgae biomass, wheat bread, sourdough, microbiota, bioactivity.

ABSTRACT

Debaryomyces hansenii is an unconventional yeast species remarkable for its attributes that holds significant promise for several biotechnological applications. Its notable capacity to grow in NaCl-rich environments, even when potassium concentrations are low, renders it especially intriguing.
The primary objective of this investigation was to compare several physiological parameters of cells cultivated in mineral media containing 1 M NaCl and those grown in the presence of 1 M KCl. Particular emphasis was made on the comparison between cells in exponential and stationary growth phase, focusing on growth kinetics, glucose utilization and transport, and intracellular concentrations of trehalose and Na+, K+, and Pi. Additionally, we assessed intracellular pH, proton efflux, and proton membrane permeability.
In terms of growth parameters, both 1 M NaCl and 1 M KCl-cultivated cells reached the stationary phase without depleting their carbon and energy sources. The response exhibited similarities for both cations, albeit with a longer latency period and slightly lower final biomass in the presence of NaCl.
Regarding other cell parameters, the behavior of 1 M NaCl and 1 M KCl grown cells varied depending on the type of cation and growth phase. These discrepancies were more pronounced for intracellular contents, proton fluxes, intracellular pH, and glucose transport. Notably, for both cations, exponential phase cells grown displayed more dissimilarities than stationary phase cells. Exponential phase cells cultivated with 1 M NaCl exhibited higher proton efflux and reduced proton influx rates, similar K+ content, and higher pHi_n compared to those grown with 1 M KCl.
The findings suggest that distinct resistance mechanisms may be present in cells at different growth stages. Additionally, they confirm that, despite its significant intracellular accumulation, sodium does not exert toxic effects and does not impede cellular metabolism, affirming the adaptability of Debaryomyces hansenii's cellular physiology to sodium presence in the growth medium.

Acknowledgements:
This work was financially supported by: Portuguese Foundation for Science and Technology (FCT), UIDB/04129/2020 of LEAF Research Center.

P5.38 - INTERACTION OF *ACINETOBACTER* SPP. AND *CANDIDA SPP.* IN DUAL MIXED BIOFILMS

Ana Patrícia Abrantes [1], Tiago Lima [1,2,3], Sara Domingues [1,2], Gabriela Jorge da Silva [1,2(*)]
[1] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[2] Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal
[3] CIVG – Vasco da Gama Research Center / EUVG – Vasco da Gama University School, Coimbra, Portugal

(*) e-mail: gjsilva@ci.uc.pt

Keywords: polymicrobial biofilm; interkingdom; virulence; fungi.

ABSTRACT

Fungal-bacterial infections are being increasingly recognized in clinical settings, and the interaction between these species in polymicrobial biofilms often lead to infections that are highly resistant to treatment. The biological relevance of microbial interactions remains largely unknown. The main objective of this study was to evaluate the microbial interaction in an interkingdom biofilm formation by using *Acinetobacter* spp. and *Candida* spp. and evaluate the effect of antibiotics.
Different clinical *Acinetobacter* and *Candida* species were used. The biomass was evaluated in single species and mixed biofilms by the crystal violet method after 24h of incubation, and also in the absence and presence of antimicrobials. *C. albicans* virulence was assessed by microscopic observation of the formation of hyphal in the absence and presence of *Acinetobacter* spp.
Biofilm production was affected by the medium and the relative quantity of cells. The results of the interaction were not uniform, and varied according the species. *C. albicans* YP0037 formed pseudohyphae in contact with *A. baumannii* 319 and *A. bereziniae* 118, while *C. tropicalis* M152540979 developed pseudohyphae both in the presence and absence of bacteria. Addition of sub-MIC of ciprofloxacin before biofilm incubation showed that mixed biofilms of *A. baumannii* 319 with *C. albicans* YP0037 and *C. glabrata* M14331 produced more biomass than single species biofilm, while with *C. tropicalis* M152540979, biofilm biomass was reduced, as well as with other Acinetobacter and Candida species. Sub-MICs of gentamicin added to an 8 hour-biofilm lead to an increase of biomass of the mixed biofilm.
Both synergistic and antagonistic interaction were observed, depending on the microbial species. Antibiotics increased the biomass. Virulence of fungi can be enhanced in mixed biofilms. Overall, the study demonstrates the complex interactions of polymicrobial biofilms that can affect efficacy of conventional therapy, urging for the study of mechanistic interactions to find new therapeutic strategies.

P5.39 - DEVELOPMENT OF GENETICALLY ENCODED BIOSENSORS FOR PLASMODIUM FALCIPARUM

Ana Pinheiro, ***Adriana Gonçalves, Pedro Ferreira***
Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

(*) e-mail: b8677@med.uminho.pt

ABSTRACT

Malaria is one of the major illnesses worldwide. The highest incidence occurs in children under five years. *P. falciparum* is the most virulent *Plasmodium spp.* associated with most of the deaths and severe cases of the disease.

Antimalarial drugs are used worldwide as a first-line defense against the disease, with the artemisinin combined therapies (ACTs) being the most effective in controlling the disease symptoms and slowing the rate of infections. However, an antimalarial resistance problem is widespread for most treatments deployed against *P. falciparum*. Therefore, urges to better understand the molecular mechanisms underlying drug resistance.

Intracellular redox metabolism is a ubiquitous system associated with most cellular processes. This metabolism is crucial for redox balance and antioxidant defense and is involved in drug action and resistance mechanisms. Besides being vital for erythrocytic stage survival, glutathione is also engaged in CQ resistance and, more recently, has been associated with ACTs resistance.

It is crucial to better understand the biology of the parasite. However, the lack of molecular tools for this organism limits the knowledge of parasite-host interactions and mechanisms of drug action. hGrx1-roGFP2 is a fluorescent genetically encoded biosensor successfully tested in *P. falciparum* redox studies. It has been described as a powerful tool in understanding the parasite redox metabolism, as it makes possible in vitro real-time studies.

Therefore, we propose developing new genetically encoded biosensors to go deeper into studying fundamental mechanisms in *P. falciparum*. We aim to export hGrx1-roGFP2 into the parasitized erythrocyte and perform parasite-host interaction studies. The signal peptide PEXEL - a trafficking motif that signals proteins to export - will be coupled to the sensor and integrated into the P. falciparum genome. We also propose to develop a biosensor with red fluorescence emission. If successfully made, these sensors will allow different analyses of the parasite redox behaviour.

P5.40 - EXPLORING OXIDATIVE STRESS PATHWAYS IN G. SULFURREDUCENS: THE REDOX NETWORK BETWEEN MACA PEROXIDASE AND TRIHEME PERIPLASMIC CYTOCHROMES

Pilar C. Portela [1, *], Leonor Morgado [1], Marta A. Silva [1], Lukas Denkhaus [2], Oliver Einsle [2], Carlos, A. Salgueiro [1]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy and UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal

[2] Institut für Biochemie, Albert-Ludwigs-Universität, Freiburg, Germany

(*) email: md.portela@campus.fct.unl.pt

Keywords: *Geobacter*, oxidative stress, cytochrome, peroxidase, electron transfer, protein-protein interactions, NMR

ABSTRACT

Geobacter sulfurreducens bacterium performs extracellular electron transfer, delivering the electrons originating from its metabolism to extracellular electron acceptors such as iron, uranium or electrodes [1]. This led to the development of Geobacter-based biotechnological applications for the bioremediation of contaminated waters or the production of electrical energy [1]. However, G. sulfurreducens' low oxygen tolerance poses a bottleneck in the improvement of biotechnological applications. *G. sulfurreducens* reclassification from strictly anaerobe to microaerotolerant offers an opportunity to overcome this by developing more oxygen-resistant *G. sulfurreducens* strains [2]. Recently, genetic studies suggested: (i) a possible involvement of all the triheme periplasmic cytochromes (PpcA-E) in the protection pathway from oxidative stress [3] and (ii) MacA's diheme peroxidase involvement in these same pathways. Evidence had already shown PpcA interacted and exchanged electrons with MacA but the exact mechanism remained elusive [4]. In MacA, the reduction of its high potential heme prompts a conformational change that displaces the axial histidine of the low potential heme, enabling it to perform its peroxidase activity for periplasm detoxification. To elucidate if all periplasmic cytochromes could establish a redox partnership with MacA, we investigated both the electron transfer reactions and the molecular interactions between each triheme cytochrome and MacA using Nuclear Magnetic Resonance spectroscopy [5]. Cytochromes possess distinct spectral features in the oxidized and reduced state and, by monitoring these features, we can detect electron transfer between two proteins. In this work, we followed the stepwise electron transfer from reduced PpcA- E cytochromes to oxidized MacA. We have observed full electron transfer from the PpcA-E hemes specifically to MacA's high potential heme that is favored by surface electrostatic complementary.

Our observations indicate that PpcA-E cytochromes can provide reducing power to MacA in oxidative stress situations, supporting the observations in genetic studies that these cytochromes are essential in oxygen detoxification pathways in *G. sulfurreducens*.

References:

[1] D. R. Lovley, et al, in Advances in Microbial Physiology, Vol. 59, 2011, pp. 1-100.; [2] A. M. Speers, G. Reguera, Biofilm 2021, 3, 100052.; [3] S. Choi, C. H. Chan, D. R. Bond, Journal of Bacteriology 2022, 204, e00322-00322; [4] J. M. Dantas, A. Brausemann, O. Einsle, C. A. Salgueiro, FEBS Letters 2017, 591, 1657- 1666.; [5] L. Morgado, C. A. Salgueiro, Metallomics 2022, 14.

Acknowledgements:
Work supported by Fundação para a Ciência e Tecnologia through the grants: PTDC/BIA-BQM/4967/2020 (CAS), 2020.04717.BD (PCP), EXPL/BIA-BQM/0770/2021 (LM), UIDP/04378/2020, UIDB/04378/2020 (UCIBIO), LA/P/0140/2020 (i4HB), ROTEIRO/0031/2013 – PINFRA/22161/2016, co-founded by FEDER through COMPETE 2020, POCI, PORL, PIDDAC. Work also supported by Deutsche Forschungsgemeinschaft (CRC 1381, project ID 403222702) (OE).

MICRO BIOTEC 23

Poster Session

Topic 6

P6.1 - DESIGN OF A SUSTAINABLE TECHNOLOGY FOR ALGAL PROTEIN EXTRACTION: EXPLORATION OF DES-BASED-SALT ATPS FOR C-PHYCOCYANIN PARTITIONING

Bruno Pereira [1]*, Carla Brazinha [1], João G. Crespo [1], Sónia P. M. Ventura [2], Cristina T. Matos [3], Luís Costa [3]

[1] LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, Caparica, Portugal.

[2] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

[3] A4F – Algae for future, Estrada do Paço do Lumiar, Campus do Lumiar, Lisboa, Portugal.

(*) e-mail: bad.pereira@campus.fct.unl.pt

Keywords: Sustainable protein sources; C-phycocyanin (C-PC); Food industry; Biorefinery strategies; Deep Eutectic Solvents (DES); Aqueous two-phase systems (ATPS).

ABSTRACT

In recent years, microalgae have become a focus of interest for their bioactive compounds with applications in nutraceutical, cosmetic, and pharmaceutical industries. [1,2] C-phycocyanin (C-PC), acts as a blue pigment-protein complex, exhibits beneficial health properties, making it valuable for pharmaceutical, cosmeceutical, and food sectors. [2,3] Conventional valorisation methods have adverse environmental and cost impacts, prompting research for sustainable alternatives. Aqueous two-phase systems (ATPS) emerged as an innovative and eco-friendly technique due to its simplicity, low energy requirements, and cost-effectiveness. [4] Among ATPS types, polymer-salt and deep eutectic solvent-salt systems were studied. Several parameters were investigated to improve the stability of the interface between ATPS, assessed by the interfacial tension (IFT) between both aqueous phases, in order to select the most effective extracting ATPS. [5,6]

The study employed various methods, including the preparation of polymer-salt ATPSs using different polymers and salt concentrations, and the synthesis of DES-based ATPSs combined with optimized salt concentrations. Partition coefficients, extraction efficiency data, and IFT measurements were determined through C-PC absorbance measurement using a UV–vis spectrophotometer and an in-built drop analyser.

Results indicated that the IFT of polymer-salt ATPSs was not measurable (Table 1), which could impact C-PC partitioning. Salt concentrations exceeding 24*wt%* resulted in C-PC precipitation, so concentrations between 14*wt%* and 24*wt%* were chosen. Three stable DES-based ATPSs were selected, and their partition coefficient and extraction efficiency for C-PC were evaluated (Table 2).

In conclusion, ATPS interfacial stability plays a vital role in achieving a stable double-phase system. DES-based ATPS showed promise as a separation technology for C-PC recovery. Further research will focus on optimizing parameters related to biomolecule integrity, moving towards a more sustainable and efficient process. The study contributes to the development of green technologies for microalgae protein extraction, addressing the global need for sustainable protein sources.

Table 1. Main results obtained for the polymer-salt ATPS considering the extraction efficiency, and partition coefficient.

Bottom-phase (wt%)	Top-phase (wt%)	Polymer A (16%)				Polymer B (16%)			
		V_R	EE	K_p	IFT (mN/m)	V_R	EE	K_p	IFT (mN/m)
Salt	14	1.1 ± 0.1	0.9 ± 0.0	12.7 ± 2.6	-	1.0 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	-
	19	0.8 ± 0.1	0.8 ± 0.0	7.3 ± 2.6	-	0.7 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	-
	24	0.7 ± 0.1	0.6 ± 0.0	2.8 ± 1.3	-	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	-

Table 2. Main results of the *DES-based-salt* ATPSs.

Bottom-phase (wt%)	Top-phase (wt%)	DES A				DES B				DES C			
		V_R	EE	K_p	IFT (mN/m)	V_R	EE	K_p	IFT (mN/m)	V_R	*EE*	K_p	IFT (mN/m)
Salt	14	0.7	0.7 ± 0.0	2.5 ± 0.1	2.7 ± 0.1	1.0	0.9 ± 0.0	12.9 ± 3.4	5.1 ± 0.1	1.0	0.98 ± 0.0	15.3 ± 0.2	4.8 ± 0.0
	24	0.8	0.9 ± 0.1	7.1 ± 1.5	1.8 ± 0.2	1.0	1.0 ± 0.0	29.4 ± 0.3	7.7 ± 0.1	1.0	0.97 ± 0.1	12.8 ± 0.6	5.5 ± 0.0

References:

[1] Â.P. Matos, The Impact of Microalgae in Food Science and Technology, JAOCS, J. Am. Oil Chem. Soc. 94 (2017) 1333–1350. https://doi.org/10.1007/s11746-017-3050-7.

[2] J. Rajesh Banu, Preethi, S. Kavitha, M. Gunasekaran, G. Kumar, Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis, Bioresour. Technol. 302 (2020) 122822. https://doi.org/10.1016/j.biortech.2020.122822.

[3] N.D. Giraldo-Calderón, R.J. Romo-Buchelly, A.A. Arbeláez-Pérez, D. Echeverri-Hincapié, L. Atehortúa-Garcés, Microalgae biorefineries: Applications and emerging technologies, DYNA. 85 (2018) 219–233. https://doi.org/10.15446/dyna.v85n205.68780.

[4] B.M. Khan, K.L. Cheong, Y. Liu, ATPS: "Aqueous two-phase system" as the "answer to protein separation" for protein-processing food industry, Crit. Rev. Food Sci. Nutr. 59 (2019) 3165–3178. https://doi.org/10.1080/10408398.2018.1486283.

[5] M. Iqbal, Y. Tao, S. Xie, Y. Zhu, D. Chen, X. Wang, L. Huang, D. Peng, A. Sattar, M.A.B. Shabbir, H.I. Hussain, S. Ahmed, Z. Yuan, Aqueous two-phase system (ATPS): an overview and advances in its applications, Biol. Proced. Online. 18 (2016) 1–18. https://doi.org/10.1186/s12575-016-0048- 8.

[6] Q. Zeng, Y. Wang, Y. Huang, X. Ding, J. Chen, K. Xu, Deep eutectic solvents as novel extraction media for protein partitioning, Analyst. 139 (2014) 2565–2573. https://doi.org/10.1039/c3an02235h.

Acknowledgements:

Bruno Pereira thanks the financial support to Fundação para a Ciência e Tecnologia/Ministério da Educação e Ciência, Portugal, for the PhD Fellow grant SFRH/BD/151221/2021. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC).

P6.2 - NEW 4-STYRYLCOUMARIN DERIVATIVES AS POTENTIALS FLUORESCENT LABELS FOR BIOMOLECULES: APPLICATION IN RNA-FISH PROBES

Raquel Eustáquio [1]*, Ana Teresa Caldeira [1,2,3], Sílvia Arantes [1], António Candeias [1,2,3], António Pereira [1,2,3]

[1] Laboratório HERCULES, Instituto de Investigação e Formação Avançada, Universidade de Évora, Évora, Portugal.
[2] Departamento de Química e Bioquímica, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal.
[3] City U Macau Chair in Sustainable Heritage, Instituto de Investigação e Formação Avançada, Universidade de Évora, Évora, Portugal.
[(*)] e-mail: raqueleustaquio98@hotmail.com

Keywords: Fluorescent labels; 4-styrylcoumarin derivatives; Biomolecules; RNA-FISH probes

ABSTRACT

Currently, fluorescence microscopy is one of the highly sensitive imaging techniques that allow visualize, detect, and track biomolecules in analytical studies in many important scientific areas as cellular biology, environmental sciences, medicine, pharmacy, among others. One of the great advantages of fluorescence microscopy is the possibility to use several fluorescent labels to detect different biomolecules and produce multicolour images allowing the identification, in vitro and in vivo, of specific components of complex biomolecular assemblies, as well analysis of their interactions. Fluorescent labels can produce chemically stable and small bioconjugates, with insignificant interference on the structure and biological functions of the unlabeled biomolecules. The amine-reactive fluorescent labels, due to the abundance of amino groups or its easy insertion into biomolecules, are the most frequently used to prepare bioconjugates for a multiplicity of biological applications as fluorescence in situ hybridization (FISH), histochemistry, cell tracing, receptor binding, or direct and indirect immunochemistry. Presently, the most widely used available fluorescent labels are very expensive and that's why coumarin derivatives can be a solution to develop low- cost new brightness fluorophores.
In this work, we developed a low cost and effective synthetic strategy to synthesize six new 4-styrylcoumarin derivatives, as potentials fluorescent labels for biomolecules, using the inexpensive 7-diethylamino-4- methylcoumarin as a starting material. New fluorescent oligonucleotide probes have been obtained, six directed to the rRNA region of eukaryotic cells (EUK516) and six to the rRNA region of prokaryotic cells (EUB338). The developed fluorescent probes were tested on microorganisms belonging to the culture collection of the Laboratory of Biodegradation and Biotechnology of the HERCULES Laboratory (University of Évora), showing effective performance as RNA-FISH probes. These findings evidenced the applicability of the new 4-styrylcoumarin derivatives in labeling of biomolecules and bioimaging.

Acknowledgements:
The authors acknowledge financial support to FCT—Foundation for Science and Technology, I.P.—within the scope of the projects UIDB/04449/2020 (HERCULES Lab), UIDB/04033/2020 (CITAB), ART3mis (2022.07303.PTDC/FCT) and Old Goa Revelations project (2022.10305.PTDC/FCT) and also through the PhD Grant UI/BD/153584/2022. Acknowledges additionally the City University of Macau endowment to the Sustainable Heritage Chair & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science supported by the Belt and Road Initiative.

P6.3 - PHYSIOLOGICAL CHARACTERIZATION AND PROMOTER ENGINEERING OF ACETOBACTERIUM WIERINGAE FOR ACETONE PRODUCTION VIA GAS FERMENTATION

João P. C. Moreira [1], Stefan Pflügl [2,3], Joana I. Alves [1,4] and Lucília Domingues [1,4]

[1] CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal.
[2] Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria.
[3] Christian Doppler Laboratory for Optimized Expression of Carbohydrate-Active Enzymes, Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria.
[4] LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

[(*)] *e-mail: jpcm9.1994@gmail.com*

ABSTRACT

The pressing need to mitigate environmental concerns has driven research into sustainable energy and chemical production methods that reduce carbon emissions. Gas fermentation offers a promising avenue for low-carbon fuel and chemical synthesis. *Acetobacterium wieringae*, particularly strain *A. wieringae* JM, has emerged as an attractive host for gas-based biorefineries due to its unique abilities, including growth in diverse gas compositions and pH ranges, and efficient growth on carbon monoxide without co-substrates.

This study focuses on enhancing the potential of *A. wieringae* for acetone production through genetic modification. A transformation protocol was developed, and the acetone production operon from *Clostridium acetobutylicum* was introduced. Novel promoters were explored to widen gene expression possibilities in *A. wieringae*. The stability of the plasmid backbone pMTL83151 carrying replicon pCB102 was assessed. Additionally, the tolerance of *A. wieringae* to gas synthesis derived from biogenic residue gasification was evaluated for potential industrial application.

Gas composition significantly influenced acetone production by *A. wieringae*, with distinct physiological effects observed between strain *A. wieringae* DSM 1911 and
A. wieringae JM. Four constitutive promoters from *A. wieringae* JM and four from *C. autoethanogenum* were successfully expressed, exhibiting stronger activity than the reference Pthl promoter from *C. acetobutylicum*. Notably, *A. wieringae* JM demonstrated robust growth in synthesis gas from biomass gasification, though with physiological variations.

This study unveils the intricate relationship between gas composition, physiological attributes, and acetone production in *A. wieringae*. The expanded promoter repertoire enhances genetic manipulation potential, propelling the strain's capacity for versatile gene expression. Moreover, the resilience of *A. wieringae* JM to gasification-derived gas synthesis highlights its viability for industrial implementation. These findings contribute to advancing the development of gas-based biorefineries, paving the way for sustainable chemical production with reduced environmental impact.

P6.4 - RECOMBINANT HPV E7 ONCOPROTEIN BIOSYNTHESIS AND PURIFICATION: A CRUCIAL INTEGRATION STEP TOWARDS TARGETED ANTI-HPV DRUG DEVELOPMENT

Diana Gomes [1,2,3]*, ***Luís A. Passarinha*** [1,2,3,4], ***Ângela Sousa*** [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*
[2] *UCIBIO–Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal.*
[3] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA, Caparica, Portugal.*
[4] *Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal.*

(*) *e-mail: dianarouca@gmail.com*

Keywords: Affinity chromatography, E7 Protein, Recombinant production.

ABSTRACT

Cervical carcinoma, the 4th leading cause of cancer-related mortality among women worldwide, is primarily attributable to lesions induced by the oncogenic activities of human papillomaviruses (HPVs), due to E6 and E7 oncogenic role. The high-risk HPV E7 protein exerts its malignancy by interacting with the retinoblastoma tumor suppressor protein (pRb), culminating in its degradation via the proteasome-mediated pathway. This pivotal event activates the E2F-mediated transcriptional cascade, thereby precipitating unregulated progression of the cell cycle. Hence, E7 has emerged as a promising target for the development of pharmaceutical agents aimed at combating HPV-related malignancies [1]. However, to conduct biointeraction studies with selected drugs, large amounts of highly purified E7 protein are required. Therefore, our aim was to employ recombinant technology to overexpress E7 protein and evaluate several capture/purification flowcharts to isolate the protein in its native folding. The biosynthesis of E7 protein was accomplished by using *Escherichia coli* in LB medium, supplemented with different glucose concentrations, and its induction was achieved by the addition of IPTG and zinc sulphate. Subsequently, protein isolation was conducted via sonication in conjunction with ice cycles, resulting in primary capture of the E7 protein with two fused tags, MBP and His_6 at N-terminal. For the purification, we have been studying a one-step or two-step protocol based on different affinity chromatography approaches. Ongoing studies encompass additional biophysical assessments, such as circular dichroism and thermofluor assays, to evaluate the secondary structure and stability of the protein obtained through distinct purification strategies.

[1] Almeida AM, et al. Cervical cancer and HPV infection: ongoing therapeutic research to counteract the action of E6 and E7 oncoproteins. *Drug Discov Today*. 24(2019)2044.

Acknowledgements:
Foundation for Science and Technology supports this work through European Regional Development Fund under Portugal 2020 Program with projects UIDB/00709/2020, UIDB/04378/2020, the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020), the research contract from the call CEECINST/00016/2021 under the scope of the CEEC Institutional 2021, and PhD fellowship 2020.06792.BD.

P6.5 - PHASE SEPARATION HYDRODYNAMICS OF CHOLINIUM CHLORIDE-BASED AQUEOUS BIPHASIC SYSTEMS (ABS) – FINDING THE BEST BIPHASIC REGION OF OPERATION

Alexandre M. S. Jorge [1(*)], João A. P. Coutinho [2], Jorge F. B. Pereira [1]

[1] *University of Coimbra, CIEPQPF, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, Coimbra, Portugal*

[2] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal*

(*) e-mail: alexandrej@eq.uc.pt

Keywords: Aqueous two-phase systems; Hydrodynamics; Cholinium chloride; Liquid-liquid extraction; Phase settling.

ABSTRACT

Aqueous biphasic systems (ABS) have been widely studied for extraction and purification of biomolecules. Although, the industrial application of these biocompatible, amenable, and sustainable separation platforms depends on a proper scaling and implementation into existing liquid-liquid extraction (LLE) operation units. For that purpose, the phase separation hydrodynamics of three ABS comprising polypropylene glycol with an average molecular weight of 400 g/ml (PPG-400), choline chloride ([Ch]Cl), tripotassium hydrogen phosphate (K_3PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were investigated. For the systems PPG-400/[Ch]Cl, [Ch]Cl/K_3PO_4 and [Ch]Cl/K_2HPO_4, the mixing time (T_m) was correlated with the phase settling time (T_s), at 25 ºC and 50 ºC. The results showed that T_s is independent of T_m, being very long for the polymer/salt ABS (T_s > 6 h) and very fast for salt/salt ABS (T_s < 150 s). The enhanced separation hydrodynamics of the salt/salt ABS is directly related with the salting-out effect of the inorganic salt. The phases' density was the most important physicochemical property in ABS' formation. A best biphasic region of operation was defined for [Ch]Cl/salt-based ABS, which can be paramount for the industrial application of ABS in the separation and purification of biomolecules using conventional mixer-settler units.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UIDB/ EQU/00102/2020 and UIDP/EQU/00102/2020. This work was partly developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). J.F.B. Pereira and A. M. S. Jorge acknowledge FCT for funding the project DRI/India/0044/2020.

P6.6 - OPTIMIZATION OF ENZYMES PRODUCTION BY *ASPERGILLUS NIGER* IN SOLID STATE FERMENTATION OF AGRO-INDUSTRIAL BY-PRODUCTS

Ana Catarina Mota [1], ***Diana Machado*** [1(*)], ***Ana Sofia Pereira*** [1], ***Ana Guimarães*** [1], ***Isabel Belo*** [1,2]

[1] *Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS - Associate Laboratory, Braga, Guimarães, Portugal*

(*) *e-mail: dianacafm.bq@gmail.com*

Keywords: solid state fermentation; *Aspergillus niger*; cellulase; xylanase; β-glucosidase; amylase; brewer's spent grain; rice husk; vine shoot trimmings.

ABSTRACT

The agri-food industry generates significant amounts of by-products that are often discarded or used in low-value applications. Solid by-products of lignocellulolytic structure have been proved to be suitable substrates in solid-state fermentation (SSF) to produce carbohydrases, that have many industrial applications [1]. In this work, the by-products brewer's spent grain (BSG), rice husk (RH), and vine shoot trimmings (VST) were used to carry out SSF experiments using *Aspergillus niger* CECT 2088 and to produce enzymes of relevance in textile industry, such as cellulases, xylanases and amylases. Factors influencing SSF performance were examined such as the substrate granulometry and the supplementation of nitrogen and phosphorous sources. In SSF experiments performed at 25ºC and 75% (w/w) moisture for 7 days, a positive effect of medium supplementation with 2% (w/w) $(NH4)2SO4$ and 1% (w/w) $K2HPO4$ was observed, leading to 2 to 10-fold increase of enzymes production using RH and BSG. Under these conditions SSF was performed with different particle sizes (1, 4 and 10 mm) and the highest enzyme activities were obtained with the highest size of BSG and RH, and with 4 mm VST. With selected granulometry of the three by-products, a Simplex-Centroid mixtures design was performed. Optimal enzyme activities were obtained with 100% BSG for xylanase (651 U/g), β-glucosidase (363 U/g), cellulase (189 U/g) and 72% (w/w) of BSG and 28% of RH for amylase (263 U/g). Besides those optimal values, the use of 50% (w/w) BSG/RH mixture resulted in high production of xylanase (553 U/g) and β-glucosidase (221 U/g), and with 50% (w/w) BSG/VST a cellulase activity similar with 100% BSG was obtained. These results indicates that SSF-based bioprocessing of BSG alone or in mixtures of VST and RH, is an effective and sustainable approach to produce enzymes of textile industry interest, contributing to boost circular economy in this sector.

References:

[1] Leite, P., Silva, C., Salgado, J. M., & Belo, I. (2019). Simultaneous production of lignocellulolytic enzymes and extraction of antioxidant compounds by solid-state fermentation of agro-industrial wastes. *Industrial Crops and Products*, *137*, 315-322

Acknowledgements:

This study was supported by the integrated project be@t – Textile Bioeconomy (TC-C12-i01, Sustainable Bioeconomy No. 02/C12-i01/2022), promoted by the Recovery and Resilience Plan (RRP), Next Generation EU, for the period 2021 – 2026; by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit; by LABBELS Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems (LA/P/0029/2020).

P6.7 - EXTRACTIVE FERMENTATION AS A PLATFORM FOR THE PRODUCTION AND PURIFICATION OF LACCASE BY *PICHIA PASTORIS*

Flávia F. Magalhães [1], Augusto Q. Pedro [1], Mara G. Freire [1], Ana Paula Tavares [1(*)]
[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

(*) e-mail: aptavares@ua.pt

Keywords: Extractive Fermentation, Recombinant Protein, Laccase

ABSTRACT

Laccases (EC 1.10.3.2) are industrial multicopper oxidases enzymes with relevant applications in wastewater treatment and the textile industry. Nowadays, extractive fermentation using liquid-liquid extraction based on aqueous biphasic systems (ABS) hold high potential to improve enzymatic bioprocesses, namely by the integration of upstream and downstream steps with consequent reduction in the number of additional purification steps required. This work aims to develop a bioprocess resorting to ABS and able to integrate the heterologous production and purification of *Trametes versicolor* laccase in *Pichia pastoris*, ultimately creating a more sustainable and low-cost process. To study extractive fermentation, the fermentation medium was supplemented with multiple ABS constituents and the microbial growth was monitored by OD_{600} (Optical density) measurement. The best growth results were achieved using polyethylene glycol (PEG) and phosphate buffer pH 6. Using the optimal conditions for microbial growth, laccase production was then monitored by the quantification of enzymatic activity by UV-Vis spectrophotometer at 420 nm measuring 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) kinetic oxidative reaction. After the fermentation stage in the most promising ABS constituents, phase separation was achieved, allowing microbial recovery for future reuse and laccase extraction with biological activity and an extraction efficiency of 100%. This work shows ABS as a promising option for integrative platform for protein production, reducing manufacturing steps, and hence process costs.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). Flávia Magalhães, Ana Tavares and Augusto Pedro acknowledge FCT, respectively, for the SPQ/FCT PhD grant SFRH/BD/150669/2020 and research contracts CEECIND/01867/2020 and CEEC-IND/02599/2020.

P6.8 - LIPASE PURIFICATION FROM *YARROWIA LIPOLYTICA* FERMENTATION BROTH USING AN AQUEOUS TWO-PHASE SYSTEM BASED ON QUATERNARY AMMONIUM COMPOUNDS

Filipe Smith Buarque [1(*)], ***Adriano Carniel*** [1], ***Bernardo Dias Ribeiro*** [1], ***Maria Alice Zarur Coelho*** [1]

[1] *Biochemical Engineering Department, School of Chemistry, Federal University of Rio de Janeiro, Brazil.*

(*) *e-mail: filipesmith@eq.ufrj.br*

Keywords: Separation process; quaternary ammonium; enzymes; *Yarrowia lipolytica*.

ABSTRACT

Aqueous two-phase systems (ATPS) are considered efficient and sustainable downstream processing techniques in extracting and separating enzymes. In this work, we evaluated the use of ATPS based on quaternary ammonium (tetrabutylammonium bromide ($[N_{4444}]Br$), tetrabutylammonium chloride ($[N_{4444}]Cl$), choline chloride (ChCl), and betaine) + potassium phosphate buffer (pH 7) to separate selective lipase and protease from the fermentation broth of *Y. lipolytica* yeast. Cultivation in YPD medium supplemented with 200 mM potassium phosphate buffer (pH 7) in the preparation of the culture medium positively influenced the production of lipase and protease, whereby led to lesser pH variations and better values of biomass production (15.29 g L^{-1}), lipolytic (455.96 U L^{-1}), and proteolytic activity (23.70 U L^{-1}). These results indicate that the minor pH variations in the fermentative medium contributed significantly to these high values. The presence of the culture medium did not influence the liquid-liquid demixing, and the phase separation followed the order of ammonium compounds hydrophobicity: $[N_{4444}]Br$ > $[N_{4444}]Cl$ > betaine > ChCl. Lipase was partitioned mainly to the ammonium-rich phase and protease migrates preferentially to the salt-rich phase in all systems studied. Remarkable extraction efficiencies of 100% for lipase and 96.87% for protease were achieved in a single step for $[N_{4444}]Cl$-based ATPS. Furthermore, a high level of purification was achieved with values of 10.55 and 1.69 for lipase and protease, respectively. According to the remarkable results, quaternary ammonium-based ATPS can be considered an alternative and efficient platform to separate lipase from protease, obtaining two high-value-added compounds selectively.

Acknowledgements:
This work was financed by national funds through the National Council for Scientific and Technological Development (CNPq); Coordination of Improvement of Higher-Level Personnel (CAPES); and Foundation for Research Support and Technological Innovation of the State of Rio de Janeiro (FAPERJ).

P6.9 - COMPUTER-ASSISTED IDENTIFICATION OF DEEP EUTECTIC SOLVENTS EFFECT ON *Y. LIPOLYTICA* METABOLIC INHIBITION USING MOLECULAR DOCKING

Filipe S. Buarque [1,2]***, Bernardo D. Ribeiro*** [1]***, Maria A. Z. Coelho*** [1]***, Mara G. Freire*** [2]***, Matheus M. Pereira*** [3(*)]

[1] *Biochemical Engineering Department, School of Chemistry, Federal University of Rio de Janeiro, Brazil.*

[2] *CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal.*

[3] *University of Coimbra, CIEPQPF, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II –Pinhal de Marrocos, Coimbra, Portugal.*

(*) *e-mail: matheus@eq.uc.pt*

Keywords: microorganism inhibition; molecular docking; enzymes; *Yarrowia lipolytica*.

ABSTRACT

Deep eutectic solvents (DESs) have been reported as an environmentally friendly alternative to organic solvents. They are formed by the association of two or more compounds by hydrogen bonding (acceptor (HBA) and donor (HBD)). The application of DES in biotechnology remains relatively limited and is restricted to enzymatic and whole-cell processes involving biotransformation reactions. However, the design of solvents for biotechnological processes playsa critical role, both for various applications and as potential inhibitors of microorganisms, like *Y. lipolytica*, by performing negative effects on metabolic pathway. Thus, selecting a DES requires the screening of different HBD and HBA to identify the most biocompatible combination. Given this context, in this work, the tolerance of *Y. lipolytica* yeast in the presence of DES based on ammonium salts + fatty acids was determined. In addition, a molecular docking analysis was carried out to identify the bind affinity of DES studied on hexokinase, the target enzyme on the microorganism metabolic pathway. The DES formed by HBA with Cl^- anion display a lower impact on lipolytic activity and cell growth, compared to their bromide counterparts. Concerning the HBD, shorter-chain fatty acids demonstrated a lower tendency to inhibit lipase activity and microbial growth. Molecular docking results showed that DES (HBAs and HBDs) act as hexokinase inhibitors (enzyme responsible for the first step of glycolysis), binding preferably into enzyme active site, decreasing the cellular energy production and consequently microorganism inhibition. Hydrophobic and electrostatic interaction between HBAs/ HBDs and hexokinase active site could be the main drive force to microorganism growth inhibition. Based on results here presented, the identification of DES impact on *Y. lipolytica* metabolism through molecular docking could be applied as a new approach to screen appropriate DES with low impact on microorganisms metabolism, thus extending the application of alternative solvents in biotechnological processes.

Acknowledgements:
This work was financed by national funds through the National Council for Scientific and Technological Development (CNPq); Coordination of Improvement of Higher-Level Personnel (CAPES); Foundation for Research Support and Technological Innovation of the State of Rio de

Janeiro (FAPERJ); and CICECO- Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020, and LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). CIEPQPF is supported by the FCT through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020.

P6.10 - OPTIMIZED PRODUCTION OF REFLECTINS AND CHARACTERIZATION OF THEIR REVERSIBLE SELF-ASSEMBLY

Iana Lychko [a,b(*)], ***Cátia Soares*** [a,b], ***Inês Padrão*** [a,b,c], ***Tomás Calmeiro*** [d], ***Arménio Barbosa*** [a,b], ***Pedro Costa*** [a,c], ***Margarida Dias*** [a,b], ***Ana Cecília Roque*** [a,b]

[a] *Associate Laboratory i4HB - Institute for Health and Bioeconomy, Chemistry Department, NOVA School of Science and Technology, Campus Caparica, 2829-516 Caparica, Portugal*

[b] *UCIBIO – Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Caparica, Portugal*

[c] *UCIBIO – Applied Molecular Biosciences Unit, Life Sciences Department, NOVA School of Science and Technology, Caparica, Portugal*

[d] *CENIMAT i3N, Department of Materials Science, Nova School of Science and Technology, Caparica, Portugal*

(*) email: i.lychko@campus.fct.unl.pt

Keywords: intrinsically disordered proteins; self-assembly; biotechnology.

ABSTRACT

Marine organisms are frequently used as a source for inspiration and development of sustainable and innovative materials. Among these sources, cephalopods play an important role, due to their unique biological features and behaviors. Special attention is dedicated to the camouflage ability of these animals. Recently, cephalopod specific structural proteins called Reflectins showed to hold great promise in biotechnology. These proteins play an essential role in cephalopods' camouflage, driven by phosphorylation events that trigger reversible self-assembly. In this work, we report an optimized recombinant production of two reflectins, one from static iridophores and one from dynamic iridophores. Moreover, we studied the structure-function interplay these proteins to understand their pH-responsive self-assembly in vitro. For this, after production, both proteins underwent characterization employing techniques such as circular dichroism (CD), dynamic light scattering (DLS), and atomic force microscopy (AFM). The experimental data were supported by molecular simulations at different pH conditions using coarse grained reflectin models. Our findings reveal that reflectins' amino acid composition profoundly impacts their biophysical properties. We also showed that reflectins from dynamic iridophores, exhibit significant enhanced reversible assembly capabilities when compared to those from static iridocytes. Additionally, using polymerase chain reaction (PCR) and Sanger sequencing, we identified a novel Reflectin protein in Octopus vulgaris, further increasing the possibilities for protein and material engineering. In summary, by unraveling the mechanisms behind reflectin self-assembly, we provide a robust foundation for engineering advanced functional bio-based materials that dynamically respond to environmental stimuli.

Acknowledgements:
This work was supported the projects Sea2Se (PTDC/BII-BIO/28878/2017), by Fundação para a Ciência e a Tecnologia, Portugal, through the PROTEIOS project (PTDC/CTM- CTM/3389/2021), Research Unit on Applied Molecular Biosciences – UCIBIO (UIDP/04378/2020 and UIDB/04378/2020) and Associate Laboratory Institute for Health and Bioeconomy – i4HB (LA/P/0140/2020). The authors thank FCT/MEC for the research fellowships SFRH/BD/147388/2019 for I.L UI/BD/151154/2021 for I.P and 2022.11305.BD for C.S.

P6.11 - SUSTAINABLE TECHNOLOGY TO EXTRACT AND PURIFY PROTEINS FROM INSECTS

Pedro Moleiro [1], Augusto Q. Pedro [1], Ana Paula M. Tavares [1(*)], Mara G. Freire [1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal.

(*) e-mail: aptavares@ua.pt

Keywords: Insects; Protein extraction; Alternative solvents; Sustainability.

ABSTRACT

Considering the growth rate of the Earth's population, demand in the meat protein would continue to grow with damaging impact in terms of sustainability leading to critical environmental impacts and resources wastefulness. Thus, currently, it is urgent to identify alternative protein sources, where insects have a high potential in contributing to food sustainability. Insects are highly enriched in protein and other nutrients sources comparing to other livestock animals and plants. The main advantage of insects over other protein sources is the low environmental and economic costs of production as well as low carbon footprint, which becomes essential to satisfy the rise in the global protein demand. Protein extraction from insects to introduce in food products undoubtedly is a viable way of increasing the acceptability of such rich sources of nutrients among consumers. Using aqueous solutions of biocompatible and bio-based ionic liquids (ILs), this work aims to develop a sustainable technology to extract and purify proteins from eligible insect flour, envisioning their application in food products. ILs will be synthesized and characterized, followed by their application as aqueous solutions in the extraction of proteins from insect flour. Operational conditions will be optimized to improve extraction yield. Proteins profile and their content will be addressed. This work will allow to create a sustainable synergy between eligible insects, proteins and food products.

Acknowledgements:
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MCTES (PIDDAC). This study was funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation "InsectERA" (Project no. 20 with the application C644917393-00000032). Ana Tavares and Augusto Pedro acknowledge FCT, respectively, for the research contracts CEECIND/01867/2020 and CEEC-IND/02599/2020.

P6.12 - ADVANCING MANUFACTURE OF HIPSC-DERIVED HEPATOCYTES WITH IMPROVED FUNCTIONALITY: A NATURE-INSPIRED BIOPROCESS

Joana I. Almeida [1,2], Pedro Vicente [1,2], Inês E. Crespo [1,2], Nikolaus Virgolini [1,2], Inês A. Isidro [1,2], Tomás Calmeiro [3], Henrique V. Almeida [1,2], Elvira Fortunato [3], Maren Calleja [4], Juan R. Rodriguez-Madoz [4], Felipe Prosper [4], Pedro M. Baptista [5], Paula M. Alves [1,2], Margarida Serra [1,2(*)]

[1] *iBET, Instituto de Biologia Experimental e Tecnológica, Portugal*
[2] *ITQB NOVA, Oeiras, Portugal*
[3] *CENIMAT i3N, Department of Materials Science, Nova School of Science and Technology, Caparica, Portugal*
[4] *CIMA/UNAV, Spain.*
[5] *IIS Aragón, Spain.*

[(*)] *e-mail: mserra@ibet.pt*

Keywords:

ABSTRACT

Hepatocyte-like cells differentiated from human induced pluripotent stem cells (hiPSC-Hep) provide unprecedented opportunities for hepatic cell-based therapies. Regrettably, more than 15 years of research in the field were not enough to convert hiPSC-Hep into an advanced therapy medicinal product. The often hybrid fetal-adult phenotype exhibited by these cells, the lack of reliable and reproducible protocols for their scalable production and the uncertainties regarding their engraftment ability are some of the reasons that hamper their clinical application. In this study, we aim to obtain relevant numbers of mature HLC for regenerative medicine applications. To address that, we designed a nature-inspired strategy and combined advanced manufacturing platforms with omics technologies and process analytical tools to better recapitulate and monitor the microenvironment of physiological liver development during hiPSC-Hep bioprocessing. hiPSC-Hep were generated as 3D cell aggregates using stirred-tank bioreactors (STBR) operated in perfusion. When dissolved oxygen was controlled at low levels, higher hiPSC-Hep production (2×10^6 cell/mL) and differentiation efficiencies (> 80% $Albumin^+$ cells) were obtained when compared to uncontrolled condition (0.6×10^6 cell/mL and <45% $Albumin^+$ cells). The generated hiPSC-Hep showed synthesis of key hepatic proteins (albumin, alpha 1 antitrypsin), urea and bile acids secretion as well as drug metabolization capacity, CYP450 activity and glycogen storage.

The hepatic maturation step was induced by culturing hiPSC-Hep with the secretome of human intestinal microbiota, recapitulating what happens in liver development [1]. hiPSC-Hep treated with microbiome secretome showed improved expression of critical hallmarks of human hepatocytes and preserved functionality.
Importantly, we used transcriptomic analysis (RNA-Seq) to confirm, for the first time, that hiPSC-Hep maturation levels modulate the “machinery” that mediates cell engraftment and identifiy the cell maturation stage that ensures efficient cell’ engraftment *in vitro* and *in vivo*, with preservation of hepatic functionality.

References:
[1] Almeida et al 2022. J Hepatol, doi: 10.1016/j.jhep.2021.10.015.

Acknowledgements:
This work was supported by a grant from ERA-NET-Rare 3research program, JTC ERAdicatPH (E-Rare3/0002/2015); iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020); FCT fellowships SFRH/BD/116780/2016 and SFRH/BD/145767/2019;
European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 813453. Please proofread your abstract carefully prior to submission.

P6.13 - PRE-SACCHARIFICATION AND HIGH SOLIDS OPERATION AS KEY STRATEGIES TO IMPROVE BIOETHANOL PRODUCTION FROM *EUCALYPTUS GLOBULUS* BARK BY SSF

Mariana S. T. Amândio [1,2], Ana M. R. B. Xavier [2], Jorge M. S. Rocha [1(*)]
[1] CIEPQPF, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal
2 CICECO – Aveiro Institute of Materials, Chemistry Departament, University of Aveiro, Aveiro, Portugal

[(*)] e-mail: jrocha@eq.uc.pt

Keywords: Bioethanol; *Eucalyptus globulus*; simultaneous saccharification and fermentation (SSF); pre-saccharification (PS); fed-batch; high solids loading..

ABSTRACT

The pulp and paper (P&P) industry is a growing sector of great importance, particularly for the Portuguese economy. Over the past years, this sector has strived to simultaneously reduce and valorize the residues generated towards a circular and bio-based economy.

The present work assessed the possibility of using bark, an abundant residue on the pulp mill floor, for cellulosic ethanol production instead of conventional burning for energy production. The bioconversion of bark is challenging and after kraft pretreatment, simultaneous saccharification and fermentation (SSF) setup was selected to decrease overall costs. The introduction of a short pre-saccharification (PS) stage (0, 1 and 4 h) in bioethanol production from pretreated *Eucalyptus globulus* bark following an integrated configuration at the bioreactor scale was evaluated. It was observed that the longer the pre-saccharification, the higher the productivity. Shifting from batch to fed-batch PS-SSF (4 h) configuration allowed an increase of the solids loading from 8 to 20% (w/v), boosting the final bioethanol concentration from 27.4 to 75.9 g L^{-1} and improving the overall productivity of around 25%.

This work proved that eucalyptus bark could be a promising feedstock for second-generation bioethanol, boosting market opportunities under the integrated biorefinery concept in the P&P sector. This alternative is aligned with the century's three main goals of the century, namely waste minimization, carbon neutrality, and climate change mitigation.

Acknowledgements:
This work was carried out under the Project InPaCTus – Innovative Products and Technologies from Eucalyptus. Project N.º 21874 funded by Portugal 2020 through European Regional Development Fund (ERDF) in the frame of COMPETE 2020 nº246/AXIS II/2017. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Authors would also like to thank the CIEPQPF - Strategic Research Centre Project UIDB/00102/2020 funded by the Fundação para a Ciência e Tecnologia (FCT). The authors are thankful to RAIZ – Instituto de Investigação da Floresta e do Papel for supplying the pretreated Eucalyptus globulus bark and all the equipment required for enzymatic hydrolysis.

P6.14 - LIPID PRODUCTION FROM NON-DETOXIFIED LIGNOCELLULOSIC BIOMASS HYDROLYSATE BY *ASHBYA GOSSYPII* PRECISION FERMENTATION

Miguel Francisco [1(*)], ***Tatiana Quinta Aguiar*** [1,2], ***Lucília Domingues*** [1,2], ***Gabriel Abreu*** [1], ***Susana Marques*** [3], ***Francisco Gírio*** [3]

[1] *CEB — Centre of Biological Engineering, University of Minho, Braga, Portugal.*

[2] *LABBELS—Associate Laboratory, Guimarães, Portugal*

[3] *Laboratório Nacional de Energia e Geologia I.P., Unidade de Bioenergia e Biorrefinarias, Lisboa, Portugal*

[(*)] *e-mail: miguelfrancisco8507@gmail.com*

Keywords: Ashbya gossypii; optimization; lipid production; bio-jet fuel; by-product valorization; biorefineries.

ABSTRACT

Single-cell oil production from renewable and abundant feedstocks offers a sustainable and eco-friendly alternative to fossil-fuels consumption, an issue that has gained more attention throughout the years and needs to be addressed in the following ones. Xylose-utilizing oleaginous A. gossypii strains have the ability to accumulate lipids using detoxified industrial wastes as feedstock. Their performance using non-detoxified Eucalyptus bark hydrolysate (EBH) as a substrate was evaluated in this work. A. gossypii strains were cultivated in synthetic medium mimicking the composition of non-detoxified EBH (SM) supplemented with 10 g/L yeast extract (YE). All strains exhibited similar growth in SM-10YE up to 48 hours but later exhibited autolysis, which influenced their growth dynamics. Despite this, more than 90% of glucose in the culture was consumed within 120 hours. Notably, strain A877 reached superior lipid accumulation, especially as oleic acid. Lipid production optimization was then performed by testing different nitrogen and micronutrient sources and quantities. At this stage, oxygen availability was also investigated as a potential cause for autolysis onset, and it became clear that a balance between biomass production, lipid accumulation and autolysis was needed to achieve maximum lipid yield. Corn steep liquor (CSL), a low-cost sustainable supplementation for EBH, showcased the best balance between all factors. In bioreactor fermentations using non-detoxified EBH and CSL, a lipid titer of 1.42 g/L was achieved at 78 hours, suggesting potential for high lipid production using low-cost and renewable substrates [1].

References:

[1] Francisco, M. et al. Single-cell oil production by engineered Ashbya gossypii from non- detoxified lignocellulosic biomass hydrolysate, Fermentation, 9(9), p. 791. (2023).

Acknowledgements:

This study was supported by Compete 2020, Portugal 2020, and Lisboa 2020 through MoveToLowC (POCI-01-0247-FEDER-046117) and by the Portuguese Foundation for Science and Technology (FCT) through the strategic funding of UIDB/04469/2020 and project ESSEntial (PTDC/BII- BTI/1858/2021). The authors gratefully acknowledge RAIZ (Forest and Paper Research Institute) for providing the Eucalyptus bark material, and Novozymes A/S for supplying Cellic® CTec3 HS. The technical assistance of STEX company (Aveiro, Portugal) on the operation of pilot-scale infrastructure of steam explosion and enzymatic hydrolysis is also acknowledged.

P6.15 - USE OF TOMATO WASTE HYDROLYSATES FOR PIGMENTS PRODUCTION USING *RHODOTORULA GLUTINIS* CELLS

Helena Ribeiro [1,2(*)], ***Boelo Schuur*** [2], ***Jorge F. B. Pereira*** [1]
[1] *University of Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal.*
[2] *University of Twente, Faculty of Science and Technology, Green Energy Initiative, Sustainable Process Technology Group, The Netherlands.*

[(*)] *e-mail: helenaribeiro270@gmail.com*

Keywords: Circular economy, Culture media, Hydrolysis, *Rhodotorula glutinis*, Tomato waste.

ABSTRACT

Food waste valorisation, through the recovery and production of valuable products in a biorefinery concept, improves the sustainability and economic competitiveness of agro-food industries. Natural colorants have attracted the industrial interest, due to their importance in food, cosmetics and pharmaceutical applications. Due to low production area requirements and the use of agro-industrial residues as low-cost substrates, the microbial production of colorants represents an eco-friendlier alternative to the synthetics [1]. Of all the microbial producers, *Rhodotorula glutinis* stands out not only for its ability to produce different carotenoids, but also for its production of lipids and enzymes [2]. Moreover, this yeast can grow using diverse substrates as carbon and nitrogen sources, including low-cost feedstocks [3-4], which make this an ideal candidate for creating a biorefinery of natural colorants. Tomato waste (including peels, pomace and seeds) contains highly valuable biological active compounds, such as carotenoids, lipids, crude fibres, carbohydrates and crude proteins [5-6]. The nutritional composition of this biomass makes them a good feedstock candidate for use in fermentative processes, contributing to the valorisation of this food waste by the direct extraction of valuable compounds and its use as a source of nutrients for the growth of microorganisms.

In this study, we evaluated the growth and pigment production of *R. glutinis* using various tomato waste hydrolysates. The tomato waste biomass underwent acid hydrolysis, employing different sulfuric acid concentrations. These diverse hydrolysates were then applied to cultivate *R. glutinis* and enhance pigment production, with subsequent optimization steps. This work contributes to the sustainability and the circular economy of a process with the incorporation of tomato waste, in addition to offer extra profits through the valorisation of the compounds extracted and the new compounds that can be converted from the nutrients-rich extracts.

References:

[1] N. Martins, C. L. Roriz, P. Morales, L. Barros, and I. C. F. R. Ferreira, "Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices," *Trends Food Sci. Technol.*, vol. 52, pp. 1–15, 2016.
[2] C. U. Mussagy, V. C. Santos-Ebinuma, M. Gonzalez-Miquel, J. A. P. Coutinho, and J. F. B. Pereira, "Protic Ionic Liquids as Cell-Disrupting Agents for the Recovery of Intracellular Carotenoids from Yeast *Rhodotorula glutinis* CCT-2186," *ACS Sustain. Chem. Eng.*, vol. 7, pp. 16765−16776, 2019.
[3] A. M. Kot, S. Błażejak, M. Kieliszek, I. Gientka, and J. Bryś, "Simultaneous Production of Lipids and Carotenoids by the Red Yeast *Rhodotorula* from Waste Glycerol Fraction and Potato Wastewater," *Appl. Biochem. Biotechnol.*, vol. 189, pp. 589−607, 2019.
[4] L. C. Mata-Gómez, J. C. Montañez, A. Méndez-Zavala, and C. N. Aguilar, "Biotechnological production of carotenoids by yeasts: An overview," *Microb. Cell Fact.*, vol. 13, no. 12, 2014.

[5] D. D. Adi, I. Oduro, and B. K. Simpson, "Biological and Microbial Technologies for the Transformation of Fruits and Vegetable Wastes," in Byproducts from Agriculture and Fisheries: Adding Value for Food, Feed, Pharma, and Fuels, 2020, pp. 403–420.
[6] R. K. Saini, S. H. Moon, and Y. S. Keum, "An updated review on use of tomato pomace and crustacean processing waste to recover commercially vital carotenoids," *Food Res. Int.*, vol. 108, pp. 516–529, 2018.

Acknowledgements:
CIEPQPF is supported by the Fundação para a Ciência e Tecnologia (FCT) through the projects UIDB/EQU/00102/2020 and UIDP/EQU/00102/2020. Helena Ribeiro acknowledges to FCT for PhD Grant (UI/BD/150909/2021).

P6.16 - SYNTHESIS AND CHARACTERIZATION OF PETASIS-UGI LIGAND FOR ANTIBODY PURIFICATION

Carolina Natal [1,2], Catarina Domingos [1,2(*)], Henrique Costa [1,2], Margarida Dias [1,2], Ana Cecília Roque [1,2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Chemistry Department, NOVA School of Science and Technology, Campus Caparica, Caparica, Portugal

[2] UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

(*) e-mail: c.domingos@campus.fct.unl.pt

Keywords: synthetic ligands; chemical synthesis; affinity chromatography; antibodies.

ABSTRACT

Synthetic mixed-mode and affinity ligands are low-cost, stable and robust alternative molecular recognition agents to used in bioseparation processes for the capture of biological targets. These ligands are rationally designed and further produced by combinatorial chemistry using simple and fast procedures as multicomponent one-pot reactions, e.g. Ugi reaction [1–3]. Our group has shown that multicomponent chemistries can be combined yielding new scaffolds for combinatorial chemistry as the tandem Petasis-Ugi reaction [2].

Our group previously reported the design and production of an affinity adsorbent, containing a synthetic ligand from one pot Petasis-Ugi reaction, for antibody purification. The adsorbent has shown to be highly selective for antibody purification from mammalian and avian sources, and Fab-derived fragments [4].

In this work, the new Pethasis-Ugi Affinity ligand was chemically synthesized in liquid-phase, purified, and characterized. Initially, the compound was synthesized with a Boc-group, following by the deprotection of this group, to expose an amine moiety that can be used for further immobilization in a chromatographic solid support. The compound was purified by High-Performance Liquid Chromatography, and characterized by 1H NMR and ESI-MS. The deprotected compound was obtained with high purity and the monoisotopic mass was confirmed.

Next steps include immobilization in a chromatographic solid support and binding assays with crude samples containing antibodies and their fragments from mammalian and avian sources.

References:

[1] Dias, A.M.G.C. et al, Biotechnol. Bioeng. 2017, 114, 481-491.
[2] Pina, A.S. et al, J. Chromatogr. A, 2015, 1418, 83-93.
[3] Batalha, Í. L. et al, J. Chromatgr. B, 2016, 1031, 86-93.
[4] Matos, M. J. B. et al, Sep. Purif. Technol, 2021, 265, 118476.

Acknowledgements:
This work was supported by the European Union's Horizon 2020 program under grant agreement No 899732 (PURE Project). The authors would like to acknowledge the financial support from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the PROTEIOS (PTDC/CTM-CTM/3389/2021) and project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences – UCIBIO, the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

P6.17 - EVALUATION OF OCHRATOXIN A (OTA) INTERACTION WITH RECOMBINANT DOMAIN II OF BOVINE SERUM ALBUMIN TOWARD THE DEVELOPMENT OF NEW OTA EXTRACTION PLATFORMS

Tatiana Q. Aguiar [1,2(*)], ***Tânia Leal*** [3], ***Diana G. Rodrigues*** [1], ***Luís Abrunhosa*** [1,2], ***Carla Oliveira*** [3], ***Lucília Domingues*** [1,2]

[1] *CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal*

[2] *LABBELS – Associate Laboratory, Braga/Guimarães, Portugal*

[3] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[(*)] *e-mail: tatiana.aguiar@deb.uminho.pt*

Keywords: Ochratoxin A; Bovine serum albumin; Domain II; Interaction; Mycotoxin capture.

ABSTRACT

Ochratoxin A (OTA) is a mycotoxin that raises food and feed safety concerns, existing legal limits worldwide for its presence in foodstuffs and beverages. Immuno-affinity columns (IACs) are typically used to capture, clean-up and pre-concentrate OTA from food samples before quantification by high- performance liquid chromatography, but their price limits their application. Serum albumins form stable complexes with OTA, emerging as cheaper alternatives to the antibodies used in IACs. They are composed of three globular domains, being the principal OTA binding site located within the domain II. Containing only 6 of the 17 disulfide bonds present in albumins, this domain should be more efficiently produce by bacteria than entire albumins.

This work envisioned the recombinant production of the bovine serum albumin (BSA) domain II in Escherichia coli, the study of is interaction with OTA and its evaluation as ligand receptor for developing new OTA extraction platforms. For that, this domain was cloned in fusion with His6 tag (BDII) or with thioredoxin A (Trx)-His6 tag-TEV cleavage site (TrxBDII), and produced using BL21 and Origami 2 DE3 strains. The improved cytoplasmic oxidizing environment of Origami 2 allowed the best production yield (18-24 mg purified protein/L culture) and fusion with Trx slighlty improved the stability of BDII. Fluorescence quenching studies indicated weaker interaction of OTA with TrxBDII than with the entire BSA, but no significant differences between TrxBDII and BDII. Circular dichroism spectroscopy confirmed that OTA induced conformational changes in TrxBDII, leading to a slight loss in the α-helical content. Immobilized TrxBDII was finally used to capture OTA from buffered solutions, allowing full retention of the mycotoxin followed by recovery upon elution.

Acknowledgements:
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and project MycoProAffinity 2022.03438.PTDC (https://10.54499/2022.03438.PTDC).

P6.18 - ENHANCED RNA CAPTURE AND RECOVERY FROM COMPLEX SAMPLES USING CARBON NANOMATERIALS

P.L. Ferreira [1(*)], ***Ana Videira*** [1], ***J.L. Faria*** [2,3], ***C.G. Silva*** [2,3], ***Fani Sousa*** [1]
[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[2] *LSRE-LCM – Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Porto, Portugal*
[3] *ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*

(*) *e-mail: pedroflf97@gmail.com*

Keywords: Carbon-based nanomaterials; Nucleic acids separation; RNA capture; RNA- based therapeutics.

ABSTRACT

The RNA-based therapeutics field has rapidly grown with exciting progress in different diseases. RNA is produced through multi-step biotechnological processes that require effective manufacturing strategies, precise quality control, and high purity for biopharmaceutical use. Among the most crucial steps in the process, the recovery and purification stand out as paramount, given the presence of numerous impurities with similar characteristics to RNA.

The use of carbon-based nanomaterials for capturing RNA emerges as a promising option mainly due to their excellent mechanical, chemical, and thermal properties. Thus, this study presents an effective method for capturing and clarifying RNA from complex bacterial lysates. Several carbon materials with different structures, dimensionalities, and surface modifications were studied. Pristine multi-walled carbon nanotubes (MWCNTs) with different diameters were first tested, and their ability to selectively interact with RNA, by applying mixtures with different ratios of RNA and pDNA, was confirmed. MWCNTs also showed an excellent adsorption capacity of 175 µg RNA/mg MWCNTs, however, the strong MWCNT-RNA interaction makes RNA desorption unattainable. On the other hand, HNO_3-treated N-doped CNTs showed promising desorption capacity, allowing the recovery of 73% of RNA. The selectivity of HNO_3-treated N-doped CNTs was also confirmed, where RNA was partially bound to the treated CNTs, while pDNA remained in solution. After the clarification process, RNA integrity was assessed by circular dichroism, and no conformational change was detected.

Overall, the results underscore the versatility of carbon nanomaterials in capturing RNA from a complex sample, facilitating the pre-purification process for potential therapeutic applications. This method stands out for its simplicity, efficiency, and reliability, all while preserving the integrity of the RNA.

Acknowledgements:
The authors acknowledge the projects UIDB/00709/2020, UIDP/00709/2020, UIDB/50020/2020, UIDP/50020/2020, and LA/P/0045/2020 (ALiCE) financed by national funds through FCT/MCTES (PIDDAC). Pedro Ferreira acknowledges FCT for the PhD fellowships (2022.13803.BD).

P6.19 - PROTEIN RECOVERY FROM BIOMASS USING INTEGRATED PROCESSES BASED ON IONIC LIQUIDS

João L. Fernandes [1], Leonor S. Castro [1], Ana F. Pereira [1], Ana M. Ferreira [1], Mara G. Freire [1], Pedro J. Carvalho [1], Augusto Q. Pedro [1*]
[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal

[(*)] e-mail: apedro@ua.pt

Keywords: Membrane proteins; Protic ionic liquid; Bioprocess integration; aqueous biphasic system; COSMO-RS.

ABSTRACT

Membrane proteins (MP) are a crucial part of current small molecule drug discovery programs. Despite their importance, MP production is still challenged by their hydrophobic nature with aqueous biphasic systems (ABS) holding the potential to integrate several downstream units (*e.g.*, cell lysis and MP capture), and thus contributing to reduce costs. The goal of this work is to investigate the ability of several ionic liquids (IL) to disrupt cells composed by an arquitecture enriched in chitin and recover MPs, ultimately creating an ABS for their extraction and clarification in a single step. Among the investigated ILs, protic ILs based on the N,N-diethylethanolammonium cation and combined with carboxylic acids of varying alkyl chain lengths as anions exhibited a promising performance both for cell disruption and MPs solubilization. Simultaneously, conductor-like screening model for real solvents (COSMO-RS) was applied for components of the biomass cell membrane and cell wall to screen the best IL chemical structures for the intended purpose. The integration of the cell disruption step and clarification of target MP using ABS is currently under investigation. Overall, it is expected that the integrated process under development will represent a step forward in the creation of effective and cost-effective (bio)processes for high-quality MPs.

Acknowledgements:
This work was developed within the scope of the projects CICECO-Aveiro Institute of materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). This study was funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation "InsectERA" (Project no. 20 with the application C644917393-00000032). Leonor Castro, Ana Pereira, and Augusto Pedro acknowledge FCT, respectively, for their PhD grants 2020/05090/BD and 2022/13247/BD, and for the research contract CEECIND/02599/2020 under the Scientific Stimulus – Individual Call.

MICROBIOTEC 23

Poster Session

Topic 7

P7.1 - BIOSYNTHESIS OF SILVER-BASED NANOPARTICLES USING SUPERNATANTS OF MICROBIAL CULTURES FOR CULTURAL HERITAGE PRESERVATION

António Carrapiço [1,2]*, Luís Dias [1,3], Elisabete Carreiro [5], Ana Teresa Caldeira [1,4], Maria Rosário Martins [1,6]

[1] HERCULES Laboratory & IN2PAST, University of Évora, Portugal
[2] Institute for Research and Advanced Training (IIFA), University of Évora, Portugal
[3] Department of Geosciences, ECT, University of Évora, Portugal
[4] Department of Chemistry and Biochemistry, ECT, University of Évora; Portugal
[5] LAQV-REQUIMTE, IIFA, University of Évora, Portugal
[6] Department of Medical Sciences and Health, ESDH, University of Évora, Portugal

(*) e-mail: d53109@alunos.uevora.pt

Keywords: biotechnology; nanotechnology; microbiology; NPs characterization; antimicrobial activity; sustainability.

ABSTRACT

Microbial contamination of Cultural Heritage (CH) materials is one of its most prevalent and impacting preservation issues that can lead to visual, structural, and chemical changes[1]. Currently, different approaches try to address this issue by employing products and techniques that can lead to structural or chemical alteration of materials[2] and pose several environmental risks[3]. For these reasons, nanotechnological solutions can constitute new greener alternatives. Studies on metal-based nanoparticles (NPs) have described their efficient antimicrobial properties and long-term effects at low concentrations[4]. However, their stability is highly dependent on surface functionalization, and their chemical synthesis methods may also raise environmental issues. The use of microbial cultures lowers the environmental impact of metal-based NPs' synthesis and stabilizes them with probable antimicrobial potentiating molecules secreted by these microorganisms[5]. In this study, we have successfully synthesized metal-based NPs using supernatants of microbial cultures. Their morphological, chemical, and physical characterization using spectrophotometric, x-ray based, and electron microscopy techniques was made, and their antimicrobial activity evaluated against several microorganisms isolated from CH. Results show good antimicrobial potential and stability of the biosynthesized NPs and suggest that the distinct supernatants result in variable NPs properties. Therefore, while further optimization of the synthesis process is necessary, and comprehensive testing using mock-ups and real Cultural Heritage materials is pending, our findings confirm that this approach is a promising alternative to the current traditional biocides.

References:

[1] Branysova, T. *et al. J. Cult. Herit.* 55, 245–260 (2022)
[2] Cappitelli, F. *et al. Microorganisms* 8, 1542 (2020)
[3] Kakakhel, M. A. *et al. Int. Biodeterior. Biodegrad.* 143, 104721 (2019)
[4] Rudramurthy, G. *et al. Molecules* 21, 836 (2016)
[5] Carrapiço, A. *et al. Microorganisms* 11 (2), 378, (2023)

Acknowledgements:
FCT projects UIDB/04449/2020 and UIDP/04449/2020 and grant UI/BD/153583/2022.
Project Sustainable Stone by Portugal, nº 40, proposal C644943391-00000051, co-financed by PRR.

P7.2 - EXPLORING THE POTENTIAL OF *S. CARPOCAPSAE* VENOM PROTEINS IN INNOVATIVE NANO-INSECTICIDES

Jorge Frias [1]*, Rui L. Reis [2], Nelson Simões [1]

[1] Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, Açores, 9500-321 Ponta Delgada, Portugal

[2] 3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, Portugal

(*) e-mail: jorge.mv.frias@uac.pt

Keywords: venom proteins, bioinsecticides, nanoformulations, nano-insecticides, chitosan nanoparticles, insect pest control.

ABSTRACT

Crop losses due to pests have a significant impact on the evolution of human society and food supply. Although recent advances in science and technology have considerably reduced the incidence and severity of plant pests, a large portion of the actual production is still lost each year. Therefore, plant protection in agriculture demands the control of several insect pests in a safe, effective, and low-cost manner.

This challenge can be an opportunity for biological control agents because they are much safer alternatives than several chemical pesticides. Therefore, the purpose of this work was to nanoformulate two biological molecules known as virulence factors of entomopathogenic nematodes, such as ScK1 peptide, a potassium channel blocker, and a cysteine-rich secretory protein from the CAP superfamily, to evaluate their potential use as bioinsecticides against insect pests.

Nanoencapsulation of these two venom proteins was achieved by the preparation of chitosan nanoparticles via the ionic gelation method.

The overall characterization of chitosan nanoparticles revealed a nanometric size for both NanoScK and NanoCAP formulations, with a mean size of 182.0 and 676.9 nm, respectively, and an initial rapid release followed by a slower release profile in both formulations. These nanoformulations allowed the delivery of venom proteins into the insect hemocoel.

The NanoScK formulation caused immediate effects in treated insects with an evident reduced locomotor activity, paralysis, and mortality, contrary to NanoCAP, where low mortality occurred later in the assays. Finally, the effects of these formulations on insect progeny were assessed. NanoScK triggered a significant reduction in the number of eggs laid by adult insects, whereas NanoCAP caused a significant reduction in adult emergence.

The results of this study confer a proof-of-concept status regarding the use of nematode venom proteins for developing new insecticidal formulations.

Acknowledgements:

JF received a doctoral grant from FCT (SFRH/BD/131698/2017). JF is grateful to his supervisor, Professor NS, for the patient guidance, encouragement, and advice and to co-supervisor Rui Reis. This work was funded by Fundo Europeu de Desenvolvimento Regional (Feder) under the project NanoNema (ACORES-01-0145-FEDER000113) approved by Autoridade de Gestão do Programa Operacional AÇORES 2020.

P7.3 - ENHANCING ANAEROBIC DIGESTION BY SIMULTANEOUS APPLICATION OF A COMBINED TREATMENT OF NANO-ZERO VALENT IRON AND MAGNETITE NANOPARTICLES

Nicolas Hoffmann [1,3,4(*)], Gonzalo Tortella [2,3], Christian Vergara [2], Gilberto Martins [4], Olga Rubilar [2,3]

[1] *Programa de Doctorado en Ciencia de Recursos Naturales, Facultad de Ciencia e Ingeniería, Universidad de La Frontera, Temuco, Chile.*

[2] *Departamento de Ingeniería Química, Facultad de Ciencia e Ingeniería, Universidad de La Frontera, Temuco, Chile.*

[3] *Biotechnological Research Center Applied to the Environment (CIBAMA), Universidad de La Frontera, Temuco, Chile.*

[4] *Centre of Biological Engineering (CEB), Campus de Gualtar, University of Minho, Portugal.*

[(*)] *e-mail:* n.hoffmann01@ufromail.cl

Keywords: Anaerobic Digestion; Iron nanoparticles, Biogas.

ABSTRACT

Anaerobic digestion (AD) is one of the most energy-efficient technologies for treating biodegradable waste since biogas can be generated as a by-product. AD is a biochemical process mediated by a consortium of microorganisms without oxygen in four stages: hydrolysis, acidification, acetogenic and methanogenesis. The addition of iron-based nanoparticles (Fe-NPs) aims to boost the AD process to enhance the biogas output by degrading biomass through interspecies electron transfer. In this work, three AD batch experiments were explored to assess the enhancement of methane production yield via the incorporation of conductive nanomaterials, zero-valent iron (nZVI), magnetite nanoparticles (Fe_3O_4-NPs), and a combination of the two nZVI/Fe_3O_4-NPs, under two concentrations (200 $mg \cdot L^{-1}$ and 400 $mg \cdot L^{-1}$). Glucose (1.5 $g \cdot L^{-1}$) was used as the main carbon source for biogas production. The granular inoculum was obtained from brew beer waste organic waste treatment. Chemical oxygen demand (COD $mg \cdot L^{-1}$) and methane production (NmL) were determined as the main responses via analytic and gas chromatography methods. After sixteen days, COD removal (%), pH, glucose reduction (%), and methane yield production ($NmL \cdot mgCOD^{-1}$) were determined. Control samples were anaerobic reactors without nanomaterials. Regarding the control, significant differences were observed in COD removal by incorporating 200 $mg \cdot L^{-1}$ nZVI, 400 $mg \cdot L^{-1}$ Fe_3O_4-NPs, and 200 $mg \cdot L^{-1}$ nZVI/Fe_3O_4-NPs: 13%, 26%, 30%, respectively. However, cumulative methane volume was not significant after eighteen days. In addition, an inhibitory methane production behavior was observed in the presence of 400 $mg \cdot L^{-1}$ nZVI. Maximum methane yield production was 0.693 $NmL \cdot mgCOD^{-1}$, 0.359 $NmL \cdot mgCOD^{-1}$, and 0.299 $NmL \cdot mgCOD^{-1}$ in the presence of 200 $mg \cdot L^{-1}$ nZVI, 200 $mg \cdot L^{-1}$ nZVI/Fe_3O_4- NPs, and 400 $mg \cdot L^{-1}$ Fe_3O_4-NPs, respectively. Considering the results, 200 $mg \cdot L^{-1}$ nZVI/Fe_3O_4-NPs showed interesting insights regarding COD remotion and methane yield production. Their combined effect should be deeply studied for medium exposure to methane production. This study is the first report to test combined nZVI/Fe_3O_4-NPs addition.

Acknowledgements:
BECA ANID 21202184 ANID/FONDAP/15130015

Universidad de La Frontera DI23-3002 Universidad de La Frontera PP23-0046 Universidad de La Frontera DI23-10002 Laboratorio de Nanobiotecnología Ambiental

P7.4 - DEVELOPMENT OF PLASMID DNA AND DRUG LIPID NANOPARTICLES: AN INNOVATIVE ROUTE AGAINST LUNG CANCER

Catarina Diniz [1(*)], Diana Pedro [1], Mariana Matias [1], Adriana O. Santos [1], Ângela Sousa [1]

[1] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.*

[(*)] *e-mail:* catarinapdiniz@fcsaude.ubi.pt

Keywords: lung cancer; multifunctional lipid nanoparticles; plasmid DNA.

ABSTRACT

Lung cancer is the first cause of cancer death worldwide, with 1.8 million deceased patients in 2020[1]. Non-small cell lung cancer (NSCLC), 85% of all lung cancer cases, is a heterogeneous group of cancer subtypes. NSCLC patients could benefit from novel therapeutic strategies that target multiple mechanisms of cancer progression.

We intend to develop an innovative and high-performance multifunctional lipid nanoparticle (LNP) combining gene therapy, targeted therapy, and immunotherapy against NSCLC. As first step, the aim of this work was to develop a novel LNP incorporating the drug of interest and its combination with plasmid DNA (pDNA).

LNP were prepared with different amounts of cationic lipid, phospholipid, cholesterol and a lipophilic drug, without or with pDNA at different nitrogen:phosphate (N:P) ratios (20, 10 and 5). Lipids and the drug were dissolved in ethanol or in another solvent (confidential) and added to the aqueous phase containing pDNA under agitation, without further size homogenization. LNP characterization included mean hydrodynamic particle size, polydispersity index (PDI) and zeta potential as well as DNA electrophoresis do access pDNA encapsulation.

The novel solvent produced formulations with smaller size compared to ethanol. Moreover, at a N:P ratio of 10, it allowed the complete complexation of pDNA, and, at optimal lipid and drug concentrations, drug precipitation was avoided. More specifically, LNP with cholesterol, N:P ratio of 10, a pDNA concentration of 50.84 µg/mL, and a drug concentration of 900 nmol/mL, had a size around 180 nm, a PDI of 0.3, and zeta potential of 27 mV, being stable at least over 3 days.

Concluding, we have obtained an innovative LNP, able to combine pDNA and the drug of interest, using a safer solvent. Furthermore, the size and homogeneity characteristics were favourable without extrusion. Further studies of development, optimization, and evaluation of the novel LNP are ongoing.

References:

1. Bade, B. C. & Dela Cruz, C. S. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med 41, 1–24 (2020).

Acknowledgements:

CICS-UBI received base (UIDB/00709/2020) and programmatic (UIDP/00709/2020) national funding through *Fundação para a Ciência e a Tecnologia* (FCT). The project was funded by "UBI/Santander young researcher award" 2023. Ângela Sousa acknowledges FCT and UBI for her Assistant Researcher Contract under scope of the CEEC Institutional 2021, funded by FCT in the call CEECINST/00016/2021. Lipoid kindly donated some of the lipids used.

P7.5 - FUNGAL LIPIDOMICS OF THE GENUS *LASIODIPLODIA*

Francisca Freitas [1,2], Susana Aveiro [3], Diana Lopes [2,4], Artur Alves [1], Ana Cristina Esteves [1(*)], Maria do Rosário Domingues [2,4]

[1] CESAM—Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

[2] CESAM—Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

[3] GreenCoLab - Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, Portugal

[4] Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

[(*)] e-mail: acesteves@ua.pt

Keywords: fungi; Lasioplodia; kingdom-host jumps; lipidomics; pathogenicity.

ABSTRACT

The genus *Lasiodiplodia* has long been recognized as a plant pathogen, but there are reports of species of *Lasiodiplodia* causing disease in humans. The phenomenon that allows pathogens to transition from infecting plants to humans, known as cross-kingdom jumps, is still largely unknown. There is still much to be learned about the mechanisms that facilitate and make this transition possible.

Lipids are known for their relevant roles in cellular functions, including cell signaling, which is tightly linked with pathogenicity. For example, glycosphingolipids are involved in the regulation of virulence in fungi. Due to the connection that lipids hold with pathogenicity, they may prove to be important tools in understanding this phenomenon, and possibly even serving as targets to treat fungal infections.

As such, the aim of this work was to study the effect of temperature on the lipidome of two species of *Lasiodiplodia*, *L. theobromae* and *L. hormozganensis*, utilizing gas chromatography–mass spectrometry and liquid chromatography-mass spectrometry.

Both strains showed an increase in the proportion of omega-3 and omega-6, which are known to be involved in regulating the fluidity of cell membranes, when growth temperature was raised from 25ºC to 37ºC. This suggests an adaptation of the fungus to higher temperature, like that of the human body. Interestingly, longer chained fatty acids were produced specifically when fungi were cultivated at 37ºC, such as lignoceric acid which was not present in the fungi grown at 25ºC.

The quantities and proportions of fatty acids of both strains showed different profiles when grown at different temperatures, which likely aided the ability to adapt to 37ºC.

As a final remark, these data help to explain how these species can colonize and infect host from different kingdoms. However, there is a major concern remaining: do the increasing temperatures facilitate human infections by these species?

Acknowledgements:

The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020).

P7.6 - VIRAL DETECTION IN WASTEWATERS: COMPARISON OF BIOINFORMATIC TOOLS FOR VIROME ANALYSIS

André Filipe Santos [1*], Mónica Nunes [2], Andreia Silva [3], Teresa Crespo [3], Victor Pimentel [1], Marta Pingarilho [1], Mafalda Miranda [1], Pieter Libin [4], Ricardo Parreira [1], Ana B. Abecasis [1], Sofia G. Seabra [1]

[1] Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

[2] Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

[3] iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

[4] Department of Computer Science, Artificial Intelligence Lab, Brussels, Belgium

[(*)] e-mail: afbo.santos@campus.fct.unl.pt

Keywords: metavirome; wastewater; viral detection.

ABSTRACT

Analyses of viral genetic material in wastewaters provide community-level data that can be the basis for early warnings, and efficient tracking of viral pathogen emergence and spread. Unlike traditional species-specific diagnostic methods, a metavirome approach enables a more comprehensive overview of emergent and new potentially pathogenic viruses. By harnessing large-scale sequence data collection with advanced algorithms, bioinformatics offers a more holistic solution to disease surveillance using wastewaters, ensuring timely interventions and enhanced public health outcomes. For viral pathogens, wastewater analysis at the different steps of wastewater treatment is also relevant to understand their effectiveness in decreasing viral content.

In this study we used a random-primer-based sequencing approach combined with next-generation Illumina sequencing to characterize the viral content of influent and effluent waters from two wastewater treatment plants. For viral classification, we used the algorithms implemented in Genome Detective, Kraken2, CZ.ID and INSaFLU and compared the outcomes. The initial results showed that sequencing throughput was highly variable between samples (ranging between 130,000 to 3,700,000 reads), that the percentage of remaining reads after quality control and human and contaminant filtering also ranged immensely (from 1.18% to 72.38%). Even using a viral enrichment amplification step in the experimental protocol, the proportion of viral reads was very low (ranging between >0.001 and 8.44%) but a large number of virus families was found, including pathogenic viruses, across all stages of wastewater systems, adverting to inefficiency of removal or degradation of viruses by wastewater treatment processes.

Acknowledgements:
Funding was provided by: AgriWWAter: "Reclaimed wastewater for agriculture irrigation: mitigating microbiological hazards for a safer food production". Refa PTDC/CTA-AMB/29586/2017 (Fundação para a Ciência e Tecnologia, Portugal), VirusFreeWater: "Reclaimed water for agricultural irrigation: overcoming the silent threat of waterborne viral infections". Refa 706, Projeto interno IBETXplore 2017, Internal exploratory Project GHTM - UID/04413/2020, WasteWaterVir (Fundação para a Ciência e Tecnologia, Portugal). S.G.S. acknowledges support from Contrato- programa 1567 (CEECINST/00102/2018, FCT, Portugal). P.L. acknowledges support from the Research council of the Vrije Universiteit Brussel (OZR-VUB) via grant number OZR3863BOF.

P7.7 - COMPLETE GENOME OF *PEDOBACTER LUSITANUS* NL19: A COMPREHENSIVE GENOMIC ANALYSIS

Gonçalo Figueiredo [1(*)], ***Hugo Osório*** [2,3], ***Marta V. Mendes*** [2,4], ***Sónia Mendo*** [1]
[1] *Departement of Biology & CESAM, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal*
[2] *i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*
[3] *Ipatimup - Institute of Molecular Pathology and Immunology of the University of Porto, University of Porto, Porto, Portugal*
[4] *IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*

[(*)] *e-mail: henriques.figueiredo@ua.pt*

Keywords: *Pedobacter lusitanus* NL19, biosynthetic gene clusters, genome mining, natural products, de novo assembly.

ABSTRACT

Pedobacter lusitanus NL19 is an extremophilic Gram-negative bacterium, which encloses in its genome a vast repertoire of genes that encode unique natural compounds with promising valuable biotechnological applications. Despite having 16 biosynthetic gene clusters (BCGs), only five of them have been studied so far, and only three of these have been characterized – two coding for lanthipeptides and one for a non-ribosomal peptide (NRP). The remaining BGC appear to be cryptic or weakly expressed in the laboratory conditions used. The use of in silico genome mining is a valid and strategic approach that will allow to unravel the biosynthetic potential of *P. lusitanus* NL19 in a more comprehensive way, allowing the discovery of new natural products.

We employed PacBio long-read sequencing to assemble the complete genome of *P. lusitanus* NL19 and, subsequently, conducted further in silico genomic analysis. The genome was annotated, and a comprehensive in silico analysis was performed using various bioinformatic tools and workflows. We explored the natural products (NPs) biosynthetic potential, and successfully identified metal resistance genes, prophage regions, genomic islands, CRISPR arrays and various resistance models. The potential of this strain to produce novel NPs, with predicted structures, and foreseen biotechnological applications was also explored and is highlighted. These findings pave the way to the expression of these BGCs either by direct fermentation or using different manipulation strategies, synthetic biology approaches or heterologous expression, aiming at the subsequent characterization of these natural products.

Acknowledgements:
Gonçalo Figueiredo was funded by national funds (ON) and community funds through the FSE, through FCT (2022.12997.BD). Thanks are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020), through national funds.

P7.8 - TACKLING NON-HODGKIN LYMPHOMA ON TWO FRONTS: DUAL-TARGETING IMMUNOLIPOSOMES AS A NOVEL DRUG DELIVERY SYSTEM

Ana Leonardo [1,2], Ana S. André [1,2], Margarida Ferreira-Silva [1,2(*)], Sandra I. Aguiar [1,2], Sara Nogueira [1,2], Marco Cavaco [3], Lurdes Gano [4], João D.G. Correia [4], Miguel Castanho [3], Manuela Gaspar [5], Luís Tavares [1,2], Frederico Aires-da-Silva [1,2&], Joana N. R. Dias [1,2&]

[1] Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

[3] Instituto de Medicina Molecular-João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

[4] Centro de Ciências e Tecnologias Nucleares, Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal

[5] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

[&] These authors contributed similarly to this work.

[(*)] e-mail: amsilva@fmv.ulisboa.pt

Keywords: Immunoliposomes; Non-Hodgkin's lymphoma; Panobinostat; Single domain antibody; Folate; Dual-targeting.

ABSTRACT

Non-Hodgkin's lymphoma (NHL) remains a major global cause of cancer-related deaths, requiring novel treatment strategies, particularly for refractory/relapsed cases. This study explores the potential of dual-targeting immunoliposomes for delivering the pan-histone deacetylase inhibitor panobinostat (PAN) using folate and a rabbit-derived single domain antibody (sdAb) for selective antitumoral targeting against canine NHL (cNHL), a model of human NHL.

An immune VL-sdAb library was constructed from a rabbit immunized with donor cNHL cells and subjected to in vitro and in vivo Phage Display. Clone binding and expression were evaluated by ELISA, and NGS was performed to select the best candidates. C5 sdAb was chosen and its binding and internalization properties were characterized by flow cytometry and immunofluorescence. C5 biodistribution was evaluated on a cNHL xenograft murine model. Folate expression on CLBL-1 cells was evaluated by immunoblotting. Immunoliposomes were developed by conjugating C5 to the liposome of untargeted liposomes and folate-targeted liposomes through biotin-streptavidin-biotin ligation method. Targeting properties of all liposome formulations were evaluated by flow cytometry and fluorescence microscopy. In vitro experiments assessed cytotoxicity and histone H3 acetylation induction.

A highly diverse sdAb library targeting cNHL was constructed and phage display screening allowed to identify highly specific sdAbs that recognize both human and canine NHL targets. The lead candidate, C5, exhibited promising binding properties and significant in vivo tumor uptake. Folate receptor overexpression in CLBL-1 was confirmed. PAN-loaded liposomes were efficiently developed, with all formulations exhibiting dose-dependent inhibitory effects on cNHL cells. Targeted liposomes demonstrated enhanced cellular uptake compared to non-targeted liposomes. While dual targeting did not significantly increase internalization, dual-targeted PAN liposomes had a lower IC50 compared to Lip-PEG-C5. All liposome formulations induced histone H3 acetylation.

Overall, this study demonstrates the potential of dual-targeting immunoliposomes using folate and

a sdAb for the treatment of NHL.

Acknowledgements:
This work was supported by the Portuguese funding agency Fundação para a Ciência e Tecnologia (FCT), (SAICT/2017/32085 and Ph.D. fellowship SFRH/BD/131468/2017 to AA). This research was funded by Génese Program, Gilead Sciences, Portugal (grant PGG/050/2019). Center for Interdisciplinary Research in Animal Health (CIISA) and the Associated Laboratory for Animal and Veterinary Science (AL4AnimalS) has provided support via Project UIDB/00276/2020 and LA/P/0059/2020-AL4AnimalS, respectively, both funded by FCT.

P7.9 - AIMING AT THE AUTOMATION OF GENOME-WIDE REGULATORY NETWORK INFERENCE IN *SACCHAROMYCES CEREVISIAE*

Diogo Couceiro [1,2,3(*)], ***Maria Pereira*** [1,2(*)], ***Carolina Parada*** [1,2], ***Romeu Viana*** [2,3], ***Miguel Cacho Teixeira*** [2,3], ***Pedro Tiago Monteiro*** [1,4]

[1] *INESC-ID, Lisboa*

[2] *iBB - Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa – Lisboa*

[3] *I4HB – Institute for Health and Bioeconomy - Laboratório Associado, Portugal*

[4] *Department of Computer Science and Engineering, Instituto Superior Técnico, Universidade de Lisboa – Lisboa*

[(*)] *e-mail: diogo.c.couceiro@tecnico.ulisboa.pt*

ABSTRACT

Metabolism is much more than a set of connected reactant-catalyst-product combinations, an assumption too often made by standard genome-scale metabolic models. Metabolism is complex and dynamic, and in close liaison with environmental conditions that control metabolism through signaling/regulatory pathways. Altogether, it's vital to consider all these players when wishing to represent cell behavior using mathematical models, in a way that they can be useful to increase our ability to optimize cell factories through transcription factor manipulation.

This work aims at developing tools for the automated inference of Boolean regulatory networks from a set of regulatory information, such as that present in Yeastract+ (containing around 200K TF-target associations for *Saccharomyces cerevisiae*) [Teixeira et al, NAR, 51: D785-D791, 2023], in several different environment conditions. Regulatory network topology provides interesting static information on the structure and hierarchy of genome-scale regulatory networks. However, further information on the impact of combinations of transcription factors (TF) in target gene (TG) expression is required to obtain a model that describes the dynamics of regulatory-metabolic systems. With that objective in mind, a set of Boolean forms was selected to be tested on global yeast regulatory data, to assess the adequacy of the chosen Boolean forms to be used as general rules of TFs-TG interactions. Further tools for the manual curation of the regulation of individual genes are being built to enable the fine-tuning of the developed models. Finally, these models will enable the prediction of the impact of environmental cues on metabolic outcome and the optimization of the use of genome-scale metabolic models in cell factory optimization through regulatory engineering.

MICRO BIOTEC 23

Poster Session

Topic 8

P8.1 - A DUAL BIOCATALYZED MICROBIAL ELECTROSYNTHESIS SYSTEM FOR CO2 SEQUESTRATION AND SIMULTANEOUS PRODUCT VALORIZATION WITH BIOCATHODE AND BIOANODE

Khurram Tahir 1*, Felipe Conzuelo 1, Inês Cardoso Pereira 1

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

[(*)] e-mail: Khurram.tahir@itqb.unl.pt

Keywords: CO2 Sequestration, Microbial Electrosynthesis, Dual Biocatalyzed, Gas Diffusion Electrode

ABSTRACT

The on-going climate crisis and accelerating production of greenhouse gases such as carbon dioxide has sparked a sense of urgency in the scientific community to come up with solutions to sequestrate and/or recycle CO_2. CO_2 is the most abundant greenhouse gas and has been the major contributor of greenhouse effect and climate change. Microbial electrosynthesis (MES) has emerged as a renewable and green technology that uses electroactive microbial catalysts to mineralize and treat different wastes/pollutants such as CO_2.

This study aims to develop a novel dual biocatalyzed MES consisting of an efficient gas diffusion biocathode for CO_2 sequestration in combination with a bioanode for simultaneous product valorization. The use of biocatalysts both at the anode and cathode in a dual biocatalytic MES can simultaneously treat CO_2 and waste pollutants along with bio production of chemicals making the process more energy and cost efficient.

The MES system is being established based on enriched microbial communities, monitoring substrate consumption and product formation. The developed system will minimize the carbon footprint mitigating climate change with a significant scientific and societal impact. The decreasing CO_2 emissions and simultaneous biotransformation of wastes in the bioanode will have a great societal impact resolving not only the long going carbon capture crisis but at the same time presenting a sustainable platform for environmental remediation and chemical production. The use of dual bio-catalyzed MES will create new knowledge in biosynthesis, power and fuel generation along with persistent pollutant treatment with bioelectrochemical systems using microbes.

Acknowledgements:
This research is supported by the Marie Skłodowska-Curie Actions Postdoctoral Fellowships 2021 (Project: 101068836 — BIOELECTRO-CO2, HORIZON-MSCA-2021-PF-01-01). and Fundação para a Ciência e Tecnologia (Portugal) through the Research Unit MOSTMICRO – UIDB/04612/2020 and UIDP/04612/2020, and Associate Laboratory LS4FUTURE – LA/P/0087/2020.

P8.2 - FROM DOGS TO DOGS: FIRST NATIONWIDE SCREENING OF GIP AND AMR BACTERIA IN OFFICIAL MUNICIPAL KENNELS

Patrícia Lopes [1.2], Inês Natal [1], Jacinto Gomes [3,4,5], Mónica V. Cunha [1,2(*)]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[2] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.*

[3] *Agrarian School of Elvas, Polytechnic Institute of Portalegre, Portalegre, Portugal*

[4] *CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal*

[5] *Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal*

[(*)] *e-mail: mscunha@ciencias.ulisboa.pt*

Keywords: municipal kennels; gastrointestinal parasites; antimicrobial resistant bacteria; One Health.

ABSTRACT

Stray dogs are fragile animal populations potentially exposed to a wide range of pathogens whose transmission cycles they might perpetuate. Their clinical history is mostly unknown and infectious disease screening is rarely performed upon admission into shelters, posing health risks to other housed animals and collaborating staff. In this work, we established a partnership with the National Association of Municipal Veterinary Practitioners and several municipalities across the country to monitor the occurrence of zoonotic gastrointestinal parasites (GIP) and antimicrobial resistant (AMR) bacteria in dogs that are officially sheltered, thus establishing baseline data for this specific animal population at the country level, for the first time.

More than 350 dog stool samples collected over a one-year period were screened by means of copro-parasitological analyses. Over 16% were GIP-positive, with *Trichuris sp., Toxocaridae, Ancylostomatidae*, and *Cystoisospora* sp. being the most observed GIP, in decreasing frequency. The number of housed dogs positively correlated with the occurrence of zoonotic *Ancylostomatidae* and *Trichuris* sp. Selected samples (n=44) from three large kennels, representing several municipalities each, were cultured in selective media, yielding more than 220 *Enterobacteriaceae* isolates, including the genera *Proteus, Escherichia, Enterobacter and Citrobacter*, with 12% of the isolates being multidrug resistant. Phenotypic resistance to gentamicin and to β-lactam antibiotics, including second-generation cephalosporins, were common, although ESBLs detection was rare. Whole genome sequencing of resistant isolates and metagenomic sequencing of original stool samples were performed aiming further taxonomic and functional data characterization.
Results from this study have been communicated to the surveyed kennels in near-real time and in webinars to inform adaptive prophylaxis, infection control and dog internal deworming practices. Altogether, our findings highlight the need to update the knowledge and practice of MVP, through laboratory support, reinforced education, training, and bridging communication among stakeholders.

Acknowledgements:
Fundação para a Ciência e a Tecnologia, IP [UIDB/00329/2020].

P8.3 - EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF PLANT ORIGIN BYPRODUCTS EXTRACTS ON BACTERIA AND YEASTS

Matheus Guilherme Lemos [1,2], Ana Ferreira [1,2], Ana Rita Barata [1,3], Maria José Saavedra [2,3,4], Gonçalo Almeida [1,5(*)]

[1] *National Institute of Agricultural and Veterinary Research, I.P (INIAV), Vila do Conde, Portugal*

[2] *Departamento das Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[3] *Escola de Ciências da Vida e do Ambiente, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[4] *Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB) & Institute for Innovation, Capacity building and Sustainability of Agri-Food production (Inov4Agro), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[5] *Center for Animal Science Studies - Institute of Agricultural and Agro-Food Sciences and Technologies (CECA-ICETA), University of Porto, Porto, Portugal*

[(*)] *e-mail:* goncalo.almeida@iniav.pt

Keywords: Antimicrobial activity; Aqueous extracts; Vegetable byproducts; Antioxidants.

ABSTRACT

Food waste presents itself as a global problem and has gained increasing importance on the public and political agenda, as it contributes significantly to various environmental, social and economic impacts. Large quantities of food byproducts are discarded and neglected without any particularity. In their composition there are bioactive compounds, known to regulate metabolic processes, through their antioxidant and anti-inflammatory activities, some of these compounds also have antimicrobial properties. In this sense, one of the strategies addressed to reduce food waste is to take advantage of the residues and add value to byproducts, exploring their applications. Thus, the objective of this work was to evaluate the antimicrobial activity of the aqueous extract of five plant origin byproducts extracts, namely, peanut shell and skin, pomegranate shell and seeds of three date fruit varieties (Alig, Kentichi and Deglet Nour) on bacteria and yeasts, using disk diffusion method. In addition, each extract was separated by thin-layer chromatography (TLC) to define their retention factors. For antimicrobial essay, bacterial and yeast suspensions were adjusted to 0.5 McFarland standard scale. Then, the suspension was spread in Petri dishes with Muller Hinton Agar medium for bacteria and Sabourand Dextrose agar for yeast. Sterile filter paper discs (9 mm) with 20 µL of the extract were placed on the agar surface. The aqueous extract with the greatest antimicrobial activity was that of pomegranate peel, which acted on the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* and on the yeast *Candida glabrata.* Extracts from peanut shell and skin, as well as extracts from the seeds of the three date varieties, acted solely on the *S. aureus* bacteria. This study contributed to better understand the chemical and antimicrobial characteristics of aqueous extracts of plant origin byproducts with the aim of their promotion in a circular and sustainable economy to reduce food waste.

Acknowledgements:
The authors thank CECA-ICETA UIDB/00211/2020 and VALICET project ref. PRIMA/0001/2020.

P8.4 - ALGARVE'S PGI CITRUS ESSENTIAL OILS: UNLOCKING THE POTENTIAL OF FOOD INDUSTRY BY-PRODUCTS

Cristina Rodrigues [1(*)], Daniela Magalhães [1], Ana A. Vilas-Boas [1], Manuela Pintado [1]

[1] *Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

[(*)] *e-mail:* civrodrigues@ucp.pt

Keywords: citrus by-products; bioactive compounds; essential oils; food preservation.

ABSTRACT

Worldwide, the processing of citrus juices generates elevated amounts of waste (around 120 million tons, annually), including peels, seeds and pulp, leading to an unprecedented environmental burden. Orange (*Citrus sinensis*) and lemon (*Citrus limon*) peels, for instance, contain valuable bioactive compounds such as polyphenols, essential oils, and fiber, which can be repurposed for novel food, pharmaceutical and cosmetic applications. Citrus peels essential oils, particularly, have gained increasing attention for their diverse applications within the food preservation area. Therefore, the present work aimed to evaluate and compare the essential oils from Algarve's Protected Geographical Indication (PGI) Citrus, namely oranges and lemons, regarding their composition by GC-MS, antioxidant activity by ABTS and DPPH assay, as well as their antimicrobial capacity against specific bacterial (Gram-positive: *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes;* Gram-negative*: Escherichia coli, Yersinia enterocolitica, Salmonella enterica and Pseudomonas aeruginosa*) and fungal (*Aspergillus niger*, *Penicillium expansum*, *Fusarium verticilloides*, *Clodosporium spp*.) strains. Orange's essential oil main compounds (w/w) were found to be D-limonene (94.75%) followed by β-myrcene (0.95%) and valencene (0.31%), while the lemon's essential oil was mostly composed (w/w) by D-limonene (59.67%), (-)-B-pinene (9.88%) and -terpinene (8.17%). Concerning the ABTS and DPPH assays, both oils exhibited antioxidant capacity. Moreover, lemon and orange essential oils demonstrated antibacterial and antifungal activity against various microorganisms, at the tested concentrations (0.90 – 459 mg/mL). However, lemon essential oil showed a greater potential as a more potent natural antimicrobial agent, presenting lower minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values.

Overall, the valorization of citrus processing by-products, exemplified by the essential oils from Algarve's PGI Citrus, not only offers promising avenues for sustainable food preservation solutions, but also underlines the importance of turning waste into valuable resources in the global citrus industry, as a circular economy approach.

Acknowledgements:
The authors would like to acknowledge the MEDISMART project—Mediterranean Citrus innovative soft processing solutions for S.M.A.R.T (Sustainable, Mediterranean, Agronomically evolved nutritionally enriched Traditional) products (reference number: 2019-SECTION2-20) and the Portuguese national funds from FCT—Fundação para a Ciência e Tecnologia (reference: PRIMA/0014/2019) for the supporting of this research. Ana A. Vilas-Boas would like to acknowledge FCT for the individual PhD grant (2020.05655.BD). In addition, the author Daniela Magalhães would like to acknowledge FCT for the individual PhD grant (2022.12417.BD).

P8.5 - EXPLORING THE ANTIMICROBIAL AND DERMATOLOGICAL POTENTIAL OF *VACCINIUM MYRTILLUS L.* BIORESIDUES

Juliana Garcia [1(*)], Daniela M. Correia [1(*)], Sandrina Heleno [2], Lillian Barros [2], Maria José Saavedra [3], Maria José Alves [1,2(*)]

[1] *AquaValor - Centro de Valorização e Transferência de Tecnologia da Água, Chaves, Portugal*

[2] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal,* [3] *CITAB – Centre for the Research and Technology of Agro-Environment and Biological Sciences/ Inov4Agro - Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

(*) *e-mail: juliana.garcia@aquavalor.pt | maria.alves@ipb.pt | Daniela.correia@aquavalor.pt*

Keywords: Circular economy; Blueberries leaves; antimicrobial activity; dermatological effects.

ABSTRACT

Due to the World Health Organization's recognition of blueberries' benefits to a balanced diet and the research supporting their ability to prevent various diseases, the production of blueberries has experienced an upward trend [1]. Indeed, significant volumes of wasted leaves were produced which could be an important source of bioactive chemicals [2]. Studies on these residues that identify phytochemicals and evaluate their biological effects have recently been conducted in our group [3]. These by-products' bioactive compounds have an intriguing phenolic content and a distinctive antioxidant activity profile. Reusing these waste residues is really advised given their nature and sustainability concerns. In this study, an effort was undertaken to assess the potential for employing this bioresidue for the prevention and treatment of skin diseases. Therefore, the antioxidant, antiaging, antimicrobial, and antibiofilm properties of aqueous extracts from Vaccinium myrtillus L. Bioresidues were assessed. Concerning antiaging properties, the extracts achieved 54.0 5.3% inhibition of tyrosinase, 55.3 7.2% of elastase, 59.3 0.6% of collagenase, and 31.5 2.9% of hyaluronidase. Bioresidues extracts also showed high antioxidant activity, reaching 87.5% free radical scavenging using Ferric Reducing Antioxidant Power Assay and 84.2% using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The antimicrobial properties against dermatologically relevant pathogens demonstrated notable broad-spectrum activities. Noteworthy antimicrobial activity against Propionibacterium acnes, Staphylococcus aureus, and Candida albicans reached MIC values of 0.0625mg/ml, 0.125mg/ml, and 0.250 mg/mL, respectively. Moreover, MIC concentrations suppressed biofilm formation by Propionibacterium acnes, Staphylococcus aureus, and Candida albicans. Overall, our findings indicate that Vaccinium myrtillus L bioresidues could be used as a leading natural source in the skin care cosmetic industry.

Acknowledgements:
Financial support from “AquaeVitae”-PROMOVE, MissãoInterface-PRR-AAC 01/C05-i02/2022 and “AquaValor”-NORTE-01-0246-FEDER-000053.

MICROBIOTEC 23

Poster Session

Topic 9

P9.1 - BUILDING A NETWORK OF BIOBANKS AND BIOLOGICAL RESOURCE CENTRES ACROSS PORTUGUESE-SPEAKING COUNTRIES: COLLABORATIVE EFFORTS FOR GLOBAL SUSTAINABILITY

Ana Paula Arez *(*) on behalf of Rede Lusófona de Biobancos* #

**Angola**: Joana Morais (Instituto Nacional de Investigação em Saúde, Luanda), Maria Madalena Chimpolo (Universidade Agostinho Neto, Luanda); **Brazil**: Aline Souto (Coordenação das Coleções Biológicas Fiocruz, Rio de Janeiro), Manuela da Silva, Carlos Nascimento (Biobanco Covid-19 da Fiocruz, Rio de Janeiro), Daiane Sertorio (Rede Fiocruz de Biobancos – material humano, Rio de Janeiro); **Cabo Verde**: Isabel Inês Araújo, Ariana Freire (Universidade de Cabo Verde, Praia), Silvânia Leal (Instituto Nacional de Saúde Pública, Praia); **Mozambique**: Gloria Matambisso (Centro de Investigação em Saúde da Manhiça, Manhiça), Elda Anapakala, Thebora Sultane, Carla Madeira (Instituto Nacional de Saúde, Maputo), Carlos Bento, Almeida Guissamulo, Daniela de Abreu (Museu de História Natural, Maputo), Iara Gomes Jaintilal, Nídia Cangi Vaz (Centro de Biotecnologia, Universidade Eduardo Mondlane, Maputo); **Portugal**: Nelson Lima (Microbial Resources Research Infrastructure-PT, Micoteca Universidade Minho, Braga), Sérgio Dias (biobanco.pt, Instituto de Medicina Molecular João Lobo Antunes, Lisbon), Ana Oliveira, Maria SM Assunção (Faculdade Ciências Médicas NOVA, Lisbon), Marta Barreto, Célia Ventura (Instituto Nacional de Saúde Dr Ricardo Jorge, Lisbon), Ana Paula Arez, Ana Tavares, Isabel Couto, Silvana Belo, Miguel Viveiros (Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa)

(*) e-mail: aparez@ihmt.unl.pt

Keywords: Biological collections; Biobanking procedures; Research collaboration; Biospecimens; Sample storage; Sample management.

ABSTRACT

Biobanks and Biological Resources Centres (BRCs) are pivotal infrastructures in biomedical, biotechnological, and environmental research, providing resources for fostering innovation in a healthy, global, and sustainable society.

Most of biobanks and BRCs are located in North America and Europe. In Africa, significant consortia have been established but none of them encompassing the Community of Portuguese Language Countries (CPLP). Furthermore, Portuguese-speaking African countries (PALOP) still lack capacity, infrastructure, regulations and trained human resources, for effectively biobank samples. Nonetheless, certain institutions have initiated the establishment of biological collections, while others have expressed a keen interest in doing so. Consequently, a significant demand emerges for the exchange of experiences related to capacity building, training, and expertise in establishing biological collections. Such collaborative efforts hold the potential to invigorate local activities, foster knowledge dissemination, and contribute to the equitable sharing of benefits. This, in turn, empowers these nations to preserve and safeguard their unique biodiversity resources.

The implementation of a "Lusophone Network of Biobanks and Biological Resources Centres" is currently in progress to address this need. The inaugural meeting of this network occurred last April at IHMT NOVA in Lisbon, bringing together representatives from 15 institutions from Angola, Brazil,

Cabo Verde, Mozambique, and Portugal. The diversity in terms of developmental stages, existing infrastructure, strategies, programs, funding sources, and governance policies among institutions and countries was clearly evident. Several thematic areas associated with biobanking activities and infrastructure were introduced, setting the stage for future collective reflection and discussion. This network aims to yield not only immediate advantages such as enhanced data management and standardisation of operating procedures and information systems across biobanks and BRCs, in the frame of ethical guidelines, but also the potential for future collaborative trans-biobank studies.

Acknowledgements:
Fundação para a Ciência e a Tecnologia for funds to GHTM - UID/04413/2020 and LA-REAL – LA/P/0117/2020