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PONTA DELGADA
SÃO MIGUEL . AÇORES

ABSTRACT BOOK

SPM Sociedade Portuguesa de Microbiologia

spbt sociedade portuguesa de biotecnologia

MB 25

Thank you for your presence and contribution!

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WELCOME

Microbiotec'25 will be hosted at University of Azores, between 4-6th December 2025.

The Microbiotec congress happens biannually since 2005 in a co-organization by the Portuguese Society of Microbiology and the Portuguese Society of Biotechnology.

This biannual congress aims to create an enthusiastic platform for discussion and collaboration between national and international researchers and stakeholders in diverse areas of Microbiology and Biotechnology, welcoming young researchers making their first steps into the scientific community. MICROBIOTEC'25 aims to promote knowledge and discussion among researchers, and between academia and industry. Is an opportunity to share and debate ideas and science during these three days focused on the innovations and challenges of microbiology and biotechnology research.

Oral and poster communications are welcome on all conference topics:

Topic #1 – Environmental Microbiology & Biotechnology

Topic #2 – Food Microbiology & Biotechnology

Topic #3 – Health Biotechnology

Topic #4 – Health Microbiology

Topic #5 – Molecular Microbiology and Microbial Physiology

Topic #6 – Bioprocess Engineering

Topic #7 – New and Emerging Technologies

Topic #8 – From Science to Society and Business

Topic #9 – Biological Resources and Networks

The congress venue is located at the University of Azores in the heart of Ponta Delgada, the capital, at São Miguel Island. Apart from the scientific program, São Miguel is a welcoming Island that offers visitors a mighty volcanic landscape, lagoons and geothermal vents.

The scientific program will consist of plenary lectures (5), keynote lectures (14), oral communications and poster presentation.

We expect the participation of around 400 congress participants.

We look forward to welcoming you in Ponta Delgada.

The MICROBIOTEC'25 Organizing Committee

CONGRESS CHAIRS

Duarte Toubarro
E-mail: duarte.nt.tiago@uac.pt
Mobile: +351 919 260 020
University of Azores

Nelson Simões
E-mail: nelson.jo.simoes@uac.pt
Mobile: +351 914 848 359
University of Azores

COMMITTEES

Local Organizing Committee

António Machado | CBA- University of Azores

Carla Cabral | CBA- University of Azores

Célia Silva | IITAA – University of Azores

Duarte Mendonça | CBA- University of Azores

Duarte Toubarro | CBA- University of Azores

Graça Silveira | CBA- University of Azores

Jorge Frias | CBA- University of Azores

Maria do Carmo Barreto | CE3C- University of Azores

Nelson Simões | CBA- University of Azores

Raul Bettencourt | OKEANOS- University of Azores

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Duarte Seixas | CBA-Ignae

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Célia Manaia | ESB, UCatólicaP

Célia Silva | ITAA, UAç

Diogo Neves Proença | MED, UAlg

Duarte Toubarro | CBA, UAç

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Hermínia Lencastre | ITQB, UNL

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Miguel Viveiros | IHMT, UNL

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Vitor Vasconcelos | CIIMAR, UP

PROGRAM

Thursday, December 4th

Auditorium

10:00 Registration

14:00 Opening and Welcome
Opening local organizers and the Official entities

14:30 Lecture 1
Hyperthermophilic and Piezophilic Thermococcales: Exploring Their Adaptation to Extreme Environments and Biotechnological Applications.
Professor Mohamed Jebbar | Brest University – CNRS, France

TOPIC 1 | SESSION 1: Environmental Microbiology & Biotechnology
Chairs: **Margarida Casal** | CBMA, U. Minho
Artur Alves | CESAM, UA

15:45 **KN1.1 – Marine fungi: unlocking diversity and novel resources for biotechnological applications.**
Artur Alves | CESAM, UA

16:15 **OP1.1 – Genomic Diversity and Phage-Based Biocontrol of Erwinia amylovora: Towards Sustainable Fire Blight Management.**
Hugo Oliveira | Centre of Biological Engineering – U. Minho

16:30 **OP1.2 – Plasmid diversity: what do we miss when we only look at the majority?**
João S. Rebelo | CE3c – U. Lisboa

16:45 **OP1.3 – Diversity of *Leptospira* spp. in biofilms, water, and soil: pathogenic DNA and viable saprophytic isolates in Lisbon, Portugal.**
Ana Armada | IHMT, U. NOVA de Lisboa

17:00 **Coffee Break and Poster Session 1**

TOPIC 1 | SESSION 2: Environmental Microbiology & Biotechnology
Chairs: **Paula Morais** | CEMMPRE – UC
Raul Bettencourt | OKEANOS

17:45 **OP1.4 – Assessing endogenous cyanobacteria and microalgae potential as a sustainable solution for agriculture.**
Pedro Cruz | CIIMAR- CIMAR-LA

18:00 **OP1.5 – Bridging Knowledge Gaps in Antimicrobial Resistance Dynamics at Animal-Environment Interfaces.**
Mónica V. Cunha | CE3C – U. Lisboa

18:15 **OP1.6 – Characterisation of the gastrointestinal microbiome from Catrina cattle and Holstein Friesian breeds under different diets.**
Ana Rita Azevedo | CBA – UAc

18:30 **OP1.7 – Heterologous expression of a new natural lactylate in the model cyanobacterium *Anabaena* sp. PCC 7120.**
Eunice A. Ferreira | CIIMAR- CIMAR-LA

18:45 **OP1.8 – Dual-Output Microbial Electrosynthesis System for Biofuel Production.**
Nuno Machado | ITQB – NOVA

19:00 Sponsor Talk

19:30 Welcome Cocktail

Room 1

10:00 **Registration**

TOPIC 4 | SESSION 1: Health Microbiology
Chairs: **Jorge Pedrosa** | ICVS – U. Minho
Margarida Saraiva | i3S – U. Porto

15:45 **KN4.1 – Understanding tuberculosis pathogenesis and immune responses through the lens of Mycobacterium *tuberculosis*.**
Margarida Saraiva | i3S – U. Porto

16:15 **OP4.1 – Uncovering the Antifungal Potential of Bacteria Isolated from the Microbiota of Marine Microalgae.**
Teresa Pissarro | ITQB – NOVA

16:30 **OP4.2 – The Dangerous Duo: Resistance and Virulence in Bacteria.**
Célia Domingues | CE3c — Centre for Ecology

16:45 **OP4.3 – Marine-Derived Antimicrobial Peptides: A Novel Source of Molecules against Antimicrobial Resistance.**
Ana Rita Bragança | CBMA – U. Minho

17:00 **Coffee Break and Poster Session 1**

TOPIC 5 | SESSION 1: Molecular Microbiology and Microbial Physiology
Chairs: **Jorge H. Leitão** | IST – U. Lisboa
Maria João Sousa | CBMA – U. Minho

17:45 **KN5.1 – Non-coding RNAs in bacteria of the *Burkholderia* cepacia complex: key players in bacterial cell biology and pathogenesis.**
Jorge H. Leitão | IST – U. Lisboa

18:15 **OP5.1 – Construction and analysis of a CRISPRi library to identify essential genes required for cell cycle progression in S. *aureus*.**
Dominik Alwardt | ITQB – NOVA

18:30 **OP5.2 – Multi-omic Data Integration and Modeling for Yeast Physiology: Case Studies in Regulation, Metabolism, and Biomass Dynamics.**
David Henriques | Acuabiotec Lab, IIM-CSIC, Spain

18:45 **OP5.3 – The great escape: loss of WTA glycosylation or L-form conversion protects against bacteriophage infection.**
Martin J. Loessner | Department of Health Science and Technology, ETH Zurich

19:30 **Welcome Cocktail**

Room 2

10:00 **Registration**

TOPIC 6 | SESSION 1: Bioprocess Engineering
Chairs: Lígia O. Martins | ITQB – NOVA
Carmo Barreto | CE3C – UAç

15:45 **KN6.1 – Experimental and Computational Enzyme Engineering for Lignocellulose Valorization.**
Lígia O. Martins | ITQB – NOVA

16:15 **OP6.1 – Near-infrared spectroscopy for real-time monitoring and optimization of polyhydroxyalkanoates production.**
Ana Marta Rodrigues | i4HB – NOVA School of Science and Technology

16:30 **OP6.2 – Inline Monitoring of Induced Pluripotent Stem Cell Growth Kinetics with Stirred-Tank Bioreactor Oxygen Uptake Modeling.**
William O. S. Salvador | iBB – IST, i4HB

16:45 **OP6.3 – Integrative process for bacterial production and separation of tellurium particles.**
Jorge F. B. Pereira | CERES, U. Coimbra

17:00 **Coffee Break and Poster Session 1**

TOPIC 3 | SESSION 1: Health Biotechnology
Chairs: **Joana Azeredo** | CEB – U. Minho
Nuno Cerca | CEB – U. Minho

17:45 **KN3.1 – The Expanding Therapeutic Horizon of Bacteriophages in Health Biotechnology.**
Joana Azeredo | CEB – U. Minho

18:15 **OP3.1 – A non-coding RNA modulates plasmid copy number and antibiotic resistance in *Listeria monocytogenes*.**
Gonçalo Filipe Moreno | ITQB – NOVA

18:30 **OP3.2 – Mechanical and biological assessment of metal nanoparticles functionalised bacterial nanocellulose (BNC) mesh.**
Liliana Melro | 2C2T – U. Minho

18:45 **OP3.3 – Exploiting Bacteriophages for Targeted Control and Rapid Detection of *Klebsiella pneumoniae*.**
Filipa Campos | CEB – U. Minho

19:30 **Welcome Cocktail**

Friday, December 5th

Auditorium

08:30 Registration

09:00 Lecture 2
Microfluidic Innovations for Global OneHealth Needs
Nuno Reis | University of Bath, UK

TOPIC 3 | SESSION 2: Health Biotechnology
Chairs: **Isabel Couto** | IHMT – NOVA
Mariana Pinho | ITQB – UNL

10:15 KN3.2 – Efflux-driven antimicrobial resistance in Staphylococci.
Isabel Couto | IHMT – NOVA

10:45 OP3.4 – Viruses by design – the future of phage therapy.
Martin J. Loessner | ETH Zurich, Switzerland

11:00 OP3.5 – Rabbit-Derived Single-Domain Antibody Fused to Zag Protein for Potent SARS-CoV-2 Neutralization and Extended Half-Life.
Isa Moutinho | CIISA – Faculty of Veterinary, U. Lisboa

11:15 Coffee Break and Poster Session 1

TOPIC 3 |SESSION 3: Health Biotechnology
Chairs: **Isabel Couto** | IHMT – NOVA
Mariana Pinho | ITQB – UNL

12:00 OP3.6 – Bacterial Nanocellulose–Kraft Lignin Composites as Antioxidant and Antimicrobial Biomaterials for Neural Applications.
Cátia Alves | 2C2T – U. Minho

12:15 OP3.7 – Discovery of a Novel Synthetic Antimalarial Class Active Against Multidrug-Resistant Plasmodium falciparum.
Pedro E. Ferreira | ICVS, School of Medicine, U. Minho

12:30 OP3.8 – Antioxidant and Neuroprotective Potential of Mushroom Biomass: Effects on ROS Production in Human Microglial Cell Line (HMC3) and Alzheimer's Disease *Caenorhabditis* elegans Models.
Helena Araújo-Rodrigues | CBQF – U. Católica

12:45 OP3.9 – Discovery and Functional Characterization of a Novel Kv1.3 Channel Modulator Derived from *Physalia physalis* Venom.
Zuzanna Tomkielska | Mesosystem, CBA – UAç

13:00 LUNCH, SPONSOR & EXHIBITOR

14:30 Lecture 3
Factors impacting the development of the gut microbiota in infants
Catherine Stanton | Teagasc, Moorepark Food Research Centre

TOPIC 1 | SESSION 3: Environmental Microbiology & Biotechnology
Chairs: **Graça Silveira** | CBA – UAç
António Machado | CBA – UAç

15:45 OP1.9 – Sustainable Production of Biopigments and Biosurfactants from Agro-Industrial Byproducts Using *Serratia* Strains.
Joaquim F. P. Pinto | CERES – U. Coimbra

16:00 OP1.10 – Flexizyme: Biotechnological production of fatty amines from side stream materials.
Annabel Serpico | ITENE, Spain

16:15 OP1.11 – Unveiling the Agronomic Potential of Extracellular Polymeric Substances Recovered from Aerobic Granular Sludge on Maize.
Sofia I.A. Pereira | CBQF – U. Católica

16:30 **OP1.12 – ShK-like Peptides from Entomopathogenic Nematodes: New Molecular Leads for Bioinsecticide Development.**
Tiago Paiva | CBA – UAç

16:45 **OP1.13 – Biological potential of insect raw materials for sustainable cosmetics.**
Ana Rita Silva | Solfarcos – Soluções Farmacêuticas e Cosméticas, Lda

17:00 **Coffee Break and Poster Session 2**

TOPIC 1 | SESSION 4: Environmental Microbiology & Biotechnology
Chairs: **Maria José Saavedra** | CITAB – UTAD
Jorge Frias | CBA – UAç

17:45 **OP1.14 – Biotechnological Production and Functional Characterisation of a Nematode-Derived Sterol-Binding Protein with Bioinsecticidal Potential.**
Jorge Frias | CBA – UAç

18:00 **OP1.15 – Unveiling PFAS-Tolerant Microbiomes in Polluted Soil and Sediment: Preservation Strategies and Bioremediation Potential.**
Asma Nawaz | Department of Biotechnology, University of Verona, Italy

18:15 **OP1.16 – Genome mining of novel antimicrobial biosynthetic gene clusters in extremophiles from high-salt biofilms.**
Inês Costa | Department of Life Sciences, U. Coimbra

18:30 **OP1.17 – Decoding Microbial Interactions with Vanadium.**
Joana Bonito Caldeira | Centre for Mechanical Engineering, ARISE – U. Coimbra

18:45 **OP1.18 – Refining Microbial Consortia to Improve Phytoremediation in Salt Marshes: Integrating Conventional Enrichment with iACME Selection.**
Margarida Pereira | CIIMAR/CIIMAR LA – U. Porto

19:00 **SCIENTIFIC SOCIETIES SESSION- SPM & SPBT**

21:00 **Gala Dinner**

Room 1

08:30 **Registration**

TOPIC 5 | SESSION 2: Molecular Microbiology and Microbial Physiology
Chairs: **Karina Xavier** | GIMM
Miguel Teixeira | IST – UL

10:15 **KN5.2 – Gut protective microbiota species promotes microbiota recovery and pathobiont clearance while preventing inflammation.**
Karina Xavier | GIMM

10:45 **OP5.4 – Intraspecific Variation in Nitrogen Utilisation of Torulaspora delbrueckii: Growth and Genomic Perspectives.**
Maria João Sousa | CBMA – U. Minho

11:00 **OP5.5 – To be or not to bi(ofilm): the influence of mediator-complex subunits in Candida biofilm formation.**
Inês Vieira Costa | iBB – IST

11:15 **Coffee Break and Poster Session 1**

TOPIC 5 | SESSION 3: Molecular Microbiology and Microbial Physiology
Chairs: **Nelson Lima** | CEB – U. Minho
Miguel Teixeira | IST – UL

12:00 **OP5.6 – Peroxisomal engineering for improved lactones sustainable biosynthesis within a circular economy.**
Miguel Francisco | CEB – U. Minho

12:15 **OP5.7 – Engineering of Dct-02 transporter to improve the biomanufacturing of succinate.**
Isabel Soares-Silva | CBMA – U. Minho

12:30 **OP5.8 – Inhibition of toxic sulfide production by sulfidogenic members of the human gut microbiota.**
Madalena Duarte | ITQB – NOVA

12:45 **OP5.9 – Structural and mechanistic insights into the central respiratory complex in DSR metabolism.**
Raquel M. Bernardino | ITQB – NOVA

13:00 **LUNCH, SPONSOR & EXHIBITOR**

TOPIC 4 | SESSION 2: Health Microbiology
Chairs: **Miguel Viveiros** | IHMT – UNL
Carla Cabral | CBA – UAç

15:45 **OP4.4 – Human and retail meat Campylobacter isolates: emerging antimicrobial resistance, cluster dynamics and source attribution.**
Mónica Azevedo | National Reference Laboratory of Gastrointestinal Infections, INSA

16:00 **Bacteriocin-mediated prevention of secondary pneumococcal pneumonia by a human commensal Streptococcus mitis strain.**
Catarina Candeias | Laboratory of Molecular Microbiology of Human Pathogens, ITQB NOVA

16:15 **OP4.6 – Epidemiology of Staphylococcus aureus at the Livestock-Wildlife Interface: Phylogenetic, Resistome, and Virulome Insights.**
Mónica V. Cunha | CE3C & CHANGE – U. Lisboa

16:30 **OP4.7 – Interplay Between Ciprofloxacin Resistance, Biofilm Development, and Virulence in Acinetobacter baumannii.**
Gabriela Jorge Da Silva | Faculty of Pharmacy, U. Coimbra

16:45 **OP4.8 – Pathoadaptive genomic traits of Staphylococcus aureus from atopic dermatitis.**
Mariana Araújo | Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB – NOVA

17:00 **Coffee Break and Poster Session 2**

TOPIC 4 | SESSION 3: Health Microbiology
Chairs: **Teresa Gonçalves** | CNC-UC
Carla Cabral | CBA – UAç

17:45 **OP4.9 – Insert-Based Two-Layer Model Reproduces Spatial Coexistence of Staphylococcus aureus and Pseudomonas aeruginosa in Chronic Wounds.**
Vânia Pinheiro | LEPABE, ALiCE, U. Porto

18:00 **OP4.10 – Hijacking host immunity: c4bp recruitment by moonlighting retropepsin-type proteases in Rickettsiales.**
Ana Luísa Matos | Institute for Interdisciplinary Research, U. Coimbra

18:15 **OP4.11 – Atopic dermatitis patients in Portugal are reservoirs and key-drivers of ST398-MSSA evolution.**
Diana Caieiro | ITQB – NOVA

18:30 **OP4.12 – Unravelling P. aeruginosa-S. aureus interactions and their intracellular survival using a new in vitro model.**
Maria Olivia Pereira | CBE – U. Minho

18:45 **OP4.13 – Bacterial Vaginosis: it's not just who's there, but what they do.**
Nuno Cerca | CEB – U. Minho

19:00 **SCIENTIFIC SOCIETIES SESSION – SPM & SPBT**

21:00 **Gala Dinner**

Room 2

08:30 **Registration**

TOPIC 2 | SESSION 1: Food Microbiology & Biotechnology
Chairs: **Catarina Geoffroy Prista** | ISA – U.Lisboa
Célia Silva | ITAA – UAç

10:15 **KN2.1 – Fermentation as a strategy to produce sustainable and clean-label food products.**
Catarina Prista | ISA – U.Lisboa

10:45 **OP2.1 – Aptamer-based strategies to control foodborne toxins.**
Ricardo Oliveira | INIAV

11:00 **OP2.2 – The future of Biorecognition Tools: Using Phage-derived Receptor-Binding Proteins for Targeted Detection of *Listeria monocytogenes*.**
Filipa Oliveira | CEB – U. Minho

11:15 **Coffee Break and Poster Session 1**

TOPIC 2 | SESSION 2: Food Microbiology & Biotechnology
Chairs: **Catarina Geoffroy Prista** | ISA – U.Lisboa
Célia Silva | ITAA – UAç

12:00 **OP2.3 – Characterization of the bacteriocin(s) produced by Bacillus sp. isolated from the rumen of cows.**
Nuno M.L. Paiva | ITAA – UAç

12:15 **OP2.4 – YEASTRACT+ Goes Quantitative: Enabling Advanced Systems Biology through Integration of Transcriptomic Data.**
Luís Coutinho | iBB – IST

12:45 **OP2.6 – *Fusarium venatum* as a sustainable protein source for developing functional food ingredients.**
Manuela Pintado | CBQF – U. Católica

13:00 **LUNCH, SPONSOR & EXHIBITOR**

TOPIC 2 | SESSION 3: Food Microbiology & Biotechnology
Chairs: **António Raposo** | CBIOS – U. Lusofóna
Teresa Crespo | ITQB, UNL

15:45 **KN2.2 – Jellyfish as Food: Processing Technologies and Potential Risks of Consumption.**
António Raposo | CBIOS – U. Lusofóna

16:15 **OP2.7 – Glycoside 3-Oxidases as Biocatalysts for Rare Sugar Synthesis.**
André Taborda | ITQB – NOVA

16:30 **OP2.8 – Synergistic combination of essential oil and organic acid: substitute for potassium sorbate in fruit preparation.**
Kavita sorathiya | CBQF – U. Católica

16:45 **OP2.9 – Genomes on the move: Transposable elements in the regulation of fermentation-related genes in non-*saccharomyces* yeasts.**
Fábio Pereira | CBMA – U. Minho

17:00 **Coffee Break and Poster Session 2**

TOPIC 2 | SESSION 4: Food Microbiology & Biotechnology
Chairs: **Pedro Barros Fernandes** | U Lusófona
Teresa Nogueira | INIAV

17:45 **OP2.10 – Food Safety at Home: A Longitudinal Analysis of Refrigerators in Household Environments.**
Ana Rita Barata | INIAV – Vila do Conde

18:00 **Raw Poultry Meat: hyperbaric pasteurisation/preservation at room temperature ensuring quality, safety and extended shelf-life.**
Jéssica Tavares | LAQV-REQUIMTE – U. Aveiro

18:15 **OP2.12 – Inactivation kinetics of inoculated pathogenic microorganisms in egg white and yolk using moderate pressure treatment.**
Gabriela Matos | LAQV-REQUIMTE, U. Aveiro

18:30 **OP2.13 – The role of food cultures and fermentates in the shelf life of soy-based burgers.**
Beatriz Nunes Silva | CBQF – U. Católica

18:45 **OP2.14 – Biofilm Formation by *Staphylococcus aureus* as a Driver of Cross-Contamination Between Food Handlers and Ready-to-Eat Foods.**
Joana Paiva | Department of Veterinary Sciences, UTAD

19:00 **SCIENTIFIC SOCIETIES SESSION – SPM & SPBT**

21:00 **Gala Dinner**

Saturday, December 6th

Auditorium

08:30 Registration

09:00 Lecture 4
AI-Powered Computational Mass Spectrometry for Molecular Discovery
Wout Bittremieux | Adrem Data Lab at the University of Antwerp

TOPIC 1 | SESSION 5: Environmental Microbiology & Biotechnology
Chairs: **Célia Manaia** | ESB – U. Católica
Joana Ramos | IITA – UAç

10:15 **KN1.2 – Microbes and the Changing Ocean: Small Organisms Big Impact.**
Joana Ramos | IITA – UAç

10:45 **OP1.19 – Biocalcifying potential of ureolytic *Priestia aryabhattai* 10w27b for bio-repairing of mortar cracks.**
Leonor Matos | CEMMPRE, ARISE, U. Coimbra

11:00 **OP1.20 – Microplastics and Chemical Pollutants Team Up to Facilitate the Persistence of Multidrug-Resistant Pathogens in Rivers.**
Isabel Silva | TERRA Associate Laboratory, U. Coimbra

11:15 Coffee Break and Poster Session 2

TOPIC 1 | SESSION 6: Environmental Microbiology & Biotechnology
Chairs: **Célia Manaia** | ESB – U. Católica
Joana Ramos | IITA – UAç

12:00 **OP1.21 – Development of high-biogas-affinity methanotrophic enrichments for protein-rich biomass production.**
Clara D. Moura | CEB – U. Minho

12:15 **OP1.22 – Modular Engineering of Substrate Oxidation and Electron Flow for Enhanced Bacterial Cellulose Biosynthesis in *Komagataeibacter*.**
Pedro Montenegro-Silva | CEB – U. Minho

12:30 **OP1.23 – KEGG resolved microbiome contrasts in invasive brown algal holobionts reveal complementary bioprocessing pathways and actionable mitigation strategies.**
Raul Bettencourt | FCT – UAç

12:45 **OP1.24 – Extremotolerant, Specialised Hydrocarbon-Degrading Fungi in Resin-Rich Soil.**
Ângela Pinheiro | ITQB – NOVA

13:00 LUNCH, SPONSOR & EXHIBITOR

14:00 CEREMONY – CAREER AWARDS

Award Professor Júlio Maggiolly Novais
Laureate: Professor José António Teixeira | U. Minho

Award Professor Nicolau van Uden
Laureate: Professor Nelson Lima | CEB – U. Minho

TOPIC 1 | SESSION 7: Environmental Microbiology & Biotechnology
Chairs: **Ana Teresa Caldeira** | HERCULES – UEv
Mónica Cunha | FC – UL

14:30 **OP1.25 – From Waste to Value: Sequential Recovery of Bioactive Fractions from Orange Pomace.**
Miguel Neto, RISE-Health | U. Beira Interior

14:45 **OP1.26 – Genomic insights into antifungal potential of three *Pseudomonas* strains.**
Clarisse Brígido | MED Mediterranean Institute for Agriculture, U. Évora

15:00 **OP1.27 – White grape marc extracts as modulators of turbot resilience to bacterial infections.**
Miguel Castro | Department of Biology, U. Porto

15:15 **OP1.28 – Temperature profiles and auxin production by yeasts associated with industrial microalgal cultures of *Tisochrysis lutea*.**
Madalena Matos | iBB, i4HB – IST

15:30 **OP1.29 – Viral Diversity in Native Plants from the Azores .**
Sara Luna | CBA – UAç

15:45 **OP1.30 – Class 3 resistance integrons in treated wastewater: threats that go with the flow!**
Isabel Henriques | TERRA Associate Laboratory, U. Coimbra

16:00 **Coffee Break**

TOPIC 9 | SESSION 1: Biological Resources and Networks
Chairs: **Vitor Vasconcelos** | CIIMAR – UP
Ana Paula Arez | IHMT – NOVA

16:30 **KN8.1 Biobanks and Biological Collections: driving One Health by strengthening global networks, capacity, and research equity .**
Ana Paula Arez | IHMT – NOVA

16:50 **KN8.2 The REBECA Network: Conserving Biodiversity and Driving Blue Biotechnology in Macaronesia.**
Vitor Gonçalves | CIBIO – UAç

17:10 **OP9.1 – 17 Windows to the Future: UCCCB and the Sustainable Development Goals.**
Paula Vasconcelos Morais | CEMMPRE, ARISE, U. Coimbra

17:25 **OP9.2 – Transnational Access Opportunities to Researchers at MIRRI-ERIC: The Microbes-4-Climate Project.**
Nelson Lima | CEB – U. Minho

17:40 **KN8.3 – MNOP – Microbiological Resources, Networks, Opportunities and Potentiation.**
Vitor Vasconcelos | CIIMAR, U. Porto

18:00 **PRIZES & CLOSING SESSION**

Room 1

08:30 **Registration**

TOPIC 3 | SESSION 4: Health Biotechnology
Chairs: **Gabriel A. Monteiro** | iBB – IST
Raquel Aires Barros | IST – UL

10:15 **OP3.10 – Harnessing Keratin's Versatility: From Waste to Innovative Biomedical Applications.**
Iuri Machado | CICECO, Institute of Materials – U. Aveiro

10:30 **OP3.11 –Investigating Human Host Adaptations to Infection by the Gut Bacterium *Bilophila wadsworthia* in a Multicellular Intestinal Co-culture.**
Sara Matos | ITQB – NOVA

10:45 **OP3.12 – Fungal DNA barcoding on a chip – sequence-based fungal infection diagnosis.**
Maria Zolotareva | iBB – IST

11:00 **OP3.13 – LNA-based ASO-siderophore conjugate: a new potential Trojan-horse drug to outsmart antibiotic-resistant bacteria.**
Igor Resende | LEPABE, AliCE, U. Porto

11:15 **Coffee Break and Poster Session 2**

TOPIC 3 | SESSION 5: Health Biotechnology
Chairs: **Gabriel A. Monteiro** | iBB – IST
Raquel Aires Barros | IST – UL

12:00 **OP3.14 – A ferulic acid derivative transformed by Lactococcus lactis is a potential inhibitor of endometrial cancer.**
Gabriel A. Monteiro | iBB – IST

12:15 **OP3.15 – Amino acid-based ionic liquids as the next generation of excipients for RNA with improved stability during storage.**
Ana F. Pereira | CICECO –U. Aveiro

12:30 **OP3.16 – PipeUCare: A Novel Biofilm-Targeted Disinfectant for Hospital Greywater Systems.**
Isabel Carvalho | CEB – U. Minho

12:45 **OP3.17 – Disarming and Destroying Listeria Biofilms: Cationic Dendrimers as Dual-Action Anti-Biofilm Agents.**
Sandra N. Pinto | iBB, i4HB – IST

13:00 **LUNCH, SPONSOR & EXHIBITOR**

TOPIC 6 | SESSION 2: Bioprocess Engineering
Chairs: **Lucilia Domingues** | CEB – U. Minho
Maria do Carmo Barreto | CE3C – UAç

14:30 **KN6.2 – Strain Matters: Linking Host Choice to Bioprocess Success.**
Lucilia Domingues | CEB – U. Minho

15:00 **OP6.4 – Integrated Bioconversion of Grape Pomace-Derived Sugars into 2,5-furandicarboxylic acid Using Engineered and Wild-Type Yeasts.**
Cristiana Martins | Departamento de Enxeñaría Química, Universidade de Vigo

15:15 **OP6.5 – Microbial Community Selection Under Controlled Conditions for PHA (Polyhydroxyalkanoates) Production.**
Marta Catalão | i4HB – NOVA

15:30 **OP6.6 – Be@t project: Valorization of Waste Cooking Oil and Grapevine Pruning for Sustainable Rhamnolipid Production and Application in Textile Softening.**
João Rainha | CEB – U. Minho

15:45 **OP6.7 – Valorisation of Seafood Canning Industry Residues for Biosurfactants Production.**
Joana Dias de Almeida | iBB – IST

16:00 **Coffee Break**

TOPIC 8 | SESSION 1: From Science to Society and Business
Chairs: **Duarte Toubarro** | UAç – CBA
Anabela Carvalho | PATENTREE

16:30 **KN8.1 – Creating Real Value: From Idea to IP – Protected Innovation and Value**
Anabela Carvalho | PATENTREE

17:00 **OP8.1 – MosquitoWEB – A citizen science tool for arbovirus vector surveillance.**
Luis Filipe Lopes | GHTM, LA-REAL, IHMT

17:15 **OP8.2 – Biosensors in Practice: Socioeconomic Insights from Forensics and Food Safety Monitoring in Portugal.**
Kristen Connor | CES – U. Coimbra

17:45 **OP8.4 – Techno-economic analysis and Life Cycle Assessment of Bacterial Cellulose production: a comparative study with plant derived micro and nanocelluloses.**
Fernando Dourado | CBE – U. Minho

Room 2

08:30 **Registration**

TOPIC 4 | SESSION 4: Health Microbiology
Chairs: **Maria João Amorim** | CBR – U. Católica
Susana Ferreira | RISE Health

10:15 **KN4.2 – Advances in understanding how influenza A virions assemble.**
Maria João Amorim | CBR – U. Católica

10:45 **OP4.14 – Broad-Host-Range Phages Targeting Multidrug-Resistant Pseudomonas aeruginosa – Expanding Phage Therapy Options.**
Maria João Costa | CEB – U. Minho

11:00 **OP4.15 – First report of *Candida auris* candidemia in Portugal: Genomic characterisation and antifungal resistance-associated genes analysis.**
Sofia Costa de Oliveira | Rise-Health, U. Porto

11:15 **Coffee Break and Poster Session 2**

TOPIC 4 | SESSION 5: Health Microbiology
Chairs: **Madalena Pimentel** | FF – UL
Susana Ferreira | RISE Health

12:00 **OP4.16 – A One Health Perspective on Backyard Production Systems in Portugal: Risk Factors and Antimicrobial Resistance.**
Ana Esteves | Unidade de Genómica e Bioinformática, INSA

12:15 **OP4.17 – From old drugs to novel antifungal strategies: inhibiting efflux mechanisms in *Candida albicans*.**
Liliana Rodrigues | GHTM, LA-REAL, IHMT – NOVA

12:30 **OP4.18 – Phylogenomic Discordance and Niche-Linked Structure in *Candida albicans*.**
Mariana Fernandes | RISE-Health – UBI

12:45 **OP4.19 – Identifying and characterizing enterococcal persistence in veterinary and human settings.**
Catarina Geraldes | CIISA – U. Lisbon

13:00 **LUNCH, SPONSOR & EXHIBITOR**

TOPIC 7 | SESSION 1: New and Emerging Technologies
Chairs: **Ricardo Henriques** | ITQB – NOVA
Duarte Mendonça | CBA – UAç

14:30 **KN7.1 – Towards nanoscale live-cell biology through AI-enhanced super-resolution microscopy.**
Ricardo Henriques | ITQB – NOVA

15:00 **OP7.1 – Development and validation of a nanotechnology-based medical device for treating corneal diseases.**
Patrícia Nápoles | Department of Physics, U. Coimbra

15:15 **OP7.2 – Molecular Dynamics of CexA uncovers relevant citrate binding and recognition sites.**
Francisca Ferreira | CBMA – U. Minho

15:30 **OP7.3 – Cyanobacterial extracelular vesicles as vaccination platforms for fish.**
Jorge Matinha-Cardoso | CIIMAR

15:45 **OP7.4 – Novel Phage-derived strategy for Early Detection of *Paenibacillus* larvae, the Etiological Agent of American Foulbrood.**
Renato Veloso da Mota | CEB – U. Minho

16:00 **Coffee Break**

TOPIC 7 | SESSION 2: New and Emerging Technologies
Chairs: **Ricardo Henriques** | ITQB – NOVA
Duarte Mendonça | CBA – UAç

16:30 **OP7.5 – Microfluidic techniques for microbial manipulation: studies on enrichment and detection.**
Violina Baranauskaite Barbosa | LEPABE–Laboratory – U. Porto

16:45 **OP7.6 – Exploring cellulolytic activity in compost-derived metagenomic libraries.**
Joana Sousa | CEB – U. Minho

17:00 **OP7.7 – From Images to Insights: Evaluating Cell Segmentation Strategies for Biofilm Analysis.**
Arefeh Mazarei | LEPABE, ALiCE – U. Porto

17:15 **OP7.8 – Network Dynamics as Fingerprints of Thermostability in an *In Silico*-Engineered DyP-type Peroxidase.**
Carolina Ferro Rodrigues | ITQB – NOVA

17:30 **OP7.9 – Botryosphaeriaceae early detection using a DNA-based Biosensing Platform.**
Paula Martins-Lopes | DNA & RNA Sensing Lab – UTAD

17:45 **OP7.10 – Aiming at the Automation of Genome-wide Regulatory Model Inference and Metabolic Integration in *Saccharomyces cerevisiae*.**
Diogo A. C. Couceiro | INESC-ID, Lisboa, iBB – IST

POSTERS – Session 1

Topic 5

PO1
Predicting metabolic potential with PICRUSt2 in Bathymodiolus azoricus from a whale-fall: chemoautotrophic traits and symbionts
João Moreira

PO2
Mechanical Behavior of Staphylococcus aureus in the presence of different antibiotics
Carolina Máximo

PO3
Capsular Rheological Properties of Klebsiella pneumoniae: a double-edged sword mechanism of bacterial pathogenesis
Liliane Pinto Ferrador

PO4
The Skf system of Staphylococcus aureus – role in inter-species and host interactions
Rita Sobral

PO5
COG Functional Signatures of the GIT Microbiome in Two Cattle Breeds: Towards Sustainable Ruminant Production
Ana Rita Azevedo

PO6
Exploring CAZyme-Driven Functional Potential in GIT Microbiomes of Catrina and Holstein-Friesian Cattle for Sustainable Agriculture
Maria Susana Lopes

PO7
Elucidation of the Structure-Function Relationship of the Dicarboxylate Transporter Mae1 in Saccharomyces cerevisiae
Isabel Soares-Silva

PO8
Bacterial diversity in industrial microalgal cultures and screening for Vibrionaceae-inhibiting strains
Veronica Rossetto

PO9
Profiling the metabolic responses of Candida albicans in the gastrointestinal tumor microenvironment
Rosana Alves

PO10
Glucose Transport in Torulaspora delbrueckii as a Key to Enhancing Its Potential in Winemaking
Bruna Oliveira

PO11
Breaking the mold: Alternative mechanisms of yeast arsenate detoxification
Teresa Pissarro

PO12
FunMETAromaDB: A curated database of proteins involved in fungal aromatic compound metabolism for comparative genomics
Tiago Miguel Martins

PO13
Coded to Kill: Uncovering New Families of Rearrangement Hotspot Toxins from the Bee Gut Microbiome
Margarida Moreira

Topic 1

PO14
Shell Lifetime as a Key Factor in Astaxanthin Recovery from Shrimp Waste
Rita Favas

PO15
Shrimp Shells Green Extraction for Cosmetic application
Andreia F. Peixoto

PO16
Environmental transitions recorded by bacteriohopanepolyols: implications for lake biogeochemical cycles in the Azores
Pedro Raposeiro

PO17
Microbiological control of CO2 and CH4 emissions in maar crater lakes from São Miguel (Azores)
Letícia Ferreira

PO18
Microbial dynamics and their influence on greenhouse gas emissions in shallow lakes in Corvo Island
César Andrade

PO19
Prevalence of bacterial agents of bovine mastitis in dairy farms of Terceira Island, Azores, Portugal
Fábio Pereira

PO20
Innovative PGPB-based fertilizers as a sustainable solution to promote crop resilience in degraded soils
Ana Sofia da Silva Sousa

PO21
Arthrobacter nicotinovorans EAPAA enhances must attributes: evidence from Spanish and Portuguese case studies
Ana Sofia da Silva Sousa

PO22
Arbuscular mycorrhizal fungal diversity colonizing the roots of Picconia azorica
Sara Luna

PO23
Ideal conditions of activation of agarose beads with divinyl sulfone: improving the enzyme immobilization
Diandra de Andrades

PO24
The symbiotic sphere of Cyanobacteria: their microbiome composition and chemical interactions
Adriana Rego

PO25
Antifouling Profile of Cyanobacteria-derived Nucleosides: From Efficacy to Environmental Safety
Sandra Pereira

PO26
Engineering Oral Fish Vaccines: Enhancing Mucosal Immunity by Employing SporoVaccines and Molecular Adjuvants
Bárbara Guedes

PO27
Microbial-Enhanced Phytoremediation of Cadmium and Ketoprofen in Estuarine Sediments: Microcosm and Mesocosm Validation
Rafaela Perdigão

PO28
How does the genomic diversity of CAZymes in marine fungi reflect adaptation to different marine
Artur Alves

PO29
Exploring the diversity and biological activities of wood-colonizing marine fungi
Ana Cristina Esteves

PO30
Production of antibacterial compounds by the marine-derived fungus Penicillium adametzioides
Ana Cristina Esteves

PO31
Antibacterial activity and inhibition of biofilm formation by selenium nanoparticles against bacteria isolated from diseased
Marta Tacão

PO32
Alentejo's Essential Oils and β-Cyclodextrin Complexes: A Natural Strategy Against the Biodeterioration of Heritage Assets
Maria Rosário Martins

PO33
Ultrasound-Assisted Deep Eutectic Solvent Pretreatment of Spent Coffee Grounds for Sustainable Recovery of Bioactive Compounds
Shismany de Fátima Assunção Araújo

PO34
Bacterial cellulose hydrogels as multifunctional platforms for sustainable microalgae cultivation and harvesting
Marisa Camacho Gonçalves Faria Pascoal

PO35
Alkaline extraction of phenolic compounds from Eucalyptus leaves: influence on antioxidant and antimicrobial activity
Tiago Barros Afonso

PO36
Adaptive laboratory evolution targeting caproic acid tolerance in a newly identified oleaginous Cyberlindnera jadinii
Maria João Sousa

PO37
Enantioselective antibiotic resistance: assessing fluoroquinolones stereochemistry as a driver of AMR evolution in wastewater bacteria
Alexandre van Heerden

PO38
Effects of Ocean Alkalinity Enhancement on Marine Microbial Communities
Inês de Castro

PO39
Insect Pathogen Pseudomonas sp. nov. Genome
Daniel Ladeira

PO40
Decoding Parchment Origins: An Integrated Molecular and Microbial Approach
Maria João Penetra

PO41
Protein Extracts from Pristine Bacterial Strains: a Potential Tool Against Biodeterioration in Cultural Heritage
Cátia Salvador

PO42
Enrichment of ofloxacin-degrading microorganisms from activated sludge
Joaquín Alberto Pereira Marrero

PO43
Hybrid leaching using bio-based molecules for selective copper and gold removal from printed circuit boards
Beatriz Rito

PO44
Green Biocides: Innovative Biotechnological Solutions for Cultural Heritage Protection
Cátia Salvador

PO45
Integrating Livestock Effluents into Apple Orchard Fertilization: Effects on Tree Physiology and Yield
Anton Denysov

PO46
SPATT Methology for Cyanotoxins Monitoring in Volcanic Lakes (Azores, Portugal)
Alexandra Silva

PO47
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PLENARY LECTURES

MOHAMED JEBBAR

Hyperthermophilic and Piezophilic Thermococcales: Exploring Their Adaptation to Extreme Environments and Biotechnological Applications

Mohamed Jebbar 1(*), Yann Moalic1, Trinetra Mukherjee1, Sebastien Laurent1, and Violette DA Cunha2

1 Univ Brest, CNRS, Ifremer, Laboratoire de Biologie et d'Écologie des Écosystèmes marins profonds BEEP, IUEM, Rue Dumont d'Urville, F-29280 Plouzané, France

2 UMR Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France

(*) e-mail: mohamed.jebbar@univ-brest.fr

Topic: Environmental Microbiology & Biotechnology

Background: A leading hypothesis places life's origin in the deep sea, where hydrothermal vents provided protection from early solar radiation and redox gradients that could have fueled primordial metabolism. These vents, associated with oceanic spreading centers and arc volcanoes, host diverse (hyper)thermophilic microbes. Among them, Thermococcales archaea—strictly anaerobic, hyperthermophilic, and sometimes piezophilic—are model organisms. Phylogenetic analysis of their 16S rRNA gene sequences traditionally divides them into three genera: *Pyrococcus*, *Thermococcus*, and *Palaeococcus*.

Methods: We characterized the adaptive mechanisms of Thermococcales archaea using a multidisciplinary approach, integrating omics, culture-based, physiological, biophysical, and genetic tools. This enabled a comprehensive analysis of their adaptive strategies to extreme deep-sea vent conditions.

Results: Sequencing of 69 new Thermococcales genomes, combined with existing data, yields over 100 high-quality, closed chromosomes—one of the best-represented archaeal orders. Pangenomic and bioinformatic analyses reclassify these genomes into six distinct genera, challenging current taxonomy. Integration of pangenomic data with metabolic pathway predictions clarifies the distribution, evolutionary history, and metabolic specificities across species, genera, and ancestors. Some Thermococcales, such as *Thermococcus barophilus*, thrive under high hydrostatic pressure (HHP). Comparative studies with the piezosensitive *Thermococcus kodakarensis* reveal proteome structural adaptations and distinct hydration dynamics under HHP. In *T. barophilus*, HHP triggers repression of amino acid biosynthesis genes, rendering cells auxotrophic for 17 amino acids, and upregulates energy and carbohydrate metabolism. The mechanism for amino acid acquisition under HHP remains unclear; new genetic, physiological, and omics data will address this.

Conclusions: With over 100 high-quality Thermococcales genomes, pangenomics redefines their taxonomy into six genera and reveals HHP adaptation mechanisms, including amino acid auxotrophy and metabolic shifts. These extremophiles' stable enzymes and pathways hold biotechnological potential for industrial catalysis and bioremediation under extreme conditions. Ongoing omics studies will further elucidate their adaptive and applied value.

NUNO REIS

Microfluidic Innovations for Global OneHealth Needs

Nuno M. Reis [1(*)],

1 Department of Chemical Engineering, Centre for Bioengineering and Biomedical Technologies (CBio), and Centre of Excellence In Water-Based Early-Warning Systems for Health protection (CWBE), University of Bath, United Kingdom; Centro de Egenharia Biologica, Universidade do Minho, Portugal

(*) e-mail: n.m.reis@bath.ac.uk; n.m.reis@ceb.uminho.pt

Topic: Health Biotechnology

Background: The OneHealth agenda demands integrated solutions across human, animal, and environmental health. In a number of health scenarios, there is a lack of rapid, sensitive diagnostics and monitoring tools, while in environmental microbiology, robust platforms for detecting pathogens or monitoring microbial populations remain underdeveloped. Our team has been pioneering microfluidic solutions that do not compromise on analytical performance, by delivering unmatched simplicity, portability, and scalability to tackle global challenges.

Methods: We have developed a number of microfluidic solutions centred around the unique optical properties and multiplexing capabilities of low-cost melt-extruded, microcapillary films, including gravity-driven microcapillary siphons and modular “lab-on-a-stick” devices. By functionalizing individual microcapillaries and interrogating optically through a range of imaging devices (smartphones, scanners, USB cameras) and modes (colourimetric, fluorescence, darkfield), we demonstrated world-class analytical performance on a wide number of bioassays, including immunoassays, nucleic acid detection, algae blooms, and clinical microbiology, without requiring pumps nor external instrumentation.

Results: DNA purification with magnetic beads in microfluidic siphons revealed more efficient than conventional lab-based protocol, offering integration with portable LAMP device for in-field detection of E. coli in various matrices (water, urine, and blood). Phenotypic, culture-based antibiotic susceptibility testing (AST) including minimum inhibitory concentration (MIC) revealed faster (4-10hrs), with good categoric agreement with EUCAST’s both microdilution (BMD), using both resazurin-labelled and label-free AST approaches. Microalgae growth showed maximum growth rates exceeding those previously reported for aerated bioreactors, with the ability to monitor non-invasively expansion of individual cells. In a separate study, a label-free, dipstick 1D counting of bacterial colonies and bacteriophage plaques achieved fast and robust bacterial and phage enumeration in a minimal format.

Conclusions: By combining innovative pump-free fluidics, passive mixing, and accessible detection, we demonstrated microfluidic solutions that are deployable at the point-of-need—whether for clinical diagnostics, antimicrobial surveillance, or environmental monitoring - contributing to global health and environmental equity in line with UN’s SDGs.

CATHERINE STANTON

Factors impacting the development of the gut microbiota in infants

Catherine Stanton

APC Microbiome Ireland/Teagasc Food Research Centre, Moorepark, Fermoy, P61 C996, Co. Cork, Ireland

(*) e-mail: catherine.stanton@teagasc.ie

Topic: Health Biotechnology

A diverse and complex microbial ecosystem, which is now well accepted to have a positive influence on health throughout life, begins to develop from birth in the infant gastrointestinal tract. The gut microbiota composition is initially known to be in a state of flux and consequently stabilises by 2-3 years of age, to more closely resemble that of an adult. An important factor known to impact the development of the infant gut microbiome is delivery mode and infants born by caesarean section display distinct gut microbiome profiles compared with vaginally delivered infants. Infants born by caesarean section have reduced transmission of microbial taxa from mothers to infants compared with vaginal delivery, most notably a marked depletion of *Bifidobacterium*, *Bacteroides* and *Phocaeicola* - taxa that play key roles in the early-life gut microbiome. Their delayed or absent appearance in infants born via caesarean section has been associated with altered metabolic and immune maturation and an increased risk of health conditions such as necrotising enterocolitis, asthma, atopic disorders, and ulcerative colitis. Other factors known to impact the development of the gut microbiota in infants include gestational age, feeding regime, and perinatal antibiotic usage. Thus, the microbiota of full-term vaginally born, exclusively breast fed infants, with no previous exposure to antibiotics can be considered the “gold standard” in early life. Perturbation of optimum microbiota development, as a result of these early life exposures have likely long-term implications for microbial diversity and consequent health. Thus ensuring optimal nutrition during this period is essential for long-term health and understanding the optimal nutritional regimen required for the beneficial development of the gut microbiome composition and functionality is an appealing strategy to promote health and reduce disease risk.

WOUT BITTREMIEUX

AI-Powered Computational Mass Spectrometry for Molecular Discovery

Wout Bittremieux
Adrem Data Lab at the University of Antwerp
(*) e-mail: wout.bittremieux@uantwerpen.be

Topic: Environmental Microbiology & Biotechnology

Microbial systems produce a vast and largely uncharted molecular repertoire that underpins communication, adaptation, and interactions with hosts and the environment. Mass spectrometry allows us to observe this molecular activity, but interpreting the data remains challenging due to the diversity and volume of spectra. In this presentation, I will outline how we use AI-driven computational methods to improve molecular discovery from proteomics and metabolomics data. First I will describe our work on transformer-based *de novo* peptide sequencing with Casanovo, which improves peptide identification accuracy and can also serve as a learned scoring function in database searching. Next, I will discuss our large-scale metabolomics efforts that organize millions of public mass spectra to identify related molecular families and generate "suspects" that suggest plausible structural variants, improving annotation rates and enabling applications such as drug-exposure detection. Finally, I will present SIMBA, a spectral similarity model that learns chemical relationships directly from mass spectra and facilitates systematic analog discovery. Together, these approaches aim to make mass spectrometry data more interpretable and to support the discovery of biologically and chemically meaningful molecules.

KEYNOTE LECTURES

ARTUR ALVES

Marine fungi: unlocking diversity and novel resources for biotechnological applications

Artur Alves [1(*)],

1 Centro de Estudos do Ambiente e do Mar (CESAM), Departamento de Biologia, Universidade de Aveiro

(*) e-mai: artur.alves@ua.pt

Topic: Enviormental Microbiology and Biotechnology

Fungi are abundant and ubiquitous in marine habitats but remarkably little is known about their diversity and ecological roles. Their study lags far behind that of their terrestrial counterparts. Marine fungi can be found in seawater, sand, sediment, and are capable of colonising diverse substrates such as driftwood, decaying wood and leaves, algae, and animals. Some also establish symbiotic relationships with other marine organisms. Marine environments harbour a rich diversity of fungal species, much of which is still unexplored and unexploited. Currently, over 2,250 marine fungal species have been described, representing only about 1.5% of all known fungi. Although their biotechnological potential was long overlooked, marine fungi are now recognised as a promising source of novel bioactive compounds. They produce a vast array of secondary metabolites, such as terpenes, polyketides, and peptides, with antimicrobial, antiviral, antitumour, antioxidant and anti-inflammatory activities. These compounds hold considerable potential for application across various biotechnological sectors. As natural recyclers, fungi also synthesise a wide range of enzymes, offering promising solutions for bioremediation, aiding in the degradation of recalcitrant compounds and persistent contaminants, including microplastics. Over the past decade, fungal diversity has been extensively studied in Portuguese marine and estuarine environments, leading to the description of many novel taxa, although numerous others remain to be discovered. Integrative omics approaches, particularly genomics and metabolomics, are helping to unravel the biology and ecology of these fungi and assess their biotechnological value. Marine fungi are emerging as a sustainable resource with the potential to support a circular blue bioeconomy. However, to unlock their full potential, we must deepen our understanding of these organisms and explore how best to use their metabolic capabilities to address major global challenges such as pollution, antimicrobial resistance, food security and food scarcity.

MARGARIDA SARAIVA

Understanding tuberculosis pathogenesis and immune responses through the lens of *Mycobacterium tuberculosis.*

Margarida Saraiva [1(*)],

1 i3S – U. Porto

(*) e-mail

JORGE H. LEITÃO

Non-coding RNAs in bacteria of the *Burkholderia cepacia* complex: key players in bacterial cell biology and pathogenesis.

***Jorge H. Leitão*[1(*)],**

iBB-Institute for Bioengineering and Biosciences; Associate Laboratory i4HB-Institute for Health and Bioeconomy at Instituto Superior Técnico; Department of Bioengineering. Instituto Superior Técnico, Universidade de Lisboa, 1049-001, Lisbon, Portugal. Email: jorgeleitao@tecnico.ulisboa.pt

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

TOPIC: Molecular Microbiology and Microbial Physiology

Over the last years small non-coding RNAs (sRNAs) have emerged as key regulators of post-transcriptional gene expression in bacteria [1]. Despite the identification of hundreds of sRNAs in bacteria, mainly as the result of technologies such as RNASeq, their biological functions remain to be elucidated. Bacteria of the *Burkholderia cepacia* complex (Bcc) are opportunistic pathogens, capable of causing severe and often lethal infections among cystic fibrosis patients [2]. In recent years, an increasing number of infections caused by these bacteria have been described among non-cystic fibrosis patients, particularly among patients hospitalized for prolonged periods and suffering from malignancies such as cancer [3]. Bcc bacteria harbor in their genomes 2 Hfq-like RNA chaperones [4], as well as a ProQ-like RNA chaperone (unpublished). The experimental strategy aiming at the identification of sRNAs expressed by the Bcc member *B. cenocepacia* J2315 when infecting the nematode *Caenorhabditis elegans* will be presented [5]. This strategy allowed us to identify a total of 108 new sRNAs and 31 sRNAs previously described [5]. Results will also be presented and discussed on the functional characterization of the RIT11b sRNA targeting the *dusA* and *pyrC* messengers [5], previously found as involved in biofilm formation, epithelial cell adherence, and chronic infections in other bacteria.

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LÍGIA O. MARTINS

Engineering Bacterial Oxidoreductases for Enzymatic Bioprocesses Targeting Lignocellulose Components

Lígia O. Martins [(*)],

Instituto de Tecnologia Química e Biológica António Xavier, Universidade da Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

(*) email: lmartins@itqb.unl.pt

Topic: Bioprocess Engineering

Our research focuses on bacterial oxidoreductases, a diverse class of enzymes that catalyze oxidation reactions on a wide range of substrates, including lignin and sugars. We aim to elucidate their catalytic mechanisms and to enhance and broaden their catalytic efficiency through protein engineering. These efforts are driven by the potential to develop novel biotechnological applications that contribute to sustainable processes in the pharmaceutical, food, and biocatalysis sectors.

During the lecture, I will present examples that illustrate the transformative potential of protein engineering in expanding enzyme functionality and applicability, paving the way for innovative solutions in biocatalysis and biotechnology. We have optimized laccase variants to catalyze the synthesis of several (neo)lignans, includingreaching yields of up to 100%. These compounds hold promise for medicinal chemistry and polymer synthesis. We have also enhanced the activity of pyranose 3-oxidase toward D-glucose and optimized a bioprocess to produce the natural sweetener D-allose, a rare sugar, with an overall yield of 81%. Ultimately, through computational enzyme engineering, we significantly enhanced the thermal stability of DyP-type peroxidases. The integration of dynamic modeling with protein correlation network analysis revealed dynamic interaction networks as novel indicators of protein stability, offering a new framework for rational enzyme design.

Keywords: Industrial Biotechnology, Biocatalysis, Directed Evolution, Protein Stability

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JOANA AZEREDO

The Expanding Therapeutic Horizon of Bacteriophages in Health Biotechnology

***Joana Azeredo1,2(*)*,Priscila Pires1,2, Luciana Meneses1,2, Hugo Oliveira1,2, Luís Melo1,2, and Ivone Martins1,2.**

1CEB- Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

2LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

(*)emaul: jazeredo@deb.uminho.pt

Topic: Health Biotechnology

Bacteriophages, the viruses that infect bacteria, represent a remarkably versatile but still underexplored biotechnology platform for addressing a wide range of diseases, from chronic bacterial infections to neurodegenerative disorders. By infecting and killing bacteria, phages can act as powerful natural antimicrobials, either as alternatives to antibiotics or in combination with them, in an approach known as phage therapy. Our research has shown that phage therapy is highly effective against chronic, biofilm-associated infections, which are highly tolerant to antibiotic's therapy. Moreover, combining phages with antibiotics enables complete eradication of biofilms under conditions where either treatment alone fails. These results are in line with studies demonstrating strong phage–antibiotic synergy, and our recent work further revealed that optimized dosing strategies, such as simultaneous or repeated administration, can maximize bacterial killing while minimizing resistance development. We further demonstrated that phages evolved within biofilms acquire adaptive mutations that enhance their ability to infect and disrupt P. aeruginosa biofilms, increasing their therapeutic effectiveness.

Besides their direct antibacterial action, phages also encode enzymes with therapeutic potential. Among these, capsular depolymerases are particularly promising: they can dismantle the protective polysaccharide capsules of pathogens such as Acinetobacter baumannii. We have expressed, characterized, and tested several of these enzymes, showing in preclinical models that depolymerase treatment sensitizes bacteria to both immune defenses and antibiotics, acting as an antivirulence adjunct or even as a stand-alone therapy.

Clinically, personalized phage therapy is moving closer to mainstream medical use. Portugal's recent approval of hospital-based phage therapy (November 2024) reflects this growing acceptance. Since 2022, in collaboration with the Queen Astrid Military Hospital, our group has treated 12 patients under compassionate-use protocols, achieving measurable clinical improvement in about 70% of cases, an outcome consistent with international experience.

Finally, we are exploring the potential of filamentous phages (such as M13) beyond infectious diseases. By engineering these phages to display peptides that specifically bind to amyloid-β, we have shown their ability to detect toxic oligomeric species and inhibit further aggregation in vitro. This opens new avenues for developing diagnostic and therapeutic tools targeting Alzheimer's and related neurodegenerative disorders. Together, these strategies illustrate the multifaceted potential of phages, as antibacterial agents, antivirulence tools, and molecular platforms, positioning them at the forefront of next-generation interventions in health biotechnology.

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ISABEL COUTO

Efflux-driven antimicrobial resistance in Staphylococci

Isabel Couto [(*)],

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal

(*)email: icouto@ihmt.unl.pt

Topic: Health Microbiology

Efflux systems are part of bacterial cell machinery, playing different physiological roles. These include extrusion of metabolic waste products or the export of factors essential for colonization and persistence in the host. Efflux pumps may also play a role in bacterial resistance to antimicrobials by pumping out antibiotics and biocides that are recognized as substrates. Several of these pumps can extrude antimicrobials from multiple classes, leading to multidrug resistance (MDR) phenotypes.

Several studies have now established the role played by MDR efflux systems as a first line response to the antimicrobial challenge in relevant bacterial pathogens. It was also demonstrated that exposure to sub-inhibitory concentrations of either antibiotics or biocides may promote emergence of clinically relevant antibiotic resistance that is mediated by efflux.

To date, up to 20 MDR efflux systems have been identified in Staphylococcus aureus, but only a few have been investigated in depth. Among these, NorA of the Major Facilitator Superfamily, known for already 35 years, is the best characterized. However, there are still several questions about the role played by this system in S. aureus antimicrobial resistance and virulence that remain to be fully understood. By studying NorA in S. aureus and other clinically relevant staphylococci we have been detailing its complexity and impact. In this lecture, we will address what we have learn so far on the role played by NorA and other staphylococcal MDR efflux pumps on shaping the antimicrobial resistance traits of these pathogens and discuss some of the questions that remain to be clarified. We will also discuss how this expanding knowledge may be explored in designing new strategies against efflux-mediated resistance.

KARINA XAVIER

Gut protective microbiota species promotes microbiota recovery and pathobiont clearance while preventing inflammation

Karina Xavier [(*)],

GIMM

(*) e-mai: karina.xavier@gimm.pt

Topic: Molecular Microbiology and Microbial Physiology

The mammalian gut microbiota plays a pivotal role in various aspects of host health, ranging from nutrition to immune system maturation and protection against intestinal pathogens. Many environmental factors can influence the composition and function of the gut microbiota. Notably, antibiotic treatments can inadvertently perturb the gut microbiota, rendering the host more susceptible to infections and chronic inflammation. While studying the mechanisms promoting microbiota recovery after the cessation of antibiotic treatment, we have identified a non-pathogenic *Klebsiella* gut microbe capable of restoring colonization resistance against Enterobacteriaceae following antibiotic treatment. This particular non-pneumonia *Klebsiella* species represents a keystone member of the mammalian gut microbiota found in both mice and humans. In wild-type mice, our studies demonstrated that this bacterium was sufficient for providing colonization resistance against *Escherichia coli* through a mechanism of nutritional competition. Building on this foundation, we are now expanding our research to explore the potential use of this *Klebsiella* strain as a next-generation probiotic.

Our latest findings show that in a mouse model of inflammatory bowel disease, this *Klebsiella* strain accelerates the clearance of Adherent and Invasive (AIEC) *E. coli*. It achieves this by promoting the recovery of butyrate-producing bacteria and preventing inflammation. These results underscore the importance of identifying microbes with critical microbiota functions and their potential use in developing strategies to facilitate microbiota recovery following antibiotic treatments.

CATARINA PRISTA

Fermentation as a strategy to produce sustainable and clean-label food products

***Catarina Prista*(*),**

1 LEAF (Linking Landscape Environment Agriculture and Food) Research Centre, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

(*)email: cprista@isa.ulisboa.pt

Topic: Food Microbiology and Biotechnology

Fermentation, one of the oldest known food processing techniques for food preservation, is gaining relevance as a potential strategy to address the challenges faced by today's food industry systems. In response to the growing demand from consumers for more sustainable and clean-label products, microbial fermentation provides a natural, efficient, and versatile approach to transform underutilized raw materials and valorize byproducts into nutritious, safe, and tasty foods.

Fermentation-based processes often rely on mild processing and low-energy input methods and can valorize food industry byproducts and underutilized biomass into high-value ingredients. Through the selection and optimization of microbial starters, fermentation enables the development of sustainable, clean-label food products, offering environmental and health benefits and increasing consumer trust and market differentiation. These include natural preservatives, flavor compounds, organic acids, and fermented plant-based proteins, which reduce the need for synthetic additives and enable a simpler ingredient list, thereby contributing to waste reduction, resource efficiency, and the creation of sustainable value chains.

Case studies will be presented to highlight how fermentation can be used to address key sustainability goals — such as reducing food waste, lowering carbon footprints, and enhancing nutritional profiles — while aligning with clean label requirements, bridging the gap between consumer expectations, environmental goals, and food innovation.

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ANTÓNIO RAPOSO

Jellyfish as Food: Processing Technologies and Potential Risks of Consumption

António Raposo[(*)]

CBIOS (Research Center for Biosciences and Health Technologies), ECTS (School of Health Sciences and Technologies), Lusófona University, Campo Grande 376, 1749-024 Lisboa, Portugal

(*)e-mail: antonio.raposo@ulusofona.pt

Topic: Food Microbiology & Biotechnology

Studies toward a sustainable future conducted by international organizations uniformly agree about having to change some of our present consumer behaviors. Regarding food, suggestions include eating locally farmed, less industrialized and renewable food to promote health and circularity, and limiting waste. Jellyfish are frequently sorted and discarded after being caught with fish in fishing nets and gear. In contrast, we propose utilizing this by-catch as food. Our investigation discusses the economic value and sustainability of jellyfish, the technologies used to prepare them for human consumption, their nutritional profile and health impacts and, finally, consumer acceptability and sensory evaluation of jellyfish food products. This discussion is critical for promoting jellyfish as an important aquatic resource to support blue and circular economies.

Keywords: allergenic assays; *Catostylus tagi* as food; consumers' attitudes and perceptions; food chemical analysis; food security; food technology; sensory analysis; sustainability

JOANA RAMOS

Microbes and the Changing Ocean: Small Organisms Big Impact

***Joana Ramos*(*),**

IITA – UAç

(*) e-mai:

ANA PAULA AREZ

Biobanks and Biological Collections: driving One Health by strengthening global networks, capacity, and research equity

***Ana Paula Arez*(*),**

1Global 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, Rua da Junqueira 100, 1349-008 Lisbon, Portugal.

(*)e-mail: aparez@ihmt.unl.pt

Topic: Biological Resources and Networks

Biological collections, preserved in biobanks, are critical assets for advancing innovation, preparedness, and translation in both One Health and Global Health. These collections—which may include organisms, tissues, vectors, and other biological materials—serve as powerful sources of scientific data. They underpin the identification and monitoring of pathogens, characterization of hosts, vectors and reservoirs, and tracking of diseases that impact human, animal, and environmental health. As the interconnection between these health domains becomes increasingly evident, biobanks emerge as essential tools for applied research and the development of evidence based policies. They support early outbreak detection, monitor antimicrobial resistance, uncover epidemiological patterns, and offer insights into the ecological dynamics shaping public health. In this context, biobanks are not merely repositories—they are strategic instruments for health surveillance, resilience, and security through a One Health lens. Despite their value, low- and middle-income countries (LMICs)—where the burden of infectious diseases is highest—often face major barriers in biobank development. Challenges include limited infrastructure, insufficient regulation, and a shortage of trained personnel. These constraints underscore the pressing need for knowledge exchange, capacity building, and equitable partnerships in biobanking. Collaborative networks, particularly those linking lusophone countries, offer a powerful model. By fostering the exchange of biological materials, harmonizing protocols, and enabling trans-biobank research, these alliances strengthen local scientific ecosystems. They also support fair benefit sharing in accordance with national ethical and legal frameworks Ultimately, these efforts advance scientific equity, promote sustainable research capacity, and drive global progress in tackling tropical and emerging health threats—laying the groundwork for a more interconnected, collaborative, and prepared global health community.

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VITOR GONÇALVES

The REBECA Network: Conservation Biodiversity and Driving Blue Biotechnology in Macaronesia

***Vitor Gonçalves*[1,2(*)],**

1 Faculdade de Ciências e Tecnologia, Universidade dos Açores, 9500-321 Ponta Delgada, Portugal

2 CIBIO-A Centro de Investigação em Biodiversidade e Recursos Genéticos – Polo dos Açores, InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning, UNESCO Chair—Land within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores

(*)email: vitor.mc.goncalves@uac.pt

Other authors: Rúben Luz [2], Rita Cordeiro [2], Amélia Fonseca [1, 2]

Topic: Biological Resources and Networks

The REBECA network (Blue Biotechnology Excellence Network) is a collaborative platform that unites microalgae and cyanobacteria culture collections across Macaronesia, aiming to conserve the unique insular biodiversity and promote a sustainable bioeconomy. By integrating conservation with applied biotechnology, REBECA acts as both a repository of native strains and a catalyst for innovation, fostering industrial development that strengthens regional competitiveness and quality of life.

The network comprises five major collections: the Bank of Algae and Cyanobacteria of the Azores (BACA), the Spanish Algae Bank (BEA), the Madeira Algae Bank (MAB), the Cape Verde Algae Bank (BACV), and the Mauritania Algae Bank (BAM). By connecting infrastructures and expertise, REBECA strengthens the foundations for a regional bioeconomy based on high-value applications in health, nutrition, and environmental sustainability.

As a founding member, BACA provides strains from the Azores and plays a key role in advancing scientific knowledge through taxonomy, phylogenetics, and bioactivity screening. Its research focuses on discovering bioactive compounds with pharmaceutical and cosmeceutical potential, evaluating toxicological and anti-inflammatory properties using advanced biological models, and exploring applications for sustainable aquaculture and animal nutrition.

Together, these collections provide certified biological resources, standardized cultivation protocols, and shared services for taxonomy, cytometry, biochemistry, and cultivation technologies. This integrated approach ensures quality, traceability, and accessibility of strains for research and industry, while driving innovation in sectors such as aquaculture, agriculture, biomedicine, and environmental remediation.

By linking biodiversity conservation with applied biotechnology, REBECA positions Macaronesia as a reference hub for blue biotechnology, promoting knowledge transfer, regional competitiveness, and sustainable development.

VITOR VASCONCELOS

MNOP – Microbiological Resources, Networks, Opportunities and Potentiation.

Vitor Vasconcelos [(*)],

1 CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal

2 FCUP – Faculdade de Ciências da Universidade do Porto, 4169-00, Porto, Portugal

(*) e-mail: lferreira@ciimar.up.pt

Topic: Biological Resources and Networks

Background: Studies involving microbiological resources are very important not just for conservation purposes but also for the sustainable economic exploitation. In that sense culture collections are fundamental infrastructures that should be organized and made available via the different national and international organizations and networks.

Methods/Results: At international level we should focus on the World Federation of Culture Collections (WFCC) and on the European Culture Collections Organizations (ECCO) as the main organizations that foster individual culture collections to organize themselves and give them a framework to attain excellence and great visibility internationally. MIRRI, The Microbial Resource Centre Infrastructure in an European network that allows interaction but also potentiates the access to funds at European, national and regional levels. Recently CIIMAR coordinates the Portuguese Blue Biobank that joins 9 national institutions and a similar number of Marine Biobanks, that is planned to expand in the next years and apply to be in the Portuguese Road Map of Infrastructures, to be updated soon. At CIIMAR we host the LEGE_CC, a culture collection of more than 1500 strains of cyanobacteria and microalgae, with more than 34 years old and recently accredited by the ISO 9001. LEGE_CC has been widely studied from a molecular, taxonomic and chemical approaches, with several patents being deposited regarding antimicrobial, anticancer, antiobesity, antifouling and cosmetic activities.

Conclusions: Microbiological Resources are essential for conservation and bioeconomy development. Networks at national and international level are fundamental to potential access to funding. Portuguese Blue Biobank needs to enlarge its range. Opportunities in science and industry are enormous.

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LUCILIA DOMINGUES

Strain Matters: Linking Host Choice to Bioprocess Success

Lucilia Domingues [(*)],

1 CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

(*)e-mail: luciliad@deb.uminho.pt

Topic: Bioprocess Engineering

Biotechnological production of lignocellulosic-based biofuels and biochemicals offers a sustainable alternative to petroleum-derived processes, with microbial conversion or fermentation at its core. However, in most bioprocess development pipelines, strain engineering and process design remain poorly integrated. Equally limiting is the widespread assumption that metabolic engineering strategies are universally transferrable across strains of the same species. Growing evidence shows this is not the case: the choice of host—its genetic background, evolutionary history, physiological traits, and overall "chassis identity"—profoundly shapes engineering outcomes, process robustness and, ultimately, bioprocess performance[1].

Strain heterogeneity within a single species remains an underexplored resource, despite the biological diversity it offers in stress tolerance and productivity[2]. Extending this perspective, a growing repertoire of non-conventional microorganisms from diverse species is emerging as promising platforms for next-generation bioprocesses[3]. As our understanding of microbial biodiversity deepens, new hosts with unique metabolic capabilities continue to surface, offering opportunities for tailored applications.

Another key message is the importance of integrating strain development from the earliest stages of bioprocess conception — "start with the end in mind." Host selection cannot be decoupled from feedstock characteristics. Our work, together with that of others, consistently shows that metabolic engineering outcomes depend not only on the chosen chassis but also on the physicochemical properties of the biomass substrates used in lignocellulosic biorefineries.

This keynote will illustrate these concepts through several examples, including recent work on the bioconversion of furan derivatives, showing how aligning host and strain selection, metabolic engineering strategies, and process design leads to more resilient and efficient bioprocesses. Ultimately, embracing microbial diversity, both between species and within species, and choosing the right strain for the right process will be essential for unlocking the full potential of sustainable biomanufacturing.

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ANABELA CARVALHO

Creating Real Value: From Idea to IP – Protected Innovation and Value

Anabela Carvalho[(*)]*,*

PATENTREE

(*) e-mail

MARIA JOÃO AMORIM

Advances in understanding how influenza A virions assemble

Maria João Amorim [(*)],

Catolica Biomedical Research Centre, Universidade Católica Portuguesa

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

TOPIC: Health Microbiology

Many viruses form biomolecular condensates *de novo* as part of their replication programmes. Influenza A virus is an important human pathogen that has the genome divided into eight different RNA segments. Interestingly, each infectious particle contains no more than eight RNA segments and one of each type. Here, we show that during infection influenza forms liquid condensates named viral inclusions where the eight RNA segments accumulate. Viral inclusions are formed with a single RNA type, suggesting that these structures are formed before the genomic complex assembles and raises the hypothesis that these are specialized sites for the formation of influenza epidemic and pandemic genomic complexes. We will debate how viral inclusions are formed and provide proof of the concept that condensate hardening blocks viral infection. Since native or engineered transitions affect condensate behavior, phase transitions may offer novel antiviral opportunities for influenza, as well as for many other viruses that utilize biomolecular condensates during their lifecycle. In addition, we will discuss how influenza A virus genome assembly is dependent on host cell membranes and explore how the virus remodels the lipidic landscape during infection.

RICARDO HENRIQUES

Towards nanoscale live-cell biology through AI-enhanced super-resolution microscopy

Ricardo Henriques[(*)],

ITQB – NOVA

(*) e-mail

CRISTINA SILVA PEREIRA

Filamentous Fungi in the Built Environment: Pollution, Virulence, and Hidden Associations

Cristina Silva Pereira [1(*)]

1 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal

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

Other authors: Pedro Crespo[2], João Jorge[2], Tiago Martins[2]

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal

Topic: Environmental Microbiology & Biotechnolog

Fungal communities, though ubiquitous in the built environment, remain understudied compared to their bacterial counterparts despite surging threats to public health. Recent decades have seen an unprecedented rise in severe fungal infections; for example, chronic pulmonary aspergillosis and related invasive diseases now affect over 6.5 million people, with an estimated mortality of 3.8 million [1]. Our research reveals how environmental drivers characteristic of human-built systems, especially anthropogenic pollution, select for resilient soil fungi such as *Aspergillus* spp., thereby raising new public health concerns. Metagenomic and phenotypic analyses of polluted soils confirm a narrowing of belowground diversity but highlight the emergence of *Aspergillus* as a dominant and stress-adapted genus [2]. Airborne spores from these environments show enhanced virulence, establishing a mechanistic link between environmental disturbance and the elevated burden of fungal disease in urban spaces [3].

Alongside this, our new investigations using transcriptomics, advanced imaging, and targeted microbiology assays have revealed a hidden dimension to *Aspergillus fumigatus*: the presence of core endohyphal bacteria within clinical and environmental isolates [4]. These previously overlooked partners demonstrate metabolic cooperation, potential modulation of stress and pathogenicity, and, in selected cases, may directly influence on virulence and antifungal resistance. This advances our conceptual understanding: rather than acting solely as solitary pathogens, fungi operate as dynamic consortia, with microbial partnerships shaping their ecology and clinical significance.

Our research highlights how built environment dynamics and cryptic microbial relationships mutually reinforce fungal adaptation and risk. Our data prompt key questions for future work: How do pollutants drive fungal virulence trajectories? To what extent do endobacteria alter the infection ecology of *A. fumigatus*? Addressing these gaps is essential for rethinking the risks posed by fungi in the built environment.

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ABSTRACTS

TOPIC #1: Environmental Microbiology & Biotechnology

ORAL COMMUNICATIONS

ID215 | Modular Engineering of Substrate Oxidation and Electron Flow for Enhanced Bacterial Cellulose Biosynthesis in *Komagataeibacter*

Author: Pedro Montenegro-Silva
Centre of Biological Engineering, University of Minho, Braga, Portugal
E-mail: montenegro.silva.pedro@gmail.com

Other authors: Fernando Dourado1,2, Miguel Gama1,2, Lucília Domingues1,2
1Centre of Biological Engineering, University of Minho, Braga, Portugal
2 LABBELS–Associate Laboratory, Braga/Guimarães, Portugal

Background: Bacterial cellulose (BC) is a high-performance biopolymer with broad utility in various fields (e. g. materials science, biomedicine, and electronics). However, the bioprocess is often not economically viable due to inefficient carbon use (e.g. byproduct formation). In Komagataeibacter spp., periplasmic oxidation of glucose to gluconate via PQQ-dependent dehydrogenases diverts carbon from cellulose biosynthesis and acidifies to suboptimal values. Ethanol supplementation has been associated with improved BC yields, partially by supporting respiration-linked ATP generation. This work explores modular metabolic engineering strategies to optimize substrate routing and electron transport activity in Komagataeibacter sucrofermentans.

Methods: In this work, several respiration- and biosynthetic-related targets were knocked out and/or overexpressed (e.g. membrane-bound PQQ-dependent dehydrogenases) in K. sucrofermentans ATCC700178. Mutants were cultured with glucose, ethanol, or both, under static and agitated conditions. Substrate consumption, extracellular metabolite profiles, and BC production were evaluated. Ethanol gradients were used to explore how co-substrate conditions influence BC biosynthesis in the engineered strains.

Results: Eliminating PQQ-glucose dehydrogenase suppressed gluconate accumulation and enhanced BC production in glucose media. Ethanol supplementation further increased yields in this background. Conversely, deletion of the PQQ-alcohol dehydrogenase impaired the positive effect of ethanol, suggesting disrupted coupling to the electron transport chain. Acidification profiles and metabolite accumulation patterns indicate shifts in redox-linked metabolism. While intracellular fluxes were not measured directly, strain-specific responses suggest altered energy balance and carbon partitioning.

Conclusions: Targeting substrate oxidation and electron transport offers a rational strategy to enhance BC production in Komagataeibacter. This modular approach provides a framework for tuning redox metabolism and improving biosynthetic performance through combined genetic and culture-based interventions.

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ID222 | Development of high-biogas-affinity methanotrophic enrichments for protein-rich biomass production

Author: Clara Dantas Moura

Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: claradmoura@ceb.uminho.pt

Other authors: Sónia Glória Barbosa(1,2); Maria Salomé Duarte (1,2); Maria Alcina Pereira (1,2)
1 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;

2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Methanotrophic bacteria (MOB), or methanotrophs play a crucial role in the carbon cycle, reducing methane (CH4) release into the atmosphere. This CH4-oxidizing bacteria are great allies in the fight against climate change. They are also valuable for sustainable biotechnology, such as producing protein-rich biomass for animal feed. Current processes use natural gas, raising sustainability issues. However, biogas from anaerobic digestion of organic wastes is a renewable, low-cost alternative. Biogas-derived MOB production represents an alternative biogas valorisation route with potential to support a transition into a circular economy, promoting waste treatment and generating products valuable for feed (e. g. aquafeed). Developing microbial cultures able to use raw biogas and optimizing mass transfer rate are the key steps toward an economic and environmental viability.

Methods: Aiming high biogas affinity, four MOB enrichments were developed at 30 ºC using DNMS medium and a headspace mixture of biogas and air (50/50 %) at the moderate pressure of 200 kPa for better mass transfer between gas and liquid phases. Four different inocula were used: anaerobic suspended sludge (SS), and sediment samples of three lagoons from the São Miguel Island (Azores, PT): Seven Cities (7C), Fire (FO) and Furnas (FU) Lagoons. MOB enrichments were characterized in terms of performance, protein content, and microbial communities' composition (16S rRNA sequencing).

Results: The developed enrichments have differentiated microbial communities. SS is 97.7 % enriched in Type I MOB from the genus Methylococcus spp. being taxonomically close to strain Methylococcus geothermalis IM1. 7C, FO, and FU are respectively 98.7 %, 41.1 % and 46.5 % enriched in Type II MOB, but only FO was taxonomically identified (Methylosinus sporium). All enrichments have similar protein content (close to 50 %, g protein g-1 dry weight).

Conclusions: Four promising methanotrophic enrichments were developed for microbial protein production intended for aquafeeds. All show strong adaptation to biogas as a carbon and energy source. Their distinct microbial compositions highlight the potential to magnify underutilized microorganisms for inclusion in aquafeeds and enhance process productivity.

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ID289 | Diversity of *Leptospira spp.* in biofilms, water, and soil: pathogenic DNA and viable saprophytic isolates in Lisbon, Portugal

Author: Ana Armada

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal

E-mail: aarmada@ihmt.unl.pt

Other authors: 1Ana Rita Nascimento, 2Priscyla dos Santos Ribeiro, 3Angela Lopes, 3Fernanda Simões, 2Paula Ristow, 1Maria Luísa Vieira, 1Ana Armada

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal;

2 Amsterdam University Medical Center, Leptospirosis Reference Center, Department of Medical Microbiology and Infection Prevention, Meibergdreef 39 1105 AZ Amsterdam, The Netherlands;

3 Nacional Institute of Agrarian and Veterinarian Research, Avenida da Republica, Quinta do Marquês, 2780-157 Oeiras, Portugal

Background: Leptospirosis is a globally important zoonosis causing over 1M human cases and about 60,000 deaths annually, primarily transmitted through direct or indirect contact with urine of infected animals. The etiological agent, pathogenic Leptospira, alongside non-pathogenic species, can persist for long periods in the environment, making water and soil important transmission vehicles. In Portugal, 65 confirmed cases were reported in 2022, and the country, along with four others, accounted for nearly 80% of European cases. Despite this, environmental data on Leptospira spp. remain scarce in Portugal. Thus, this study investigated the presence and diversity of Leptospira spp. in environmental samples in Portugal. Methods: Paired samples (N=8) of biofilms, water and soil were collected at Jamor and Queluz, in Lisbon, Portugal, and cultured in EMJH medium with antibiotics. Leptospira-like cells were detected by darkfield microscopy and molecularly identified by qPCR targeting lipL32 (pathogenic Leptospira) and 16S rRNA (all Leptospira spp.). lipL32-positive cultures underwent 16S rRNA (V3–V4) illumina sequencing. Leptospires positive samples were subject to clonal isolation on EMJH agar plates, followed by WGS on the MiSeq platform, and bioinformatics analysis.

Results: Positive Leptospira-like cultures were obtained from all Queluz samples and Jamor water samples. qPCR detected lipL32 in DNA extracted from enriched, decontaminated cultures of Queluz soil and biofilm, which, according to illumina sequencing showed a high percentage of bacteria of the genus Leptospira (25,78%), followed by 0,07% of L. licerasiae (intermediate) and very low levels of L. santarosai (pathogenic) and L. idonii (non-pathogenic). All isolates (n=100) were lipL32-negative and were confirmed as non-pathogenic by sequencing. The whole genome sequencing (WGS) of selected isolates identified L. bourretii (biofilm and water) and L. biflexa (soil) in Queluz, L. bandrabouensis (water) in Jamor, and potential novel species.

Conclusions: This study reports for the first time, the isolation of Leptospira spp. from environmental samples in Portugal. The detection of pathogenic, intermediate and non-pathogenic highlights the environmental diversity of Leptospira supports biofilms as potential environmental niches. These findings reinforce the need for molecular surveillance and continued taxonomic revision of the genus.

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ID300 | Unveiling PFAS-Tolerant Microbiomes in Polluted Soil and Sediment: Preservation Strategies and Bioremediation Potential

Author: Asma Nawaz

Department of Biotechnology & VUCC-DBT, University of Verona, Strada Le Grazie 15, 37134, Verona, Italy.

E-mail: asma.nawaz@univr.it

Other authors: Marco Andreolli, Beatrice Tontini, Silvia Lampis

1,2,3 affiliation: Department of Biotechnology & VUCC-DBT, University of Verona, Strada Le Grazie 15, 37134, Verona, Italy.

Background: Per- and polyfluoroalkyl substances (PFASs) are persistent environmental pollutants posing significant ecological and health risks due to their bioaccumulation, toxicity, and resistance to degradation. Microbial-based bioremediation strategies may offer a promising solution, requiring an in-depth understanding of microbial communities in PFAS-impaired environments[1]. This study, under the SUS-MIRRI.IT project explores microbiomes from two environmentally polluted matrices - soil and sediment - focusing on their preservation, diversity, and potential for PFAS bioremediation.

Methods: Samples were cryopreserved at -80 °C with 30% glycerol as a cryoprotective agent and analyzed at four time points: T0 (pre-storage), T1 (3 months), T2 (9 months), and T3 (12 months). Both culture-dependent and culture-independent methods were employed to evaluate microbial preservation and functionality. Total microbial counts and community-level physiological profiling were assessed using Biolog EcoPlates, while metabarcoding analysis provided detailed insights into the conservation of microbial diversity. In addition, enrichment cultures with perfluorooctanoic acid (PFOA; 600 mg/kg) were performed at each time point to selectively isolate resistant strains to investigate the tolerance and potential bioremediation capacity of preserved microbiomes.

Results: The results revealed noticeable changes in microbial communities during the storage period in both soil and sediment samples. Specifically, total counts, Biolog EcoPlates, and DNA barcoding indicated that the most pronounced microbial alterations in soil occurred between T2 (8 months) and T3 (12 months). In contrast, sediment samples displayed a more consistent, gradual shift throughout the experimental period. Enrichment cultures yielded PFOA-tolerant microbial strains, with several strains recurring across T0, T1, T2, and T3 within both samples. The dominant bacterial class identified was Gammaproteobacteria, followed by Alphaproteobacteria, with fewer representatives from Betaproteobacteria, Actinomycetia, and Flavobacteria. Among the genera, Stenotrophomonas and Pseudomonas were predominant, while Brucella and Klebsiella were also detected. Notably, Citrobacter was consistently observed across all time points in soil samples.

Conclusions: Although most of the original microbiome was preserved even after one year of storage, the chosen protocol requires further improvement.

ID318 | Genomic Diversity and Phage-Based Biocontrol of *Erwinia amylovora*: Towards Sustainable Fire Blight Management

Author: Hugo Oliveira

1 Centre of Biological Engineering, University of Minho, Braga, Portugal 2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

E-mail: hugooliveira@ceb.uminho.pt

Other authors: Gabriel Abreu 3, Carlos Rodrigues 3, Miguel Leão 4, Carina Almeida 4, Luisa Moura 5, Maria Conceição 6, Leonor Cruz 4, Luis Carvalho 7

3 Centre of Biological Engineering, University of Minho

4 Instituto Nacional de Investigação Agrária e Veterinária, I. P. (polo Vairão, Alcobaça e Oeiras)

5 Instituto Politécnico de Viana do Castelo

6 Faculdade de Ciências da Universidade do Porto

7 Bioscale

Background: *Erwinia amylovora*, the causal agent of Fire Blight, is one of the most destructive bacterial pathogens of apple and pear trees worldwide. Once considered genetically uniform, recent studies have revealed previously hidden genomic variation, particularly among isolates infecting different hosts such as Amygdaloideae and Rubus. These subtle differences may influence virulence and bacteriophage sensitivity, with direct implications for disease control. As antibiotics and copper compounds face increasing restrictions, bacteriophages have emerged as promising biocontrol agents.

Methods: Over 100 *E. amylovora* isolates from Portuguese orchards were collected, sequenced, and compared with all publicly available genomes using a newly developed bioinformatic genotyping tool. In parallel, bacteriophages were isolated and screened against a vast panel of E. amylovora strains, and their receptor usage was mapped. Based on these data, a rationally designed phage cocktail targeting complementary receptors was formulated and tested in vitro and in planta. Phages were further produced in industrial bioreactors and evaluated under greenhouse conditions.

Results: Despite high overall genomic similarity (>95%), our surveillance framework tool enabled the differentiation into distinct genotypes, uncovering previously underappreciated global distribution. Subtle receptor variations (capsule, cellulose, and LPS) correlated with measurable differences in phage infectivity. The selected phage cocktail demonstrated strong synergistic activity, broad efficacy against genetically diverse isolates, and reduced the risk of resistance development. Sequential assays confirmed its effectiveness in detached flowers and significant suppression of E. amylovora infection (≈50%) in greenhouse experiments.

Conclusions: By integrating genomics with receptor-based screening, we developed a robust, broad-spectrum phage cocktail effective under greenhouse conditions, offering a sustainable strategy for Fire Blight management.

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ID323 | Decoding Microbial Interactions with Vanadium

Author: Joana Bonito Caldeira

University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal

E-mail: joanaritacaldeira@gmail.com

Other authors: Rita Branco, Paula Vasconcelos Morais

University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal

Background: Vanadium (V) is a crucial metal for the European Union,due to its importance in sustainable technologies, resource security, and supply risks. V shows different colours depending on its oxidation state: V(V), the most common and toxic form, is yellow, while reduced forms are blue, green, or lilac. Although some bacteria resist V, the specific genes for V(V) bioreduction are rarely documented. The increasing V-demand across various industries and the EU's reliance on it highlights the need for alternative recovery methods. This work aimed to explore bacterial metabolic mechanisms under V-stress, focusing on the ability to bioreduce V(V) as a potential biotool for the green recovery of critical metals.

Methods: Rhodococcus strain B2S2 3.2 3w12 isolated from a metal-contaminated environment was tested for its minimum inhibitory concentration of V(V). Different conditions (lower and higher agitation, 100 and 140 rpm, respectively) were used to evaluate the bacteria's ability to grow with V(V). After growth, media were assessed by scanning electron microscopy/energy dispersive spectroscopy for metal analysis and SDS-PAGE for protein expression.

Results: The strain was able to grow in 20 mM V(V), with optical density values at 600 nm similar to the control. Growth conditions affected the bacteria; lower agitation promoted a dark colour in the metal-bacteria medium. Under these conditions, a protein of 25 kDa was observed and identified as either a subunit of a MspA family porin or a Tellurium resistance protein TerD using genome information. Spectroscopic analysis permitted the detection of V-structures, not observed in abiotic conditions or higher agitation. The spectra revealed different ratios of V:Na; the abiotic sample showed 1V:3Na, while the dark sample exhibited an inverse ratio (3V:1Na).

Conclusions: Lowering agitation levels promoted the presence of a different profile of proteins in the medium and black-brown V-structures. This finding suggests a process of V(V)-bioreduction resulting in a protein-V structure with Na as a counterion. Understanding these interactions is relevant since they can influence the compound's solubility, absorption, tissue uptake, and toxicity.

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ID325 | Microplastics and Chemical Pollutants Team Up to Facilitate the Persistence of Multidrug-Resistant Pathogens in Rivers

Author: Isabel Silva

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456, Coimbra, Portugal

E-mail: silva.isabel.ua@gmail.com

Other authors: Inês Ataíde Soares; Isabel Henriques

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456, Coimbra, Portugal

Microplastics (MPs, <5 mm) facilitate contaminant adsorption (e.g., metals, antibiotics) and microbial colonization, potentially promoting the selection and persistence of antibiotic-resistant bacteria on aquatic systems.

We investigated the influence of different MPs, namely Polystyrene (PS), Polyethylene terephthalate (PET), Polyethylene (PE), and biodegradable Polylactic acid (PLA), on the persistence of multi-drug resistant pathogens in river water. Additionally, we evaluated the combined effects of selected MPs (PET or PLA) with copper (Cu^2n; 12.7 µg/L) or the antibiotic cefotaxime (CTX; 500 ng/L and 5 µg/L). A microcosm experiment was conducted using cefotaxime-resistant strains, representing critical priority pathogens, previously isolated from MP-associated biofilms: Klebsiella pneumoniae A33, Escherichia coli A31, and E. coli B4. Sand particles were used as a natural substrate for comparison of bacterial persistence.

Whole-genome sequencing of the selected strains confirmed species-level identity and revealed a broad repertoire of antibiotic resistance genes (ARGs), including extended-spectrum β-lactamase (ESBL) genes as blaCTX-M-15 and blaCTX-M-32, consistent with multi-drug resistance phenotypes. Genes associated with copper tolerance were also identified, as well as multiple virulence factors, supporting strains classification as potential human pathogens. Among the tested polymers, PET most strongly favored the bacterial persistence over 28 days. For K. pneumoniae A33 and E. coli B4, CFU counts on PET were significantly higher (1 to 2 log units) compared to PE and PS. However, significant differences between PET and sand were not observed under these conditions. In the presence of chemical contaminants, PET and PLA further enhanced bacterial persistence compared to sand. Specifically, under copper and cefotaxime exposure, CFU levels on PET and PLA were 1 to 2 log units higher than on sand.

Our findings demonstrate that MPs, including biodegradable variants, can promote the environmental persistence of multidrug-resistant pathogens, particularly under co-exposure to chemical pollutants. This highlights an underrated dimension of MP pollution, with potential implications for antibiotic resistance dissemination and public health.

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ID327 | Class 3 resistance integrons in treated wastewater: threats that go with the flow!

Author: Isabel Henriques

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, Portugal

E-mail: isabel.henriques@uc.pt

Other authors: 1,2 - Rafael D. S. Tavares; 1 - Elsa T. Rodrigues; 2 - Marta Tacão

1 - Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, Portugal;

2 - Centre for Environmental and Marine Studies (CESAM) and Department of Biology, University of Aveiro, Portugal

Background: Integrons are important players in the spread of antimicrobial resistance (AMR). However, most studies focus on class 1 integrons, while little is known about class 3 integrons and their role in AMR. Given that Wastewater Treatment Plants (WWTPs) are pivotal entry points of resistance from human to environmental settings, we aimed to characterize the class 3 integron pool of wastewater.

Methods: Effluent samples from a Municipal WWTP were collected weekly throughout 2020 (n = 52 samples). DNA was extracted and used for PCR amplification of class 3 integrons using two primer sets. Amplicons were pooled by sampling month and sent for high-throughput PacBio sequencing.

Results: We obtained a total of 233,948 class 3 integron reads, which represented 72,591 unique arrays. From the 12 monthly pooled samples, we recovered between 4,181 and 18,212 unique class 3 arrays per sample, while the estimated richness was between 36,891 to 183,200. The gene cassettes (GCs) identified mostly encoded proteins with unknown functions (74.5%). The most abundant functions were associated to defence mechanisms (7.3%) and amino acid transport and metabolism (7.2%). A total of 25,179 unique resistance class 3 integrons [i.e., carry at least 1 AMR-encoding gene cassette (ARGC)] were identified. Aminoglycoside (e.g., aadA10) and beta-lactam resistance (e.g., blaGES, blaOXA) were the most abundant AMR mechanisms in class 3 integrons across the year. GCs conferring beta-lactam resistance predominantly encoded extended-spectrum beta-lactamases (ESBLs) or carbapenemases. The most abundant and ubiquitous resistance class 3 integron carried an aadA10 gene and a cassette encoding a putative vicinal oxygen chelate (VOC) protein (associated to the detoxification of xenobiotics).

Conclusions: Treated wastewater constitutes a reservoir of class 3 resistance integrons. The presence of cassettes encoding ESBL and carbapenemase genes constitutes a risk to the interconnected domains of One Health, while the co-location of ARGCs with genes related with niche adaptation functions can lead to co-selection of AMR mechanisms without a direct antibiotic selective pressure.

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ID331 | Sustainable Production of Biopigments and Biosurfactants from Agro-Industrial Byproducts Using Serratia Strains

Author: Joaquim Filipe Pereira Pinto

University of Coimbra, CERES, Department of Chemical Engineering, 3030-790, Coimbra, Portugal

E-mail: joaquimfp99@gmail.com

Other authors: Paula Vasconcelos Morais2 and Jorge Fernando Brandão Pereira1

1 University of Coimbra, CERES, Department of Chemical Engineering, 3030-790, Coimbra, Portugal;

2 University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, 3000-456, Coimbra, Portugal

Background: Olive oil production is a strategic agro-industrial sector in the Mediterranean basin, but it generates over 30 million m³ of olive mill wastewater (OMWW) annually. OMWW is an acidic red-black emulsion containing 85-92% water and poses significant environmental challenges, including soil and water pollution and inhibition of plant growth. However, OMWW also contains more than 50 phenolic compounds with high economic value and biological activity. Current treatments (physical, chemical, biological) are costly, while alternative practices such as municipal waste disposal or agricultural use are limited by its phytotoxicity. An emerging approach is biotechnological strategies using microorganisms to produce value-added bioproducts such as enzymes, pigments and biosurfactants, representing one of the most promising avenues for OMWW valorization.

Methods: Serratia strains from the University of Coimbra Bacteria Culture Collection (UCCCB) were screened for their availability to produce biosurfactants and biopigments. Biosurfactants were evaluated through emulsification index and surface tension measurements, while biopigments were quantified using spectrophotometric calibration curves.

Results: Selected strains produced biosurfactants with an emulsification index (E24, %) of 56.66 ± 0.01% (comparable to synthetic surfactants) and prodigiosin at 0.291 ± 0.004 g/L, displaying strong antioxidant activity (3.398 ± 0.006 µg/mL Trolox equivalents). In synthetic OMWW enriched with phenolics to mimic real effluent, strains produced prodigiosin (1.9 mg/L) and biosurfactants at satisfactory levels.

Conclusions: Serratia sp. Arv.22-2.5c exhibited the highest potential for commercial-scale production of prodigiosin and biosurfactants in both optimized conditions and OMWW-mimicked solutions. Serratia strains grew in real OMWW effluent while producing biosurfactants, highlighting their suitability for biotechnological valorization. This work supports the principles of circular economy by reducing OMWW-related environmental impacts, valorizing waste streams, and converting available carbon sources into valuable bioproducts.

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ID333 | Genome mining of novel antimicrobial biosynthetic gene clusters in extremophiles from high-salt biofilms

Author: Inês Costa

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456 Coimbra, Portugal

E-mail: icosta@uc.pt

Other authors: Isabel Henriques, Igor Tiago

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456 Coimbra, Portugal

Antimicrobial resistance is one of the 21st century's major global concerns, with microorganisms acquiring resistance to existing antibiotics at an alarming rate. Thus, discovering novel therapeutic strategies and exploring new antimicrobial compound sources is paramount.

Extremophiles, microorganisms adapted to extreme environments, thrive and survive by using unique strategies. These may include the ability to form biofilms and producing uncommon secondary metabolites, some with antimicrobial activity. These characteristics further enhance their survival and competitive advantage over competitors. As such, many of these extremophiles are promising sources for novel antibiotics awaiting discovery.

Our study focused on biofilms from the cloister chapels of The Old Cathedral of Coimbra. The monument's high-salt rocks foster ideal conditions for the establishment of halophilic and halotolerant microbial communities. Biofilms were sampled and bacteria were isolated to determine the diversity of the cultivable aerobic microbial population. From a total of 714 isolates, 6 isolates were identified as potential new species. Their novelty was confirmed using a comprehensive approach that included phenotypic, biochemical, phylogenetic, chemotaxonomic, and genome-based analyses. These strains were determined to be taxonomically related to species belonging to genera Alteribacillus, Caldalkalibacillus, Deinococcus, Nocardioides, Oceanobacillus and Paenibacillus.

Genomic analyses using antiSMASH revealed the presence of 27 Biosynthetic Gene Clusters (BGCs): 4 PKS, 2 RiPPs, 3 betalactones, 7 terpenes and 11 related to other molecules. We identified 8 of these BGCs as the most promising due to their low or no similarity to known BGCs and the presence of complete functional pathways for synthesis, modification, and transport. The genomic data also revealed the presence of resistance genes, regulators for antibiotic production, and virulence factors. The genomes most closely related to the genera Caldalkalibacillus and Alteribacillus were particularly notable, each containing 2 of these high-potential BGCs.

Our work highlights the importance of combining traditional culture-dependent techniques with modern genome mining methods. This integrated approach is an excellent starting point to explore bacterial potential in the discovery of new and unique *compounds.**This work was supported by FCT, through a PhD scholarship (2022.12620.BD).*

ID353 | Biocalcifying potential of ureolytic *Priestia aryabhattai* 10w27b for bio-repairing of mortar cracks

Author: Leonor Matos

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal

E-mail: leonormatos98@gmail.com

Other authors: Carina Coimbra 1, Pedro Farias 1, Ricardo Duarte 2, Fernando G. Branco 3, António A. S. Correia 4, Paula V. Morais 1

1 University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal;

2 University of Coimbra, Department of Civil Engineering, 3030-788 Coimbra, Portugal;

3 University of Coimbra, ISISE, ARISE, Department of Civil Engineering, 3030-788 Coimbra, Portugal;

4 University of Coimbra, CERES, Department of Civil Engineering, 3030-788 Coimbra, Portugal

Microbial Induced Calcium Carbonate Precipitation (MICP) is a process where bacteria precipitate calcium carbonate (CaCO3) through pathways such as ureolysis, carbonic anhydrase CO2 fixation, and denitrification. Concrete is widely used due to its low cost, durability, and strength. However, it is prone to cracking, and conventional repair methods are costly and environmentally damaging, and MICP offers a more sustainable alternative. So far, the number of MICP organisms is limited, and this work aimed to study efficient MICP bacterial strains.

Priestia aryabhattai 10w27b, isolated from Panasqueira mine, is spore-forming and capable of surviving in alkaline environments, ideal for biocementation. Following a positive result in Christensen's urea-based agar medium, confirming ammonia production, a genome analysis revealed the presence of both the urease operon, along with 3 carbonic anhydrase genes.

Ammonia production was quantified in B4 medium through the indophenol method. Sporosarcina pasteurii LMG07130 was used as a positive control due to its high ureolytic activity. Compared to the strain LMG07130, which reached peak activity after 10 hours with 147mM NH4, strain 10w27b produced only 3mM NH4 after 1 day. Despite this, CaCO3 precipitation was observed.

To evaluate strain 10w27b's potential for long-term biocementation assays, an 8-week-long experiment was conducted using pre-made cracked mortars. Weekly, a bacterial suspension (ODnnn=1) was applied to cracks, followed by the addition of a nutrient medium supplemented with 0.5M CaCl2/urea, 2 days later. Mortar samples were sealed and stored at 98% relative humidity in a temperature-controlled room at 20°C. SEM-EDS analysis was employed to visualize and confirm the presence of bacterial cells and CaCO3 crystals. Flexural strength tests assessed whether biocementation enhanced the mortar's resistance to bending. Samples were collected in triplicate after 2 weeks, 1 month, and 2 months.

The presence of both strain 10w27b and CaCO3 crystals inside cracks was detected after 2 weeks. Compared to the control, there was an increase in strength of 53%, 14%, and 17% after 2 weeks, 1 month, and 2 months, respectively.

These results show strain 10w27b's ability to promote CaCO3 precipitation and enhance mortar strength, indicating potential for long-term biocementation applications.

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ID378 | Dual-Output Microbial Electrosynthesis System for Biofuel Production

Author: Nuno Machado

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: nuno.machado@itqb.unl.pt

Other authors: Inês Cardoso Pereira, Américo Gonzalez Duarte and Felipe Conzuelo

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Greenhouse gas emissions are increasing to unprecedented levels, having a severe impact on our planet and the life in it. To mitigate their increase, it is urgent to establish sustainable processes to produce alternative non-polluting fuels. Microbial electrosynthesis has emerged as an attractive alternative for the sustainable production of biofuels. Desulfovibrio vulgaris Hildenborough (DvH) is a sulfate reducer carrying highly active enzymes in the periplasm, namely hydrogenases that catalyze the interconversion of protons to molecular hydrogen and formate dehydrogenases that reversibly convert carbon dioxide to formate, two highly valuable fuels for the energy sector.

Here, DvH was used as a catalyst coupled to an electrochemical unit, where a carbon cloth working electrode serves as the support where bacteria are immobilized and provided with electrons to drive the production of formate and hydrogen. All experiments were performed in a batch electrochemical reactor, using a three-electrode system. DvH's electrochemical communication with the electrode and H2/formate production rates were evaluated through cyclic voltammetry and amperometric experiments, followed by product quantification.

DvH is not an electroactive organism, limiting the use of exogenously provided electrons for the production of biofuels by its enzymes. Methyl viologen (MV) was used as redox mediator transferring electrons from the electrode surface, diffusing across DvH's outer membrane and delivering them to the periplasmic enzymes. The wild-type strain produced significant concentrations of both formate and hydrogen when MV was present. However, little or no production was observed in the absence of redox mediator, suggesting that as long as electrons can be conducted from the extracellular space into the periplasm, DvH is able to produce the valuable biofuels. Because MV is a toxic and expensive chemical, alternative strategies are being investigated to ensure a sustainable and cost-effective solution for electron transfer. One example is through biosynthesis of metal nanoparticles in the outer membrane of the bacteria. Although electrodes modified with DvH integrating biosynthesised nanoparticles were not able to produce detectable amounts of hydrogen, significant production of formate was observed, similar to immobilized DvH cells with MV allowing to avoid the toxic redox mediator in solution while converting carbon dioxide into a valuable product

ID384 | Temperature profiles and auxin production by yeasts associated with industrial microalgal cultures of *Tisochrysis lutea*

Author: Madalena Matos

iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

E-mail: madalena.matos@tecnico.ulisboa.pt

Other authors: Mónica A. Fernandes 1,2, Tamára F. Santos 3, Natacha Coelho 4,5, Alexandre Rodrigues 4,5, João Varela 3,6 and Isabel Sá-Correia 1,2,7

1- 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 - Centre of Marine Sciences, Campus Gambelas, University of Algarve, Faro, Portugal;

4 - Necton S.A., Belamandil, Portugal;

5 - MED—Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Universidade do Algarve, Faro, Portugal;

6 - GreenCoLab—Associação Oceano Verde, Campus Gambelas, University of Algarve, Faro, Portugal;

7 - Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Among marine microalgae, *Tisochrysis lutea* has emerged as a promising candidate for industrial-scale aquaculture and biotechnological applications. *T. lutea* is widely used in hatcheries for larval nutrition and has applications in functional foods and nutraceuticals due to its rich composition of omega-3 fatty acids, sterols, and carotenoids. Among the microbiomes associated with industrial microalgae cultures, yeasts represent a particularly interesting group (1). Yeasts may enhance algal productivity by providing essential growth factors or signaling molecules. Through microbiome engineering, co-cultivation strategies, or targeted microbial management, it may be possible to harness beneficial microbiome-microalgae interactions while minimizing negative impacts. Recent results from a study envisaging the isolation of the culturable yeast population in the same industrial setting but in association with Microchloropsis gaditana, revealed a very high percentage of red yeasts with the capacity of producing lipids and biosurfactants and suggested their potential use as probiotics in microalgae production (1). *T. lutea* large-scale cultivation is still constrained by environmental and physiological factors that limit biomass yield and biochemical productivity. In this context, the use of phytohormones such as indole-3-acetic acid, a naturally occurring auxin, has gained interest as a strategy to enhance microalgal growth and metabolism. Although the effects are complex, emerging evidence suggests that auxins also play growth-promoting roles in microalgae by stimulating photosynthesis, pigment and lipid production and stress tolerance in both freshwater and marine algal species. Yeasts capable of auxin production are promising for enhancing microalgal growth and metabolite production through natural, biologically mediated hormone delivery. This study is dedicated to the isolation and identification of yeast culturable diversity associated with industrial cultures of *T. lutea* at Necton S.A. facilities, Olhão, Portugal, and to the characterization of temperature profiles, effects of synthetic sea water on growth and auxin production by the isolates obtained. This is a first step towards the exploitation of a co-cultivation alga-yeast strategy for improving bioprocess productivity.

1. Matos, M et al., J. Fungi 2025, 11, 228. https://doi.org/10.3390/jof11030228 In the framework of the Blue Bioeconomy Pact Project (WP5 Algae Vertical).

ID395 | Plasmid diversity: what do we miss when we only look at the majority?

Author: João S. Rebelo

CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal

E-mail: joaorebelo_4@hotmail.com

Other authors: Célia P. F. Domingues1,2, Luís Borda-de-Água3,4,5, Teresa Nogueira1,2, Francisco Dionisio1

1 CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal;

2 INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal;

3 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig; Puschstraße

4, 04103 Leipzig, Germany; 4 Institute of Biology, Martin Luther University Halle-Wittenberg; Am Kirchtor 1, 06108 Halle (Saale), Germany;

5 Centro de Ecologia Aplicada Professor Baeta Neves (CEABN), Instituto Superior de Agronomia; Tapada da Ajuda, 1349-017 Lisboa, Portugal

Background: In bacterial communities, a few dominant species typically coexist with a large number of rare taxa. Interestingly, these rare bacteria often perform critical functions within the community, with impacts that extend far beyond their low abundance. In some cases, essential community-level functions are carried out exclusively by these rare taxa. While bacterial abundance dynamics are well characterized, the distribution of plasmids within such communities remains largely unexplored.

Methods: In this study, we analyzed a dataset comprising approximately 53,000 plasmids from the curated RefSeq database. For each plasmid, we characterized the diversity of coding regions, resistance and virulence genes, mobility type, and the phylogeny of their hosts. Plasmids were clustered into Plasmid Taxonomic Units (PTUs), in which genetically similar plasmids are grouped together.

Results: Plasmids also exhibit a highly uneven distribution, with a few PTUs being highly prevalent while the majority remain rare. This distribution does not directly mirror that of their hosts: some plasmids from common hosts are rare, while others from less common hosts are common. Interestingly, although plasmids are not living organisms, their distribution patterns resemble those reported for other groups, including insects and arachnids. The success of plasmids within common PTUs can be partly attributed to their higher mobility. On the other hand, plasmids from rare PTUs display greater genomic diversity, including virulence genes, and have a broader host range.

Conclusions: Our results suggest that rare plasmids may function as a genetic reservoir, potentially harbouring genes of relevance for health, biotechnology, and other fields. Due to their diversity, these plasmids can be rapidly selected and increase in frequency under changing environmental conditions, thereby playing a crucial role in bacterial evolution.

ID402 | Heterologous expression of a new natural lactylate in the model *cyanobacterium Anabaena sp.* PCC 7120

Author: Eunice A. Ferreira

CIIMAR/CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

E-mail: eferreira@ciimar.up.pt

Other authors: Francisco Camacho1, Diana Sousa1,2, Paulo Oliveira1,3, Pedro N. Leão1

1 CIIMAR/CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal;

2 ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Portugal;

3 FCUP - Faculty of Sciences of the University of Porto, Portugal.

Cyanobacteria are ubiquitous photoautotrophic microorganisms known to produce diverse and valuable natural products. The culture collection at CIIMAR, LEGEcc, contains a unique phylogenetic diversity of over 1000 cyanobacterial strains, representing untapped biological resources to discover new compounds. Natural product biosynthesis relies on enzymes encoded by consecutive genes called biosynthetic gene clusters (BGCs). However, the majority of BGCs available in genomic data are considered orphan, i.e., are not linked to the corresponding metabolite, meaning that most of the natural products are still to be discovered. Deorphanizing cyanobacterial BGCs through genetic engineering is a slow and challenging process, if not impossible, since most of the strains are not genetically tractable. To circumvent this challenge, cyanobacterial BGCs have been deorphanized using heterologous hosts. In this context, and for the first time, we used the model cyanobacterium Anabaena sp. PCC 7120 to express a BGC from a LEGEcc cyanobacterium. The cly BGC was previously identified as putatively related to the production of chlorosphaerolactylates, which are industrially important compounds, used as emulsifiers in food and cosmetics. In this work, genomic DNA from the cyanobacterium Nodularia sp. LEGE 06071 was extracted, and the clyA-F genes were amplified by PCR, assembled with characterized genetic tools and transformed into Anabaena sp. PCC 7120. A comparative metabolomics approach was applied and the new unsaturated chlorosphaerolactylate E was identified. This compound is an ester of dichlorinated dodecanoic acid and L-lactic acid with an unsaturation, possibly in the β,γ position. With this study, we experimentally connected a BGC with its respective compound and established the proof-of-concept to implement a pipeline for discovery of other new natural products and their associated biochemical diversity for future biotechnological applications.

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ID430 | White grape marc extracts as modulators of turbot resilience to bacterial infections

Author: Miguel Castro

1 Department of Biology, Faculty of Sciences of the University of Porto, Portugal.

2 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

E-mail: miguelcastro505@gmail.com

Other authors: Rafaela Santos1; Rui Magalhães 2; Ana Couto1,2; Roberto Bermúdez 3,4; María Isabel Quiroga 3,4; Maria Celeiro 5; Marta Lores 5,6; Aires O.Teles 1,2; and Claudia Serra1,2

1 Department of Biology, Faculty of Sciences of the University of Porto, Portugal.

2 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

3 Department of Anatomy, Animal Production and Veterinary Clinical Sciences, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Galicia, Spain.

4 Aquaculture Institute, University of Santiago de Compostela, Santiago de Compostela, Spain.

5 LIDSA, Department of Analytical Chemistry, Nutrition and Food Science, University of Santiago de Compostela, Spain.

6 i-Grape Laboratory, Santiago de Compostela, Spain.

Background: Aquaculture is one of the fastest-growing food production sectors and this growth has been accompanied by an increase in the occurrence of infectious diseases and antimicrobial use, contributing to antimicrobial-resistant bacteria and drug residues. In the absence of effective strategies to fully control bacterial pathogens, there is an urgent need for alternatives to ensure advanced and integrated healthcare for humans, animals, and the environment. In this context, the present study evaluated the use of AquaGrape extracts (glycol (G), and ketone (K)) derived from white grape marc (a polyphenols-rich residue from the wine industry, with antioxidant and antimicrobial properties), as functional feed additives to assess their potential protective effects in turbot (Scophthalmus maximus) upon bacterial challenge with Vibrio anguillarum.

Methods: Fifteen groups of 20 turbot (initial weight of ~20 g) were assigned to five experimental diets; each tested in triplicate. Diets were isolipidic (14%) and isoproteic (54%), formulated to meet turbot nutritional requirements, and included glycol and ketone AquaGrape extracts at concentrations of 450 and 900 ppm (G450, G900, K450 and K900). A control diet (CTRL) without extract was also included. Fish were fed for 88 days. Post-growth trial, fish from all groups were intraperitoneally injected with 100 µL of 3x107 CFU mL-1 V. anguillarum or 100 µL of PBS (placebo control). Mortality and clinical signs were monitored over a 12-day challenge period. Dead or moribund fish were removed daily for analysis and weighing.

Results: No mortalities were observed in the non-challenged (PBS-injected) fish. Following V. anguillarum challenge, cumulative mortality ranged from 33.3% to 73.3% across treatments. The highest mortality occurred in fish fed the G900 diet (73.3%), followed by K900 (60.0%), CTRL (53.3%), K450 (46.7%), and G450 (33.3%). Although differences were not statistically significant compared to the control, fish receiving the G450 and K450 diets showed improved resistance to infection, with comparatively higher survival rates (66,7% and 53,3%, respectively).

Conclusions: At low incorporation levels (450ppm), AquaGrapes additives are promising modulators of aquaculture fish resilience against bacterial pathogens.

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ID435 | Refining Microbial Consortia to Improve Phytoremediation in Salt Marshes: Integrating Conventional Enrichment with iACME Selection

Author: Margarida Pereira

1- CIIMAR/CIIMAR LA, 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: margarida.pereira@ciimar.up.pt

Other authors: Joana P. Fernandes 1,2; Dmitrii Deev 3; Maja Zugan 3; Tomaž Rijavec 3; Aleš Lapanje 3; C. Marisa R. Almeida 1,2; Ana P. Mucha 1,2

1 - CIIMAR/CIIMAR LA, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal;

2 - FCUP – Faculty of Sciences, University of Porto, Porto, Portugal;

3 - Jožef Stefan Institute (JSI), Ljubljana, Slovenia.

The modelling of enhanced microbial consortia for efficient bioremediation has proven to be a complex and challenging endeavour. To boost the development of efficient microbial consortia, we explored the combination of more conventional techniques, such as culture enrichment from contaminated matrices, followed by innovative approaches to assemble microbial consortia from isolated strains, including advanced iACME (interspecies Aggregation, Combinatorics, Microfluidics, and Entrapment) selection tools. Firstly, rhizosediments from the salt marsh Sporobolus maritimus, collected in the Lima River Estuary, were subjected to culture enrichment in the presence of copper (Cu) and the antidepressant venlafaxine (Vfx), both of which have been previously detected in the estuary. After enrichment, Cu and Vfx removal from solution ranged from 70% to 90%, individually or in combination, through a synergy of biological and physicochemical mechanisms. This enrichment approach allowed the isolation and characterisation of 57 bacterial strains.

To assess the most effective combinations among the 57 isolated strains using the iACME approach, bacterial strains were mixed in equal proportions and randomly aggregated into consortia through electrostatic surface modification. As a result, 704 bacterial consortia were encapsulated in alginate beads and cultivated aerobically for 3 days with either Cu and Vfx, or Vfx alone, with or without sodium acetate as a co-metabolic enhancer. Growth curves identified the 12 most promising consortia, which were then sent for bacterial community structure characterization by 16S rRNAhigh-throughput sequencing .

The 12 most efficient consortia were grown and tested for their pharmaceutical (Vfx) degradation abilities, minimum inhibitory concentration assay (Cu) and biofilm production, a very vital PGP trait. Integrating these latest results will guide our choice of the most effective consortia for estuarine plant microcosm trials. The consortia tested presented Vfx degradation percentages between 15-31%, MIC for Cu between the intervals 0-1 mg/L and 64-128 mg/L, with 10 out of 12 consortia presenting biofilm formation abilities. The next step will involve inoculating the salt marsh plants with the best-performing microbial consortia to assess their combined potential for pharmaceutical detoxification and metal immobilization within estuarine ecosystems.

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ID441 | Genomic insights into antifungal potential of three *Pseudomonas* strains

Author: Clarisse Brígido

1 MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Universidade de Évora, Largo dos Colegiais 2, 7004-516, Évora, Portugal

2 Santarém Polytechnic University, School of Agriculture, Quinta do Galinheiro - S. Pedro, 2001-904 Santarém, Portugal

Email:ccb@uevora.pt

Background: Fungal phytopathogens significantly reduce crop yields worldwide. Although chemical fungicides are effective, their intensive use promotes resistance, impacts non-target organisms, and raises ecological and food safety concerns. Beneficial bacteria with antifungal activity represent sustainable alternatives for plant disease management.

Methods: To uncover the genetic basis of antifungal activity, the genomes of Pseudomonas protegens MH2, P. koreensis CNI3, and Pseudomonas sp. R3, previously shown to inhibit Phomopsis sp., Botryosphaeria sp., and Harpophora maydis (unpublished data), were sequenced and annotated. Secondary metabolite biosynthetic gene clusters (BGCs) were predicted using antiSMASH, and comparative genomic analyses were performed to identify candidate metabolites potentially responsible for antagonistic activity.

Results: Strain MH2, which strongly inhibited Phomopsis sp. and Botryosphaeria sp. (≥70%), harbored diverse BGCs, including those for 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, orfamide-type lipopeptides, phenazines, hydrogen cyanide, and multiple siderophores. CNI3, active against H. maydis and Botryosphaeria sp., carried clusters for NRPS, PKS, WLIP-like lipopeptides, betalactones, siderophores, and hydrogen cyanide. R3, effective only against H. maydis, possessed a smaller set of BGCs, mainly pyoverdine-like siderophores, hydrogen cyanide, arylpolyenes, and betalactones. Unique NRPS/PKS clusters in MH2 and CNI3 likely explain their broader antifungal activity.

Conclusions: Genomic data highlight MH2 as the most promising candidate for biocontrol due to its extensive biosynthetic potential and broad antifungal activity. These findings establish a foundation for further transcriptomic, metabolomic, and functional studies to support biotechnological applications in sustainable agriculture.

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ID464 | Unveiling the Agronomic Potential of Extracellular Polymeric Substances Recovered from Aerobic Granular Sludge on Maize

Author: Sofia I.A. Pereira

Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327 4169-005 Porto, Portugal

E-mail: sapereira@ucp.pt

Other authors: Catarina Miranda1, Ana S.S. Sousa1, Philipp Wilfert2,3, Mark van Loosdrecht2, António Martins4, Paula M.L. Castro 1, Catarina L. Amorim1

1Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327 4169-005 Porto, Portugal

2Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van der Maasweg 9, Delft, 2629 HZ, The Netherlands

3University of Applied Sciences Lübeck, Urban Water Management, Mönkhofer Weg 239 in 23562 Lübeck, Germany

4Águas do Algarve S.A., 8000-302, Faro, Portugal

Background: Extracellular polymeric substances (EPS) recovered from waste aerobic granular sludge (AGS) generated during wastewater treatment represent a promising route for resource recovery. As valuable biopolymers, EPS have garnered special attention across multiple sectors, particularly agriculture, although their benefits in this field remain understudied. This study assessed the agronomic potential of EPS recovered from three AGS sources, including two full-scale wastewater treatment plants in Utrecht (EPS_U) and Faro (EPS_F), and a laboratory-scale reactor (EPS_R), as soil amendments.

Methods: A greenhouse pot experiment was conducted to evaluate the effects of two EPS doses (0.5% and 1% w/w) on maize growth, nutrient uptake, and soil enzyme activities, compared with vermicompost at the same doses and a non-amended soil (control).

Results: Both the source and dose of EPS strongly influenced their performance, with EPS often outperforming vermicompost. Notably, application of 1% of EPS, regardless of the source, markedly enhanced maize chlorophyll content, with EPS_R at 1% increasing it by 62% compared to 1% vermicompost and by 105% compared to the control. Similarly, applying 1% of EPS_R significantly increased shoot fresh weight and stem thickness. Nutritionally, 1% of EPS_R and 1% of EPS_F enhanced magnesium and potassium contents in shoots by 46% and 34%, respectively. Regarding soil enzymatic, EPS_U_1% greatly stimulated urease activity and fluorescein diacetate hydrolysis, while EPS_R_1% maximized acid phosphatase activity, underscoring the role of EPS in fostering a healthy rhizosphere.

Conclusions: Using EPS as soil amendments offers a viable alternative to conventional fertilizers, while their recovery for agricultural use supports circular economy principles within the water sector.

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ID470 | Biological potential of insect raw materials for sustainable cosmetics

Author: Ana Rita Silva

Solfarcos - Soluções Farmacêuticas e Cosméticas, Lda., Avenida da Imaculada Conceição, 4700-034, Braga, Portugal

E-mail: ars@solfarcos.com

Other authors: Diana Guimarães1, Joana Cunha1 e Artur Cavaco-Paulo1

1 Solfarcos - Soluções Farmacêuticas e Cosméticas, Lda., Avenida da Imaculada Conceição, 4700-034, Braga, Portugal

Background: Cosmetic industry is seeking sustainable alternatives to replace petrochemical ingredients and support the green transition. Insects offer a promising source of biodegradable bioactive compounds that align with environmental goals while providing skin benefits. This study evaluated the biological properties of Acheta domesticus (AD) extracts and Hermetia illucens (BSF) chitosan microparticles for cosmetic applications. This study is part of the InsectERA Mobilizing Agenda.

Methods: AD extracts (whole-ground and flour) were obtained by mechanical homogenization with four green solvents, followed by filtration. BSF chitosan (90% deacetylation, 95% purity, and medium molecular weight) was prepared at 8 mg/ml under the same conditions; commercial chitosan and the solvents alone were used as controls. BSF chitosan microparticles were characterized by dynamic light scattering. Antioxidant activity was assessed using BQC Redox voltammetric system. Cellular assays were performed on human keratinocyte (HaCaT) and fibroblast (BJ-5ta) cell lines for the AD extracts and on HaCaT and human dermal papilla (HFDP) cell lines for chitosan microparticles. Cell viability and proliferation were evaluated by MTS assay after 48h. Wound healing and collagen production of AD extracts were assessed in BJ-5ta cells by a 48h migration assay. Anti-inflammatory activity was evaluated by ELISA, measuring IL-6 and IL-8 secretion after 24h.

Results: AD extracts showed no cytotoxicity in HaCaT cells up to 100 µg/mL, stimulated collagen production similarly to TGF-β, and exhibited antioxidant activity comparable to vitamin C. No anti-inflammatory activity or enhancement of wound healing activity was observed. Only the BSF chitosan formed positively charged microparticles with an average size of 1180±192 nm and a polydispersity index > 0.3. BSF chitosan showed no cytotoxicity at low concentrations nor antioxidant or proliferative effects. BSF chitosan in solvent 1 (1% v/v) reduced IL-8 and IL-6 secretion in HFDP cells by 21.6% and 37.9%, respectively, and in solvent 3 (0.25% v/v) inhibited IL-8 by 27.3% in HaCaT cells.

Conclusions: Overall, AD extracts and BSF chitosan showed biological potential, combining antioxidant, collagen stimulating, and anti-inflammatory properties. These findings highlight insects as raw material that can be subjected to green processes to yield bioactive and sustainable ingredients for cosmetic formulations.

ID474 | Flexizyme: biotechnological production of fatty amines from side stream materials

Author: Annabel Serpico

Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE) Carrer d'Albert Einstein, 1, 46980 Paterna, Valencia, Spain

E-mail: annabel.serpico@itene.com

Other authors: Maria Carmen Tronch,; Javier Bucho; Rosa Domenech

Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE) Carrer d'Albert Einstein, 1, 46980 Paterna, Valencia, Spain

FLEXIZYME is a CBE-JU funded project (2024–2028) that demonstrates how industrial biotechnology can unlock sustainable and economically viable pathways for the production of fatty amines (FAs) from renewable biowaste [1]. FAs are key intermediates in cosmetics, detergents, coatings, and agriculture [2,3], yet their conventional fossil-based “nitrile route” is resource-intensive and environmentally damaging [4–6]. FLEXIZYME proposes a biotechnology-based alternative positioned at the interface of green chemistry and circular bioeconomy [7–9].

The project integrates enzyme discovery with process design to develop a robust biocatalytic platform. Central to this approach is a custom-designed carboxylic acid reductase–transaminase (CAR–TA) cascade capable of converting underutilised feedstocks into high-value FAs under mild, scalable conditions [10]. Early screening of 26 CARs with 11 fatty acid substrates (C4–C18) has already identified promising enzyme–substrate pairs, enabling selective and efficient biochemical transformations through tailored reactor engineering.

Feedstocks are sourced from agrifood and oleochemical residues, including palm oil distillate, high-oleic sunflower oil, coconut oil distillate, and protein-rich co-streams from lentil and chickpea processing. Their valorisation supports waste minimisation, resource efficiency, and new bio-based value chains [2]. If successful, the project will deliver a process achieving up to 80% conversion yields from bio-based side streams, while reducing greenhouse gas emissions by 30% and waste generation by 40% compared to conventional methods [1].

Preliminary life cycle assessment (LCA) results confirm the superior environmental performance of the enzymatic route over both the nitrile process and waste-to-energy options [5,6]. Additionally, secondary process residues are repurposed into new products, reinforcing the project’s biocircular approach [2].

By bridging enzyme innovation, process scalability, and circular feedstock integration, FLEXIZYME exemplifies how bio-based industries can replace petrochemical pathways with sustainable, high-performing alternatives. The project highlights the potential of industrial biotechnology to strengthen European resilience in chemical manufacturing and accelerate the transition towards a climate-neutral bioeconomy [1,9,10].

ID491 | From Waste to Value: Sequential Recovery of Bioactive Fractions from Orange Pomace

Author: Miguel Neto

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã Portugal; Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125, Turin

E-mail: miguel.leite.neto@ubi.pt

Other authors: Carlotta Valle2, Giorgio Grillo2, Ângela Sousa1, Silvia Tabasso2, Joana Rolo1

1 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã Portugal;

2 Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125, Turin

Background: Orange pomace, the main solid residue from citrus juice production, accounts for 50–60% of the fresh fruit mass and represents a large-scale agro-industrial waste with underexploited potential as a source of valuable volatile and phenolic compounds. With global orange production exceeding 70 million tons per year, the management of this vast residue poses significant environmental challenges, including waste disposal and greenhouse gas emissions. Valorising orange pomace through sustainable extraction of bioactive compounds can contribute significantly to waste reduction and resource efficiency.

Methods: An integrated green extraction strategy was developed for sequential recovery, enrichment, and biological evaluation of bioactive fractions from orange pomace. Microwave-assisted hydrodistillation (MAHD) was used to extract essential oil (EO) and hydrolates. The aqueous phase from maceration (water maceration, WM) and the residual biomass were processed using Microwave-assisted Subcritical Water Extraction (MASWE) and Ultrasound-Assisted Extraction (UAE), with systematic optimization of key parameters such as temperature, pH, and extraction time. Extracts were characterized by GC-MS and LC-MS, while enrichment of polyphenols was performed using adsorbent resins.

Results: The EO yield was 0.59% (w/w, dry basis), with limonene as the major component, while hydrolates presented linalool as the predominant volatile. Preliminary evaluation of total phenolic content (TPC) and antioxidant activity (DPPH assay) revealed superior results for MASWE extracts

compared to UAE. Resin treatment effectively reduced sugar content and enriched flavonoids, increasing polyphenol concentration, as revealed by LC-MS. Preliminary cytotoxicity assays in HeLa and HaCaT cell lines demonstrated a favourable safety profile for the enriched extracts.

Conclusions: This integrated extraction approach provides a sustainable and scalable route to valorize orange pomace by recovering multifunctional natural ingredients. The strategy supports circular economy principles and promotes environmentally responsible innovation in agro-industrial waste management, with potential applications in pharmaceutical, cosmetic, and food industries.

ID576 | Extremotolerant, Specialised Hydrocarbon-Degrading Fungi in Resin-Rich Soil

Author: Ângela Pinheiro

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), 2780-157 Oeiras, Portugal

E-mail: apinheiro@itqb.unl.pt

Other authors: Tiago Martins1, Adélia Varela2, Patrícia Domingos1, Rita Escórcio1, Artur Bento1, Isabel Martins1, Carlos A M Afonso3, Cristina Silva Pereira1

1 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), 2780-157 Oeiras, Portugal;

2 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), 2780-157 Oeiras, Portugal;

3 Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Background: Resin-rich soils constitute chemically selective environments where high concentrations of resin acids act as potent filters. These niches remain largely unexplored for their fungal diversity, despite potential as reservoirs of novel lineages with biotechnological relevance. We investigated whether five decades of Pinus resin contamination in soil from an abandoned resin refinery, had shaped the fungal community by selecting for hydrocarbon-degrading taxa.

Methods: Resin-rich soil (RS) was collected from the refinery location and compared with adjacent forest soil (FS). Physicochemical and microscopic analyses were conducted to evaluate resin content, nutrient status, and fungal structures. Resin acids were quantified by nuclear magnetic resonance (NMR), and the degradation of abietic acid was tested in synthetic soil pre-inoculated with RS fungal extracts. High-throughput ITS2 sequencing was used to characterise fungal communities, and predictive tools were used to assign taxonomic groups and make functional inferences.

Results: RS showed strong chemical limits, with high concentration of organic carbon, abundant resin acids, and reduced water activity. Despite these conditions, scanning electron microscopy (SEM) and incubation assays showed the presence of metabolically active fungi. RS fungal extracts degraded abietic acid in vitro, suggesting a potential for hydrocarbon metabolism. Sequencing revealed a fungal community in RS comprising 620 operational taxonomic units (OTU) across six phyla, with a significant share of poorly classified or novel taxa. Key taxa comprised resinicolous specialists such as Sorocybe and Sarea, and hydrocarbon-associated fungi, including the abundant Candida keroseneae and the ecologically significant degrader Amorphotheca resinae. Diversity analyses highlighted marked taxonomic turnover and clear compositional differences between RS and FS. Comparisons with global soil datasets placed RS at the lower boundary of fungal diversity yet compositionally distinct, with minimal overlap with regional soils.

Conclusions: Long-term Pinus resin contamination generates a taxonomically unusual mycobiome enriched in resin-tolerant and hydrocarbon-degrading taxa. Resin-rich soils, and possibly resin-rich environments in general, emerge as chemically selective hotspots of fungal novelty and resilience, with implications for understanding microbial adaptation to toxic environments.

ID579 | ShK-like Peptides from Entomopathogenic Nematodes: New Molecular Leads for Bioinsecticide Development

Author: Tiago Paiva

Center of Biotechnology Azores, 9500-321, Portugal

E-mail: tmdp92@gmail.com

Other authors: Duarte Toubarro 1, Jorge Frias 1, João Vicente 2 and Nelson Simões 1

1 Centro de Biotecnologia dos Açores, Universidade dos Açores. Ponta Delgada;

2 Applied Protein Biochemistry, Universidade Nova de Lisboa. Lisboa

Background: Virulence factors of entomopathogens are a source for insecticides. In our laboratory we have a genetic library of the complex nemato-bacteria, Steinernema carpocapsae – Xenorhabdus nematophila, that is currently used in biological control. This library contains genes encoding for adhesion, invasion, immune evasion and toxic factors. We are exploring cysteine-rich peptides that are well represented among the toxic factors with the goal to design peptides more specific to a target pest.

Methods: Characterization of the molecular sequence and 3D structures predicted by AlphaFold. The structures of D. melanogaster ion channels were predicted with SWISS-MODEL. Molecular docking was carried out using rigid-body docking with ZDOCK and semi-flexible docking with HADDOCK 2.4. Heterologous expression in E. coli and site-directed mutagenesis were used to produce the K20A and K31A mutants. Insecticidal activity was tested in Drosophila via injection. Sublethal effects were monitored using the Ethoscope platform.

Results: In this experimental approach we choose Drosophila as the target insect and the cysteine-rich peptide, named Sc-ShK3, from our library, that was predicted to be toxic by the ToxiPred and confirmed in vivo using recombinant peptide. Docking analysis allowed the identification of Shaker between the eight Kv channels in Drosophila as the target. In vivo assays, the treatment of Drosophila with Sc-ShK3 induces phenotypic modifications that resemble those of Drosophila with knock out Shaker. Thus, we assumed that our peptide is active in Shaker channel. Furthermore, docking analysis identified two residues of the peptide, residues Lys 20 and Lys31, interacting with the pore of the Shaker with a binding energy of -7.5 kcal/mol and -8.0 kcal/mol, respectively. To validate this protein – protein interaction we produced Sc-ShK3 mutated in the Lysine of the two clusters. In vivo essays comparing the activity of mutants with wild peptide we showed the reduced toxicity of the mutants: wild peptide induced 74.1 ± 20.56% mortality, whereas the K21A and K31A mutants induced 52.2 ± 20.53% and 48.2 ± 16.78% mortality, respectively. Furthermore, the mutation in the Lys31 reduces more significantly toxicity than Lys 20, in accordance with the calculated binding energy.

Conclusions: These results confirm the value of our library to exploit bioactive molecules and support our goal in engineering insecticidal molecules to improve toxicity and specificity.

ID588 | KEGG resolved microbiome contrasts in invasive brown algal holobionts reveal complementary bioprocessing pathways and actionable mitigation strategies

Author: Raul Bettencourt

Faculty of Science and Technology. University of the Azores, 9900-862 Horta, Portugal

E-mail: raul.s.bettencourt@uac.pt

Other authors: João Moreira

Faculty of Science and Technology. University of the Azores, 9900-862 Horta, Portugal

Background: Marine macroalgae host diverse and dynamic microbial communities collectively referred to as the algal microbiome. These microbiomes play essential roles in modulating algal physiology, mediating nutrient cycling, supporting host health, and sustaining ecosystem functioning. The structure and composition of algal-associated microbial communities are shaped by host identity, environmental variables, and physicochemical conditions, resulting in species-specific assemblages with functional diversity. Yet, these algal microbiomes remain understudied compared to planktonic microbes, especially in the context of invasive macroalgae and their biotechnological potential.

Methods: We investigated the microbiomes of two invasive brown algae—Rugulopteryx okamurae and Sargassum spp.—through shotgun metagenomic sequencing of surface-associated DNA, followed by functional annotation using KEGG, a global catalog of biological pathways. Microbial genes were grouped into KEGG pathways, and relative abundance (TPM) was computed separately per host. To prioritize biotechnology-relevant functions, we curated a literature-informed shortlist of KEGG pathways linked to marine applications (e.g., polysaccharide degradation, quorum sensing, vitamin Bnn biosynthesis, detoxification). We then ranked the Top 25 pathways per holobiont, with conservative KO handling to avoid overrepresentation. Functional presence was interpreted as capacity—not guaranteed activity or yield.

Conclusions: The Rugulopteryx holobiont is energy-forward, enriched in oxidative phosphorylation, propanoate metabolism, and carbon fixation—traits that enable efficient ATP production and carbon capture, supporting dense, persistent biofilms and bioprocessing routes such as PHA (bioplastic) and ethanol production. In contrast, the Sargassum holobiont is interface-and movement-optimized, showing elevated expression of flagellar assembly, secretion systems, and porphyrin metabolism—enabling host colonization, motility, and adaptation across habitats. Both holobionts share core metabolism and invasion-enabling traits (e.g., quorum sensing, motility, nutrient cycling, vitamin Bnn supply, xenobiotic tolerance), but differ in specialization: Rugulopteryx suits sugar-based bioproducts, while Sargassum favors alginate valorization and biocatalysis. These KEGG-resolved contrasts inform microbiome-aware mitigation and circular bioeconomy strategies in coastal environments.

ID605 | Characterisation of the gastrointestinal microbiome from Catrina cattle and Holstein Friesian breeds under different diets

Author: Ana Rita Azevedo

University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal

E-mail: ana.rf.azevedo@uac.pt

Other authors: Artur da Câmara Machado1, Ricardo Franco Duarte2, Alfredo Borba3, Duarte Mendonça1, Maria Susana Lopes 1

1 University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal;

2 Centre of Molecular and Environmental Biology (CBMA), Biology Department, Minho University, Campus de Gualtar, 4710-057 Braga, Portugal;

3 University of the Azores, Institute of Agricultural and Environmental Research and Technologies (IITAA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal

Background: The gastrointestinal (GIT) microbiome houses a highly complex microbiota responsible for plant biomass degradation and energy production for ruminants. Comparative studies between native and high-production breeds that assess how diet and breed influence microbial functions are rare. The present study evaluates the GIT microbiome of two breeds, under the influence of two distinct feeding regimes: the Catrina, a rustic native Azorean breed; and the Holstein-Friesian (HF), a high-productivity dairy breed.

Methods: Whole-genome shotgun sequencing was used to characterise microbial communities in rumen and faecal samples, under two diets: 1) a pasture-based diet; 2) a total mixed ration including corn and grass silage. Throughout the bioinformatics pipelines, the most consistent results were selected for further analysis.

Results: Our data reveal a highly complex and dynamic GIT microbiome whose composition was significantly structured across host, diet, and sample type. Read-based profiling provided a broad taxonomic overview, detecting 361 phyla, 457 classes, 927 orders, 2370 families, 3555 genera, and 3121 species. Metagenome Assembled Genome (MAG)-derived reconstructions identified 2 domains, 11 phyla, 18 classes, 23 orders, 34 families, 46 genera, and 74 species. At species level, clear breed-specific differences were observed. Catrinas were enriched in methanogenic and fibrolytic taxa, e.g. Methanobrevibacter spp., Fibrobacter succinogenes, and Ruminococcus spp. In contrast, HF were characterised by higher abundances of Bifidobacterium, Prevotella, alongside protozoan like Babesia bigemina. MAGs reinforced breed-specific signatures, in particular with Methanobrevibacter thaueri being exclusive to the Catrinas. On the other hand, HF were characterised by significantly higher levels of Bifidobacterium spp..

Conclusions: This study elucidated the complex interplay between host genetics and the microbial community structure in the bovine GIT. The Catrina breed, particularly under semi-natural pasture, harboured a more methanogenic microbiome diversity, potentially linked to lower CH4 production. While HF exhibited signatures of saccharolytic and propionate-producing bacteria under mixed diets, supporting improved energy capture. These distinct microbial signatures are stable across diets and sample types, suggesting strong breed-specific microbiome structuring with potential implications for rumen function and CH4 emissions.

ID658 | Bridging Knowledge Gaps in Antimicrobial Resistance Dynamics at Animal-Environment Interfaces

Author: Mónica V. Cunha

Center for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

E-mail: mscunha@fc.ul.pt

Other authors: Inês Mendes1, Daniela Pinto1§

1Center for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal.

§Current affiliation: Phage Biology Research and Infection Control (PhaBRIC), Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Background: Antimicrobial resistance (AMR) is a growing global health challenge. While AMR dynamics have been well characterized in intensively managed livestock, less is known in extensive husbandry systems and wildlife. Escherichia coli, a commensal bacterium with zoonotic potential, serves as an ideal model organism to study AMR across diverse hosts and environments. This study thus aimed to enhance our understanding of *E. coli* transmission dynamics at the animal-environment interface.

Methods: A total of 178 samples were collected from cattle, wild boar, red deer, and environmental sources across Portugal. E. coli was isolated and analyzed for colonization rates, pathogenicity, and AMR profiles. Antibiotic susceptibility was assessed for a range of clinically relevant antibiotics. Whole-genome sequencing (WGS) was performed on selected isolates to examine their resistome, virulome, and mobile genetic elements (MGEs). Phylogenetic analysis investigated genetic relatedness and transmission pathways, integrating data from different hosts and regions.

Results: *E. coli* was detected in 92.4% of the samples, with a low prevalence of Shiga toxin-producing *E. coli* (STEC) strains (2.9%). Phenotypic AMR was infrequent, with azithromycin and tetracycline resistance being the most common. Multidrug resistance (MDR) was limited, but resistance to critically important antibiotics such as colistin was sporadically detected. WGS revealed several resistance genes, including β-lactamases, and virulence genes associated with pathogenic *E. coli* (e.g., ehxA, stx1, stx2, eae). Phylogenetic analysis indicated a high degree of genetic relatedness between E. coli isolates from different hosts and regions, suggesting that transmission is facilitated by animal movement, human activity, and environmental contact.

Conclusions: This study underscores the complex transmission dynamics of E. coli at the livestock-wildlife-environment interface, where both commensal and pathogenic strains coexist. Despite the overall low prevalence of AMR and pathogenic E. coli in wildlife and extensively managed cattle, resistance to critical antibiotics like colistin and the detection of transmission events across hosts and environments advocate for integrated AMR surveillance across ecosystems.

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ID666 | Assessing *endogenous cyanobacteria* and microalgae potential as a sustainable solution for agriculture

Author: Pedro Cruz

1 CIIMAR- CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research , University of Porto, Matosinhos, Portugal;

2 FCUP – Faculty of Sciences of the University of Porto, University of Porto, Porto, Portugal;

3 CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro,Vila Real, Portugal

E-mail: pcruz@ciimar.up.pt

Other authors: Guilherme Scotta Hentschke1, André Silva1, Conceição Santos2,4, Vítor Vasconcelos 1,2

1 CIIMAR- CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research , University of Porto, Matosinhos, Portugal;

2 FCUP – Faculty of Sciences of the University of Porto, University of Porto, Porto, Portugal;

4LAQV/REQUIMTE, Faculty of Sciences of University of Porto, Porto, Portugal

Background: Global population is estimated to reach 9.8 billion by 2050, significantly increasing the demand for food production. To meet this growing need and mitigate losses caused by abiotic and biotic stresses, agriculture has relied on chemical products. However, the widespread and prolonged use of these inputs has contributed to soil degradation, water contamination, and the emergence of resistant pathogenic microorganisms. In response to these challenges, European Union has stopped approving chemicals for agricultural, pressuring the need for sustainable, biologically based solutions. Cyanobacteria and microalgae are photosynthetic organisms, rich bioresources of natural compounds with great potential for different applications in agriculture.

Methods: Soil and tree bark samples were collected from crops in Northern Portugal and enriched in different culture media. Cyanobacteria and microalgae were isolated, and some were selected morphologically to evaluate their potential. The selected strains were scaled up, centrifuged, and freeze-dried. To assess biostimulant potential, the biomass of 18 strains was resuspended in water and sonicated. Initial assays were conducted in vitro using Petri dishes, Solanum lycopersicum seeds, and crude extracts applied at 10 µg/mL. After 7 days of incubation, roots were photographed and measured. For biocontrol assays, biomass from 27 strains was extracted using Accelerated Solvent Extraction under pressure and temperature with three solvents. The resulting extracts were dried and resuspended in either water or dimethyl sulfoxide. Xanthomonas euvesicatoria, Pseudomonas syringae pv. actinidiae, and Erwinia amylovora were exposed to the extracts at 100, 200, and 400 µg/mL in 96-well plates.

Results: Around 100 strains of microalgae and cyanobacteria were isolated. Some crudes showed biostimulant potential resulting in bigger roots than a biostimulant on the market. 12 out 81 extracts reduced bacterial viability over 50% but only one of them had this effect on the three bacteria.

Conclusions: Cyanobacteria and microalgae are a promising sustainable solution for agriculture either as biostimulants or biocontrol agents. Future assays comprise in planta assays to analyse other parameters for biostimulants and antifungal assays.

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ID673 | Viral Diversity in Native Plants from the Azores

Author: Sara Luna

CBA-Biotechnology Centre of Azores, University of the Azores, Angra do Heroísmo, Azores, Portugal

E-mail: sara.pb.luna@uac.pt

Background: The Azores archipelago, characterized by its unique biodiversity, is home to numerous native plant species that play a crucial role in the island ecosystems. However, this rich botanical heritage faces significant threats from various factors, including habitat loss, climate change, and the increasing prevalence of plant viral diseases, which can lead to the decline or extinction of vulnerable species. Viruses are widespread in wild ecosystems, however, the role of introduced and indigenous viruses on native plant communities remains understudied and has not been taken into consideration in conservation programs.

Methods: Angelica lignescens, Azorina vidalii, Deschampsia foliosa, Rubus hochstetterorum and Vaccinium cylindraceum, were selected from several populations in two islands of the Azores, Terceira and Flores. Leave samples were collected, without regarding to symptoms, and used for total RNA extraction and deep-sequencing of virus-derived small RNAs. Bioinformatic tools were used to remove plant-derived reads, to perform DeNovo assembly of reads into contigs, whichwere compared with NCBI database (Blastn, BlastX). The resulting viral genomes were used to design primers for diagnostics by RT-PCR, to confirm their presence and distribution, and whenever needed Sanger sequencing and RACE were used to obtain partial or complete sequences of the viruses. Multiple nucleotide and amino acid sequence alignments were performed to infer their phylogenies.

Results: A. vidalii is infected with crop viruses such as Cucumber mosaic virus and Tomato spotted wilt virus and a few putative novel viruses (Marafivirus) (Luna et al., 2023); A. lignescens (Apiaceae) is infected with a putative novel chordovirus closely resembling a virus known to infect wild carrots, to which we named Angelica chordovirus (Luna et al., 2025); other putative novel viruses are being molecularly characterized in D. foliosa and R. hochstetterorum.

Conclusions: Our results provided for the very first-time insights into the presence of viruses in Azorean endemic plants, including important viruses in the agroecosystems. In addition to the apprehension about the risk to native plant biodiversity, these plants could potentially serve as reservoirs, playing a role in the emergence of novel viruses in crops. This emphasizes the necessity for continued research to unravel the dynamics of these novel viruses' transmission, its repercussions, and potential broader ecological implications

ID217 | Plasmid diversity: what do we miss when we only look at the majority?

Author: João S. Rebelo

CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal

E-mail: joaorebelo_4@hotmail.com

Other authors: Célia P. F. Domingues1,2, Luís Borda-de-Água3,4,5, Teresa Nogueira1,2, Francisco Dionisio1

1CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal;

2 INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal;

3 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig; Puschstraße 4, 04103 Leipzig, Germany;

4 Institute of Biology, Martin Luther University Halle-Wittenberg; Am Kirchtor 1, 06108 Halle (Saale), Germany;

5 Centro de Ecologia Aplicada Professor Baeta Neves (CEABN), Instituto Superior de Agronomia; Tapada da Ajuda, 1349-017 Lisboa, Portugal

Background: In bacterial communities, a few dominant species typically coexist with a large number of rare taxa. Interestingly, these rare bacteria often perform critical functions within the community, with impacts that extend far beyond their low abundance. In some cases, essential community-level functions are carried out exclusively by these rare taxa. While bacterial abundance dynamics are well characterized, the distribution of plasmids within such communities remains largely unexplored.

Methods: In this study, we analyzed a dataset comprising approximately 53,000 plasmids from the curated RefSeq database. For each plasmid, we characterized the diversity of coding regions, resistance and virulence genes, mobility type, and the phylogeny of their hosts. Plasmids were clustered into Plasmid Taxonomic Units (PTUs), in which genetically similar plasmids are grouped together.

Results: Plasmids also exhibit a highly uneven distribution, with a few PTUs being highly prevalent while the majority remain rare. This distribution does not directly mirror that of their hosts: some plasmids from common hosts are rare, while others from less common hosts are common. Interestingly, although plasmids are not living organisms, their distribution patterns resemble those reported for other groups, including insects and arachnids. The success of plasmids within common PTUs can be partly attributed to their higher mobility. On the other hand, plasmids from rare PTUs display greater genomic diversity, including virulence genes, and have a broader host range.

Conclusions: Our results suggest that rare plasmids may function as a genetic reservoir, potentially harbouring genes of relevance for health, biotechnology, and other fields. Due to their diversity, these plasmids can be rapidly selected and increase in frequency under changing environmental conditions, thereby playing a crucial role in bacterial evolution.

POSTERS

ID195 | Impact of selected parabens and related disinfection by-products in biofilms formed by bacteria isolated from drinking water

Author: José Carlos Teixeira

1LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
2ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

E-mail: up202410671@up.pt

Other authors: Manuel Simões1,2, Inês Bezerra Gomes1,2

1LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
2ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Background: Water is a very valuable asset for human life, but high-quality drinking water is becoming scarce due to the presence of biological and chemical contaminants, including emerging contaminants[1] (ECs). Disinfection by-products (DBPs) are commonly formed in water treatment plants due to the oxidative action of disinfectants, such as chlorine. The reaction of ECs with these agents may also result in the formation of parabens-related DBPs (p-DBPs), which have a higher impact on human health than the parent compounds. The presence of biofilms in drinking water distribution systems is unavoidable, but limited scientific knowledge on the effects of parabens and their products of disinfecting by-products (p-DBPs) on microbial communities, especially in drinking water, is scarce[2].

Methods: Strains of Stenotrophomonas maltophilia isolated from drinking water distribution systems in Portugal were used to assess their behaviour in planktonic and biofilm states (using polyvinyl chloride–PVC as an adhesion surface) from the exposure to Methylparaben (MP), Ethylparaben (EP) and p-DBPs such as para-hydroxybenzoic acid (pHBA), 3,5-dichloroethylparaben (3,5diClEP), and 3,5-dichloromethylparaben (3,5diClMP) at 50ng/L. Biofilm was analysed for culturability by CFU, cell density by DAPI, EPS content by polysaccharides and protein quantification. For planktonic analysis, a growth curve was done in the presence of parabens and p-DBPs during 24h at 610nm.

Results: pHBA increases polysaccharides (10.5µg/cm2), followed by EP (10.37µg/cm2) and MP (9.98µg/cm2). PHBA also raises protein (6.5µg/cm2), but less than EP and MP (7.07 and 8.01µg/cm2, respectively). 3,5diClMP and 3,5diClEP show decreased polysaccharides but increased proteins, with 3,5diClEP having the highest protein (16.6µg/cm2) and a significant cell density reduction (-0.07 log cells/cm2), followed by pHBA. In planktonic mode, parabens boost bacterial growth; biomass rises ~30%, and growth rate ~10% with parabens and p-DBPs as carbon sources. 3.5diClMP and 3.5diClEP undergo more significant changes than their parent compounds. This is concerning, given that they are produced during chlorine water treatment.

Conclusions: Paraben exposure affects biofilms of S. maltophilia isolated from drinking water. The impact of parabens on microbial communities becomes more significant in planktonic form raises concerns about the potential threat to the microbiological quality and safety of drinking water.

ID211 | Biofouling and Buoyancy: Assessing How Marine Colonization Alters Plastic Debris Dynamics

Author: Patrícia Branco

BIORG—Bioengineering and Sustainability Research Group, Universidade Lusófona, Av. Campo Grande 376, Lisboa, 1749-024, Portugal

E-mail: patricia.branco@ulusofona.pt

Other authors: Rúben Almeida; Hilda de Pablo

Universidade Lusófona, Av. Campo Grande 376, Lisboa, 1749-024, Portugal; BIORG—Bioengineering and Sustainability Research Group, Universidade Lusófona, Av. Campo Grande 376, Lisboa, 1749-024, Portugal

Background: Plastic pollution in marine environments poses a major threat to ocean ecosystems, yet the role of biological colonization—known as biofouling—in altering the physical properties of plastics remains underexplored. Biofouling can affect plastic buoyancy, degradation, and transport dynamics, influencing how and where plastics accumulate in the ocean. Understanding these interactions is essential to better predict the fate of marine plastics and develop effective mitigation strategies.

Methods: This study aimed to evaluate the impact of biofouling on the buoyancy of plastics by submerging PVC discs in the Marina of Oeiras, Portugal, over varying time periods. Visual inspection, stereoscopic and microscopic observations, weight measurements, DNA analysis for organism identification, and density assays using a density column were conducted to assess the extent of biological colonization and its physical effects.

Results: Results revealed a clear ecological succession: initial colonization by generalist species was followed by more specialized and complex organisms, leading to a progressive increase in the discs weight. After two months, the dry weight of colonized discs increased by an average of 17.75% compared to uncolonized controls. Sanger sequencing analysis identified known plastic-degrading microorganisms, including Pseudomonas, Raoultella (bacteria), Fusarium and Cladosporium (fungi), confirming active microbial interaction with the plastic surface. Although no visible change in density was observed during the study period, all discs maintained a density below 0.8 g/mL, suggesting that longer exposure may be required to detect significant buoyancy changes

Conclusions: These findings underscore the importance of biofouling in modifying plastic properties in the marine environment and highlight the need for continued research to better understand its implications for plastic persistence and distribution in ocean ecosystems.

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ID225 | "Actinomycetota from Caves in Bosnia and Herzegovina: Diversity and Bioactivity Assessment through the OSMAC Approach"

Author: Sofia Correia

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

E-mail: sofia.correia@ciimar.up.pt

Other authors: Lada Bilela 3 , Ralph Urbatzka1 and Maria de Fátima Carvalho1,2

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 UNSA - University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina

Members of the phylum Actinomycetota, commonly known as actinobacteria, are prolific producers of bioactive compounds. In recent years, cave-dwelling actinobacteria have attracted significant interest as promising sources of novel bioactive molecules, due to the unique characteristics and microbial diversity of these isolated ecosystems. Actinomycetota are well known for harboring multiple biosynthetic gene clusters (BGCs) within their genomes, enabling the production of diverse natural products (NPs), though many remain silent under standard laboratory cultivation conditions. In recent years, the OSMAC (One Strain–Many Compounds) approach has emerged as a powerful strategy to activate these cryptic BGCs by altering cultivation parameters, and has led to the discovery of numerous novel bioactive compounds.

This study focused on investigating the diversity and bioactive potential of Actinomycetota associated with cave environments across Bosnia and Herzegovina. Biodiversity was assessed through culture-dependent methods, by treating the samples at 60 ºC for 15 min before being cultured on selective media for actinobacteria, namely SCN and TWYE. Taxonomic identification of the isolates was performed through 16S rRNA gene sequencing.

To investigate the bioactive potential, 10 selected Actinomycetota strains were subjected to the OSMAC approach. Three different growth conditions were applied to each isolate: i) addition of Escherichia coli lysate, ii) addition of lanthanum chloride solution and iii) adjustment of the medium to pH 10 . The cultures obtained under these conditions were extracted twice with a mixture of acetone and methanol and the resulting extracts were analysed for their antimicrobial, anticancer and anti-inflammatory.

A total of 208 actinobacterial strains were isolated from 11 samples collected in caves across Bosnia and Herzegovina, spanning 17 genera, including several rare genera, with nine of these strains representing potential novel species.

Bioactivity assays of the 10 Actinomycetota strains grown under OSMAC conditions, revealed antimicrobial, anticancer, and anti-inflammatory potential in 35 extracts.

This study underscores the potential of caves as rich reservoirs of diverse and novel actinobacteria, and shows that the OSMAC approach promoted the antimicrobial, anticancer and anti-inflammatory activities of the studied strains, supporting its value in the search for bioactive molecules with biotechnological relevance.

ID228 | Assessment of Extracellular Polymeric Substances in Activated Sludge Using India Ink, Image Analysis, and Neural Networks

Author: Daniela Mesquita

CEB – Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal; LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

E-mail: daniela@ceb.uminho.pt

Other authors: Antonio Melo, Joana Costa, Eugénio Campos Ferreira

CEB – Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal; LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Extracellular polymeric substances (EPS), including polysaccharides (PS), proteins (PN), and humic acid substances (HAS), that compose loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), are crucial for the structural and functional properties of activated sludge (AS). Traditional methods for EPS quantification are time-consuming and labour-intensive. This study explores an innovative approach that combines India Ink staining, quantitative image analysis (QIA), and artificial neural networks (ANNs) to efficiently assess EPS concentrations across various sample preparation methods.

Methods: AS samples were used fresh, washed with PBS solution, and preserved with PBS and ethanol before India ink staining. Quantitative features extracted from QIA were used as input variables for ANNs. The models were trained and validated on separate datasets, with performance evaluated using mean absolute error, mean squared error, root mean squared error, standard deviation, coefficient of determination (R^2), and residual prediction deviation (RPD).

Results: Fresh samples showed the best performance for all EPS components (Global R^2=0.80-0.93, RPD=2.14-3.72), particularly for PN-TB (R^2=0.92, RPD=3.72). Preserved samples exhibited moderate results (R^2=0.70-0.86, RPD=1.81-2.63), while washed samples had the least consistency (R^2=0.56-0.86, RPD=1.50-2.50). The ANN achieved excellent PS assessment in fresh samples (PS-LB R^2=0.89, RPD=3.18) but showed limitations for HAS in preserved samples (HAS-TB R^2=0.70, RPD=1.81). LB, TB, and Total EPS prediction were most reliable in fresh samples (R^2=0.86-0.90, RPD=2.86-3.20) versus washed samples (R^2=0.60-0.78, RPD=1.50-2.12) and preserved samples (R^2=0.74-0.84, RPD=1.95-2.43). Conclusions: The use of India Ink staining, QIA, and ANNs provides a promising, rapid method for EPS quantification, with fresh samples yielding the best results. Sample preservation ensures acceptable accuracy for the majority of EPS components, while washing the samples compromises reliability. This approach can enhance EPS monitoring by offering a rapid and reliable alternative to traditional methods, reducing sample preparation time. Future work should focus on standardizing and refining protocols to ensure reliable EPS analysis while preserving matrix integrity.

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ID239 | Unraveling Cyanobacterial Mats and Microbiomes from the Bolivian Altiplano through Metabarcoding and Culture-Based Approaches

Author: Guilherme Scotta Hentschke

1CIIMAR/CIMAR-LA- Terminal de Cruzeiros do Porto de Leixões, Av. Gen. Norton de Matos, 4450-208, Matosinhos, Portugal

E-mail: guilherme.scotta@gmail.com

Other authors: Miguel Semedo1, Jimmy Ciancas2, Claudia Hoepfner3,4, Daniel Guzman3, Daniela S. Rivera4, Vitor M. Vasconcelos1,5

1CIIMAR/CIMAR-LA- Terminal de Cruzeiros do Porto de Leixões, Av. Gen. Norton de Matos, 4450-208, Matosinhos, Portugal.

2Fundación PROINPA - Av. Elías Meneces s/n Km. 4, PO Box: 4285 Cochabamba – Bolivia

3Center of Biotechnology, Faculty of Sciences and Technology, Universidad Mayor de San Simon, Cochabamba – Bolivia

4GEMA Center for Genomics, Ecology and Environment, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile.

5Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4069-007 Porto-Portugalfiliation

Background: The Bolivian Altiplano presents extreme environmental conditions, including high altitudes, intense UV radiation, low precipitation, freezing temperatures, and saline–alkaline habitats. Despite these harsh conditions, cyanobacteria thrive in the region; however, their diversity remains largely unexplored. This study aimed to investigate the morphological and 16S rRNA gene diversity of cyanobacterial mats and their associated microbiomes from the Bolivian Altiplano.

Methods: Samples were collected from saline and freshwater environments, and 16S rRNA gene amplicons were sequenced. Additionally, 40 cyanobacterial strains were isolated and the full length of their 16S rRNA gene was sequenced. Morphological analysis was carried out with light microscopy.

Results: Morphological analysis of the mats identified seven cyanobacterial morphotypes belonging to the Nostocaceae, Coleofasciculaceae, Rivulariaceae, and Microcoleaceae families. Illumina sequencing yielded 4,113 amplicon sequence variants (ASVs) for the bacterial community, including 310 ASVs identified as Cyanobacteria. Of these, 134 ASVs were classified by SILVA as Cyanophyceae, representing 32 genera. However, ML phylogeny and identity comparisons revealed 42 Cyanophyceae genera. Notably, 30 ASVs fell within 16 clades unrelated to any known cyanobacterial genus, suggesting they may represent novel taxa. Additionally, from the 40 cyanobacterial strains isolated from the natural samples, two were already identified as representatives of new cyanobacterial genera, and the others need further analyses to confirm their classification. The microbiomes associated with the mats were dominated by Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, Clostridia, Cyanophyceae, and Campylobacteria. Functional predictions based on 16S rRNA gene data indicated prevalent metabolic capabilities, including phototrophy, chemoheterotrophy, sulfur respiration, nitrogen fixation, nitrate and nitrite reduction, and fermentation. Nitrogen-fixing cyanobacteria were abundant, along with bacterial groups showing potential for bioremediation.

Conclusions: This study represents the first comprehensive survey of cyanobacterial mats and their microbiomes in Bolivia. It reveals the presence of numerous undescribed cyanobacterial taxa and highlights the remarkable, yet largely untapped, biotechnological potential of Bolivia's extreme ecosystems.

ID241 | Construction of a synthetic metabolic pathway for production of threonine from one-and two-carbon alcohols

Author: Cláudio J.R. Frazão

Institute of Natural Materials Technology, TU Dresden, 01062 Dresden, Germany

E-mail: claudio.frazao@tu-dresden.de

Other authors: Nils Wagner, T.A. Stefanie Nguyen, Thomas Walther

Institute of Natural Materials Technology, TU Dresden, 01062 Dresden, Germany

A central goal of bioeconomy consists in reducing our dependence on fossil resources by enabling sustainable biomanufacturing of bio-based fuels and chemicals1. In this context, the prospection and use of one- or two-carbon compounds, such as methanol or ethylene glycol, which can be electrochemically derived from CO2 are of high interest as alternative carbon sources2,3,4 to conventional glucose-based fermentations.

In this study, we disclose a new-to-nature metabolic pathway for the conversion of one- or two-carbon compounds into threonine, an amino acid of industrial relevance. Apart from methanol5, our pathway is compatible with the use of ethylene glycol3 due to the characteristic glycolaldehyde intermediate which is shared by pathways for the assimilation of both alcohols. This route extends the previously disclosed synthetic threose-dependent glycolaldehyde assimilation (STEGA) pathway6 for the synthesis of 2-oxo-4-hydroxybutyrate with three additional reaction steps catalyzed by homoserine transaminase, homoserine kinase, and threonine synthase. We first validated the functionality of the new pathway in an Escherichia coli strain auxotrophic for threonine, which was also employed for discovering a better-performing D-threose dehydrogenase enzyme activity. Subsequently, we transferred the pathway to producer strains and used 13C-tracer experiments to improve threonine biosynthesis starting from glycolaldehyde. Finally, extending the pathway for ethylene glycol assimilation resulted in the production of up to 6.5 mM (or 0.8 g L−1) threonine by optimized E. coli strains at a yield of 0.10 mol mol−1 (corresponding to 20 % of the theoretical yield).

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ID246 | Fast-Tracking a Slow Pathogen: Development of a Novel Culture Medium for *Xylella fastidiosa* Growth

Author: Eduardo da Costa Camilo

1 UCIBIO Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2 Associate Laboratory i4HB Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

E-mail: ec.camilo@campus.fct.unl.pt

Other authors: Tomás Martins (3); Cláudia Carvalho (1, 4); Rita G. Sobral (1, 2); Carla Pinheiro (1, 2)

1 UCIBIO Applied Molecular Biosciences Unit, Department of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;
2 Associate Laboratory i4HB Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;
3 NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;
4 PYCC – Portuguese Yeast Culture Collection, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

Background: *Xylella fastidiosa* is a xylem-limited, phytopathogenic bacterium and a growing global threat. It is listed on the EPPO A2 List of pests recommended for regulation as a quarantine organism and is responsible for serious plant diseases such as Pierce's disease in grapevines and olive leaf scorch. It causes hydraulic failure by forming biofilms that obstruct xylem vessels [1]. Once confined to the Americas, its detection in Europe (2013) and Portugal (2023) highlights the urgent need for improved research tools. The study of this pathogen is challenging due to its extremely low and restrictive growth rate in laboratory conditions (7-14 days), a limitation that hinders antimicrobial testing and physiological studies [1].

Methods: We evaluated several solid and liquid culture media to identify conditions that could support rapid and reproducible growth of *X. fastidiosa* (PYCC 9740). Bacterial proliferation was assessed over time by optical density and confirmed by PCR. The selected medium was further tested for compatibility with standard serial dilution protocols for antibiotic susceptibility profiling.

Results: Among the tested formulations, one novel medium consistently supported *X. fastidiosa* growth allowing liquid cultures to reach the stationary phase, and visible colonies to form on solid medium in just three days. Growth curves demonstrated reproducible proliferation patterns, and the purity of the culture was determined by 16S PCR. Antibiotic susceptibility testing conducted using the new medium produced results that were comparable and in some instances superior, to those reported in the literature, thereby validating its robustness [2].

Conclusions: We report a breakthrough culture system that overcomes one of the major technical barriers in *X. fastidiosa* research. This novel medium allowed to significantly increase the growth rate of these fastidious bacteria in liquid culture, facilitating physiological studies and antibiotic testing. Our findings provide a valuable tool for advancing understanding and control strategies for this emerging plant pathogen.

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ID254 | Real-time quantification of S-EPS in cyanobacterial cultures: a fast and reproducible alternative to extraction-based methods

Author: Filipa Rodrigues

Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal; CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal; CICECO- Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: ana.f.rodrigues@staff.uma.pt

Other authors: Ivana Mendonça1,2,3; Marisa Faria1,2; Juan Luis Gómez Pinchetti4; Artur Ferreira3; Nereida Cordeiro1,2

1 Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal;

2 CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal;

3 CICECO- Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal;

4 BEA- Spanish Bank of Algae, Institute of Oceanography and Global Change (IOCAG), University of Las Palmas de Gran Canaria, 35214 Las Palmas de Gran Canaria, Spain

Background: The quantification of soluble extracellular polymeric substances (S-EPS) in microalgal and cyanobacterial cultures is essential to understand adaptation mechanisms and explore biotechnological applications. However, currently used methods rely on extraction, isolation, and purification of S-EPS, making the process time-consuming, technically demanding, and poorly suited for real-time monitoring. Additionally, these methods require large culture volumes, limiting their applicability in frequent or small-scale monitoring assays. This study presents a simple, sensitive, and reproducible method for the direct quantification of S-EPS in culture medium, without any pretreatment, based on its interaction with the cationic dye Alcian Blue (AB). Although widely used, AB is often applied empirically and without optimization for the systems in question.

Methods: The protocol was optimized and validated using various cyanobacterial cultures, including Cyanocohniella rudolphia (BEA 0786B) and Anabaena minutissima (BEA 0300B). Optimization began with the preparation of the dye solution, highlighting the importance of low speed stirring and confirming the dye's stability for up to 61 days at both 4 °C. From these optimized conditions, critical variables affecting in situ quantification were evaluated, particularly those related to the matrix in which S-EPS is dissolved: distilled water vs. culture medium, pH (3–12), salinity (0–37 ‰), and the ionic strength of the culture medium.

Results: Results revealed significant matrix effects, with ionic strength being the most impactful factor, while pH and salinity had minimal influence. Recovery assays with pure S-EPS demonstrated high precision, with all results falling within the 95% confidence interval. In addition, this study demonstrates that S-EPS derived from different microalgal sources interact differently with AB, rendering the use of generic standards, such as xanthan gum, inappropriate. Therefore, species- and medium-specific calibration curves are crucial for ensuring accurate and reliable quantification.

Conclusions: This methodology offers a robust and efficient solution for the real-time quantification of S-EPS directly in live cultures, providing time savings, reduced sample requirements, and adaptability to a wide range of environmental and biotechnological applications.

ID262 | Uncovering Microbial Drivers of Cellulosic Textile Degradation in Composting Environments

Author: Liliana Almeida

CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal

E-mail: liliana.almeida@ciimar.up.pt

Other authors: Diogo A. M. Alexandrino1,3, Rui S. Oliveira5, Maria de Fátima Carvalho1,2, Marisa Freitas1,3

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal;

2 ICBAS - School of Medicine and Biomedical Sciences, University of Porto, 4050-313 Porto, Portugal;

3 ESS, Polytechnic of Porto (ESS|P.PORTO), 4200-072 Porto, Portugal;

4 CR Group Lda., Rua de Santa Maria 2647, 4800-675 Guimarães, Portugal;

5 Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal.

Background: The exponential growth of global textile production, coupled with inadequate waste management infrastructure, has intensified the environmental burden of textile waste. Of particular concern is the cellulose-rich fraction, comprising natural and regenerated fibres, which remains largely excluded from effective recycling pathways. Its high abundance and untapped potential for microbial degradation underscore the need for targeted strategies. Leveraging the metabolic capabilities of specialised microbial communities within composting environments offers a promising route to address this gap, enabling sustainable bioconversion of cellulosic textiles into value-added products. This study investigates the potential of composting as a biologically driven strategy for the sustainable treatment of cellulosic textile waste. Methods: Cotton and lyocell fabrics, in both whole and shredded forms, were subjected to industrial (55 days) and community (90 days) composting processes conducted at a urban waste treatment and recovery Association (LIPOR, Portugal). Upon completion of the composting process, compost and textile samples were collected for downstream analyses, including culture-based and metagenomic approaches. Results: Degradation efficiency, determined through dry mass weight loss, was significantly higher in shredded samples (p 93% mass loss for both fabrics. Shotgun metagenomic analysis revealed microbial communities predominantly composed of Actinomycetota, Bacillota and Ascomycota. Functional annotation highlighted a rich repertoire of carbohydrate-active enzymes (CAZymes), notably glycoside hydrolases (GH), with the highest abundance detected in lyocell samples from community composting. Notably, cellulosome-associated domains were exclusively identified in lyocell samples. Complementary culture-based screening yielded 48 cellulolytic isolates across genera such as Bacillus, Aspergillus, and Neurospora. Conclusions: These findings position composting as a promising strategy for valorising cellulosic textile waste and as a potential foundation for advancing biotechnological innovations in textile waste management.

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ID265 | Ureolytic activity and biocementation potential of Pseudomonas aeruginosa HST244P

Author: Carina Coimbra

University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, Coimbra, Portugal

E-mail: cgcoimbra1992@gmail.com

Other authors: Pedro Farias and Paula V. Morais

University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, ARISE, Department of Life Sciences, Coimbra, Portugal

Concrete is the most widely used construction material globally, yet, its production, particularly of Portland cement, contributes approximately 7–8% of global COn emissions, raising serious environmental concerns. One sustainable alternative involves using bacteria capable of inducing calcium carbonate (CaCOn) precipitation to enhance concrete durability and reduce maintenance. This study aimed to characterize a bacterial strain with high ureolytic activity for biocementation applications.

Bacteria from the UCCCB collection harboring urease genes were screened on Christensen's urea medium, which indicates urease activity via pH changes caused by urea hydrolysis. Urease-positive strains were then evaluated for ureolytic performance by measuring ammonium (NHnn) production in liquid media containing 2% urea. Ammonia was quantified using the indophenol method at intervals over 48h. Growth (ODnnnnm) and pH were also monitored. Among the tested strains, Pseudomonas aeruginosa HST244P exhibited remarkable ureolytic activity, reaching 126.87 mM NHnn after 24 hours, comparable to that of the most extensively studied microorganism in ureolytic biomineralization, Sporosarcina pasteurii (146.96 mM). In both cases, an increase in pH from neutral to alkaline was observed.

Subsequently, both strains were cultured in the presence of 2% urea and 2.5% calcium chloride to assess their potential for CaCOn precipitation. Ammonium and total carbonate/COn were quantified using LCK kits® (LCK 304 and LCK 388, respectively). The strains exhibited significant NHnn production, pH increase, and COn release, creating favorable conditions for carbonate ion formation and CaCOn precipitation. Visible precipitation confirmed ureolytic activity.

These results demonstrate that P. aeruginosa HST244P has strong potential for microbial-induced calcium carbonate precipitation and CO2 sequestration, providing a promising and sustainable alternative for concrete biocementation and self-healing applications.

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ID267 | Variations in hospital microbiome from human occupancy and desinfection practices: persistence of UV-resistant Blastomonas strain

Author: Pedro Farias

University of Coimbra, CEMMPRE, Department of Life Sciences, 3000-456 Coimbra, Portugal

E-mail: pfarias@uc.pt

Other authors: Miguel Batalha 1, Joseph Nesme 2; Søren Sørensen 2; Paula V Morais 1

2 Department of Biology, Section of Microbiology; University of Copenhagen, Denmark

1 University of Coimbra, CEMMPRE, Department of Life Sciences, 3000-456 Coimbra, Portugal

This study aims to evaluate the microbiome of a newly constructed clinical environment to determine the impact of regular use by human activity and alternative disinfection practices on the environmental microbial diversity.

One hospital was sampled after construction, when it was fully equipped to receive patients and staff and after it had regular human occupancy. Nearly 70 samples were collected across four time periods. DNA was extracted from each sample, and bacterial diversity was assessed using Illumina sequencing of the 16SrRNA gene. Bacterial load was measured in the collected samples. Strains were isolated and identified. During hospital construction, the dominant genera, independent of surface/ space, are Geobacillus, 39.7

±9%, Acinetobacter, 19±7% and Pseudomonas, 13±3.4%. The temporal analysis of inter-sample diversity reveals that human occupancy increases uniformity in the microbiome, with overall increased abundance in Gammaproteobacteria, 43±18%. At the genus level, the most relevant increase is in Bradyrhizobium and Rhodanobacter. UV-c disinfection was effective in reducing microbial load, mainly in water points, between log10 -2.3 and -1.7. The analysis of species richness highlights that differences in UV-C treatment time produce different results. A UV-C resistant Blastomonas strain was recovered from a tap before and after decontamination.

Before human use, the microbial load in the space was low, and the bacterial genera present were commonly found on surfaces, such as Geobacillus, or early colonizers, such as Pseudomonas and Acinetobacter. After regular human use, an increase in abundance is observed in Bradyrhizobium, a genus more characteristic of the hospital surrounding rural environment, and Rhodanobacter, another early colonizer of man-made surfaces. UV-C treatment decreased diversity as a result of increased abundance of the same genera in most samples. In our study, Blastomonas strain HCA006a was re-isolated from the same surface after UV-C. Since this strain is known to survive typical disinfection by chlorination, it is critical to assess which disinfection practices should be used, or the materials used to prevent its colonization.

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ID272 | Harnessing Microbial Power: Degradation of Ketoprofen and Venlafaxine by Activated Sludge Bacterial strains

Author: Inês C. Leal

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

E-mail: ileal@ciimar.up.pt

Other authors: Ana P. Mucha (1,2), C. Marisa R. Almeida (1,2) and Joana P. Fernandes (1,2)

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal;

2 Faculty of Sciences, University of Porto, Porto, Portugal

Bioremediation exploits the metabolic capabilities of microorganisms to degrade organic pollutants and emerges as a promising strategy to improve pharmaceutical removal in wastewater treatment plants (WWTPs), mainly when applied in tertiary or quaternary treatment stages.

This study aimed to evaluated the ability of fifteen bacterial strains isolated from activated sludge [1] to biodegrade two pharmaceuticals of environmental concern: ketoprofen (KPF), an anti-inflammatory drug commonly detected in wastewater, and venlafaxine (VFX), an antidepressant listed among the twelve priority substances in the updated European Union Urban Wastewater Treatment Directive (Directive (EU) 2024/3019). Biodegradation tests involved exposing each strain to 1 mg/L of each compound in 60 mL of mineral salts medium supplemented with sodium acetate (500 mg/L) over four weeks under static conditions. Weekly analyses included assessment of microbial growth through optical density measurements at λ= 600 nm and quantification of pharmaceutical concentrations using High-Performance Liquid Chromatography with a Diode Array (DAD) detector for KPF and a fluorescence detector (FLD) for VFX.KPF removal ranged from 48 % to 71 % after 4 weeks of incubation, while VFX removal ranged from 8 % to 21% after the same period. The most promising strains will be evaluated in spiked wastewater microcosms (collected after secondary biological treatment), simulating more realistic treatment conditions, in which it will be evaluated: i) the removal of KPF and VFX, and other relevant pharmaceuticals, throughout time; ii) changes in the wastewater microbial community structure after the addition of each selected strain; iii) measurement of BOD, COD and nutrients before and after the experiment.

This work will provide more knowledge regarding the pharmaceuticals' biodegradation by activated sludge bacterial strains in synthetic media and real wastewater, supporting the development of biotechnological solutions for pollution control and regulatory compliance.

[1] Fernandes, J. P., P. Duarte, C. M. R. Almeida, M. F. Carvalho and A. P. Mucha, Journal of Environmental Chemical Engineering, 2020, 8(4): 103881.

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ID273 | Microbial Profiling and Fungal Control in Heritage Paintings: An Interdisciplinary Study from the MNAA

Author: Inês Silva

HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

E-mail: d51300@alunos.uevora.pt

Other authors: Cátia Salvador1,2, Ana Z. Miller3,1, Luís Dias1, Sara Valadas1, António Candeias1,2,4, Ana Teresa Caldeira1,2,4(*)

1 HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

2 Chemistry and Biochemistry Department, School of Sciences and Technology, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

3 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain

4 City University Macau Chair in Sustainable Heritage, Institute for Advanced Studies and Research, University of Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal. (*) atc@uevora.pt

Paintings are key elements of Cultural Heritage and require proactive preservation due to their susceptibility to biodeterioration. Their heterogeneous composition, rich in both organic and inorganic materials, offers ideal conditions for microbial colonization, particularly by fungi capable of causing permanent damage [1].

This study investigated microbial contamination in selected paintings from the Museu Nacional de Arte Antiga (MNAA), which houses some of Portugal's most significant public artworks. In certain paintings, visible signs of biodeterioration, such as black, brown, and whitish staining, were observed, raising concerns about ongoing microbial activity.

To characterize the microbial communities involved, we employed Next-Generation Sequencing (NGS), culture-based techniques, and Scanning Electron Microscopy (SEM). SEM analysis revealed extensive fungal colonization, with clear evidence of spores and hyphae. NGS identified a predominance of fungi from the phylum Ascomycota, with Aspergillus emerging as the dominant genus. Meanwhile, Cladosporium and Paralloneottiosporina were the most frequently isolated taxa in culture. Several biocidal products were tested for antifungal efficacy. Results underscored the need to base treatment choices on scientific validation and material compatibility to ensure safe application in conservation contexts. Finally, this study highlights the critical role of interdisciplinary collaboration, bridging microbiology, conservation science, and art history, in developing effective and sustainable strategies to preserve cultural heritage paintings.

ID293 | γ-Decalactone bioproduction from *Ashbya gossypii*: Process design, Techno-Economic evaluation and Environmental assessment

Author: María Teresa Moreira

CRETUS, Department of Chemical Engineering, School of Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

E-mail: maite.moreira@usc.es

Other authors: Aroa Carballido (1), Eduardo Coelho (2), José Maria Oliveira (2), Gumersindo Feijoo (1), Lucília Domingues (2)

(1) CRETUS, Department of Chemical Engineering, School of Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

(2) CEB – Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal

γ-Decalactone (GDL) is a valuable aroma compound whose demand is growing in the food, cosmetics, and fragrance industries due to consumer preference for natural and sustainable ingredients. Biotechnological production of GDL from renewable substrates is a promising alternative to traditional extraction and chemical synthesis methods. However, developing sustainable processes requires an evaluation of their environmental and economic feasibility. This study explores the conceptual design and sustainability assessment of a sustainable de novo GDL bioproduction process using Ashbya gossypii, considering every stage from inoculum preparation to final purification. A life cycle assessment (LCA) was conducted using a cradle-to-gate approach, incorporating both mass-based and economic allocation to account for the GDL and the residual biomass, the latter being valued as a co-product for animal feed. During the fermentation stage, the main environmental hotspots were linked to the consumption of KHnPOn, sucrose, and myo-inositol. In the purification phase, the primary contributors to environmental impact were ethanol and the adsorption resin, particularly in categories such as human toxicity and ecotoxicity. Sensitivity analysis indicated that moderate reductions in key inputs such as KHnPOn or resin could lead to significant improvements in the environmental performance. From a techno-economic perspective, the process was modeled under industrial-scale conditions, resulting in a total capital investment (TCI) of 59.5 million $, an annual production capacity of 78.27 tonnes, and a unit production cost of $773.75 per kg. Although the process yields moderate profit margins, the production cost remains below the current commercial price of natural GDL, which often exceeds 1,600 $/kg. This suggests strong potential for competitiveness in specialized, high-value markets. Overall, the results highlight the importance of addressing both environmental and economic factors when designing biotechnological processes for high-value compounds.

ID305 | The effect of the gut microbiome on the abdomen's lipid profile of honey bees (*Apis mellifera)*

Author: Matilde Amador

Gulbenkian Institute for Molecular Medicine, Rua da Quinta Grande 6 2780-156 Oeiras, Portugal

E-mail: matilde.m.amador@gmail.com

Other authors: Waldan Kwong, Cláudia Campos

Gulbenkian Institute for Molecular Medicine, Rua da Quinta Grande 6 2780-156 Oeiras, Portugal

Background: Host-associated microbiomes can have a large impact on its host's health, by contributing to the immune system, digestion, detoxification and more. The honey bee (Apis mellifera) gut microbiome is no exception, being involved in helping digest toxic pollen compounds, protecting against pathogens, and producing short-chain fatty acids and other metabolites that can be uptake by the host or gut bacteria. The absorption of these metabolites might influence the lipid composition in the honey bees, which in turn may affect host health as abdominal fat is important for bee energy homeostasis and immune cell production. In this study, we quantify the effects of the gut microbiome on bee lipid and protein content and identify genetic factors that may be responsible for shifts in these metabolites.

Methods: Newly emerged bees were kept with and without gut microbiota for 3 and 7 days. Abdominal protein content was assessed using a BCA assay, and lipid species profiles were generated by HPLC-MS/MS lipidomics. Gene expression was quantified using bulk RNA sequencing of abdomens. Bacterial community analysis and absolute copy numbers of the gut were conducted by 16S rRNA gene sequencing and qPCR, respectively.

Results: Comparing the lipid profile of the bees, we find a decrease of triacylglycerides and phosphatidylcholine and an increase in lysophosphatidylcholine in the colonized bees compared to the non-colonized bees, at both 3 and 7 days. From the transcriptomics results, a gene that encodes a triacylglyceride lipase, responsible for triacylglycerol and glycerophospholipid degradation, involved in lipid metabolism, was upregulated. Though the same shift in lipid composition was not observed across all experimental replicates. Examining the bacterial community composition, it appears that its structure could be a factor behind the differences in lipid and protein composition across our samples. Experiments with bees colonized with a single bacteria (mono-colonized) support the idea that different strains may drive different outcomes in abdominal protein composition.

Conclusions: The honey bee gut microbiota influences the abdominal lipid profile of bees by altering host gene expression of lipid metabolism pathways. However, the direction of these changes likely depends on the composition of the microbiota, which suggests that particular microbial species could have significant impacts on host physiology beyond the immediate colonization site of the gut.

ID306 | New Insights into the Catalytic Mechanism of the W-FdhAB Enzyme from *Nitratidesulfovibrio vulgaris* Hildenborough

Author: Guilherme Martins

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

E-mail: gdc.martins@itqb.unl.pt

Other authors: Rita Rebelo Manuel , Inês Cardoso Pereira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

Background: The high levels of CO2 in the atmosphere and the deleterious effects it causes to our planet created the need to use COn as a feedstock in the circular economy. This is a rapidly growing area of research, particularly the biocatalytic reduction of COn to formate. Formate is a value-added product and is being considered as a potential alternative liquid fuel. The formate dehydrogenase from Nitratidesulfovibrio vulgaris Hildenborough (FdhAB) is a tungsten-containing enzyme primarily responsible for COn reduction to formate in this organism. FdhAB is an excellent model system to study this reaction due to its relatively simple structure, comprising only two subunits, its stability under aerobic conditions and its exceptionally high COn-reducing activity. Despite its importance, the catalytic mechanism of COn reduction by FdhAB remains not completely understood.

Methods: To gain deeper insight into this process, we engineered single-point mutations in specific, highly conserved residues of the enzyme. The impact of these mutations on both formate oxidation and COn reduction was assessed through kinetic assays.

Results and Conclusion: Our findings highlight the essential role of these residues in the catalytic efficiency of the enzyme and support their critical involvement in the enzymatic mechanism of FdhAB. Computational and saturation mutagenesis studies can be developed in the future to aim at improving the stability and catalytic properties of this protein

ID315 | Sustainable Cotton Dyeing with Curcumin:Effects of Eutectic Solvents and Mordants on Color and Antimicrobial Properties

Author: Alexandre Miguel Santos Jorge

University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal

E-mail: alexandrej@eq.uc.pt

Other authors: Mafalda Gomes Carraco1,2; Jorge Fernando Brandão Pereira1

1 University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal;

2 University of Coimbra, FCTUC, Department of Physics, Rua Larga, Polo I, 3004-516 Coimbra, Portugal

Background: The textile sector is vital to the global economy but has a significant environmental impact due to water use and chemical pollution. Dyeing and finishing processes are the main sources of contamination, primarily from synthetic dyes. Natural dyes offer a more sustainable alternative, but their application, but their application is limited by low solubility, poor fiber fixation, and weak resistance to washing and external agents. To address these issues, in this study we evaluated the use of eutectic solvents (ES) to achieve sustainable and high-performance cotton dyeing.

Methods: Cotton fabrics were dyed with curcumin using both water and ES. For water-based dye baths, the optimal pH was determined by comparing curcumin exhaustion and fabric color. Mordants (ZnSOn, CuSOn, and chitosan) were tested in both systems to assess improvements in dye uptake. Dyed fabrics were analyzed for color, washing fastness, and antimicrobial activity against E. coli and S. aureus before and after washing.

Results: Curcumin exhaustion reached ~90% at pH 2, 8, and 10; pH 8 was identified as optimal due to superior color retention after washing. Compared with water, ES (glycerol/urea with 20% water) showed lower exhaustion but achieved similar color strength, indicating more efficient dye-fiber interactions. Mordants generally did not improve exhaustion, except CuSOn, which enhanced both exhaustion and color. In antimicrobial tests, curcumin reduced S. aureus growth (~40%) but had no effect on E. coli. Chitosan inhibited only S. aureus, with improved effects when combined with curcumin, but activity was lost after washing. ZnSOn inhibited both bacteria, with stronger effects on S. aureus; however, its activity against E. coli decreased after washing due to the loss of metal ions and curcumin. CuSOn completely inhibited both bacteria, and this effect persisted after washing.

Conclusions: Cotton dyeing with curcumin was optimal at pH 8. Eutectic solvents reduced dye exhaustion but enhanced dye-fiber interactions, resulting in color strength comparable to water-based baths. Mordants provided limited improvements in color, while antimicrobial activity varied: curcumin and chitosan selectively inhibited S. aureus ZnSOn offered broader but less durable effects, and CuSOn provided complete, wash-resistant inhibition of both bacteria. Overall, eutectic solvents and selected mordants enhance both the dyeing efficiency and functional performance of curcumin-dyed fabrics.

ID316 | Eco-Friendly Cotton Dyeing Using Natural Dyes and Eutectic Solvents

Author: Alexandre Miguel Santos Jorge

University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal

E-mail: alexandrej@eq.uc.pt

Other authors: Helena de Fátima Ribeiro1; Jorge Fernando Brandão Pereira1

1 University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal

The textile sector is a significant source of wastewater, largely due to dyeing processes that discharge synthetic dyes and hazardous chemicals into water systems. Eutectic solvents (ES) have emerged as promising green alternatives, valued for their low volatility, non-flammability, and chemical and thermal stability. In this study, three ES combinations—cholinium chloride/glycerol (ChCl/Gly), cholinium chloride/urea (ChCl/U), and glycerol/urea (Gly/U)—were assessed for their efficiency in cotton dyeing. Among them, Gly/U proved the most cost-effective and resource-efficient option. Using an optimized Gly/U mixture (1:1 molar ratio with 20% water), the dye bath achieved 30% curcumin exhaustion and a ΔE of 24.0 over five successive dyeing cycles, while also enhancing colorfastness relative to traditional methods. The process demonstrated consistent performance across cycles, with stable colorimetric outcomes and fastness. These results indicate that Gly/U-based dyeing offers a reproducible, environmentally sustainable method for cotton, reducing chemical usage, energy demands, and wastewater output. Eutectic solvents offer a sustainable and efficient alternative for cotton dyeing, enabling reduced chemical use, improved dye–fiber interactions, and the development of environmentally friendly textile processes.

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ID326 | Exploring Portuguese *Macroalgae* and *Halophytes* as Sources of Antimicrobial Agents Against Multidrug-Resistant Pathogens

Author: Isabel Henriques

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456, Coimbra, Portugal

E-mail: isabel.henriques@uc.pt

Other authors: Salomé Almeida, Elsa Rodrigues, Miguel Pardal, Leonel Pereira, Isabel Silva

Department of Life Sciences, Centre for Functional Ecology, Associate Laboratory TERRA, Faculty of Sciences and Technology, University of Coimbra, 3000-456, Coimbra, Portugal

Antimicrobial agents are increasingly important due to their wide-ranging applications, particularly in the fight against emerging antibiotic resistance.

This study evaluated Portuguese macroalgae (23 species; 39 field samples) and halophytes (6 species; 12 samples) as sources of phytochemicals with antimicrobial activity. Crude extracts were prepared using a green ultrasound-assisted method and two aqueous-based solvents: 100% ultrapure water and 10% DMSO in ultrapure water. The extracts were tested by disc diffusion method against test strains: Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 6538; multidrug-resistant (MDR) strains representing priority pathogens: Citrobacter freundii BCR2, E. coli B4, Enterobacter kobei ACR1, Acinetobacter baumannii 4P3.5, and Pseudomonas aeruginosa E67; strains representing animal pathogens: Aeromonas hydrophila CECT 839, Salmonella spp. F9, Vibrio alginolyticus V9F, and V. parahaemolyticus V9A; as well as a fungal strain: Candida albicans SC 5314. Crude extracts prepared from three macroalgae species: Bifurcaria bifurcata, Fucus spiralis and Gongolaria baccata, and two halophytes: Halimione portulacoides and Sarcocornia perennis, showed activity against at least one microorganism. The most promising extract was derived from B. bifurcata and prepared with 10% DMSO, displaying broad spectrum activity against test strains, MDR strains and animal pathogens. Thus, this extract was further tested against C. freundii BCR2 and E. coli B4, for its anti-biofilm activity and synergistic interaction with the antibiotic cefotaxime. Minimum inhibitory concentrations of 3.0 and 17.0 mg/mL and minimum bactericidal concentrations of 3.0 and 21.0 mg/mL were recorded for C. freundii BCR2 and E. coli B4, respectively. While biofilm inhibition was not significant, a significant biofilm stimulation was observed for E. coli B4. Concerning synergy, when combined with cefotaxime, the extract significantly enhanced antibiotic activity against E. coli B4. For example, the combination of 100 µg/mL of cefotaxime with 9 mg/mL of extract resulted in a reduction of 99.8% in CFUs/mL, compared to a reduction of 20.4 % achieved with the antibiotic alone.

These findings highlight the potential of Portuguese coastal macroalgae, particularly B. bifurcata, as sources of antimicrobial agents, especially for enhancing antibiotic efficacy against MDR strains.

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ID329 | Agricultural Residue–Basidiomycete Biocomposites: Sustainable Alternatives to Expanded Polystyrene for Packaging and Construction Applications

Author: Francieli Colussi

Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

E-mail: fcolussi@ceb.uminho.pt

Other authors: Márcia Araújo1, Michele Michelin1; 2, José Teixeira1; 2

1 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;

2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

The increasing environmental concerns surrounding petroleum-derived materials have driven the search for biodegradable and renewable alternatives. This study investigates the development of biocomposites produced by solid-state cultivation of basidiomycete fungi on agroforestry residues, aiming to replace conventional expanded polystyrene (EPS) in packaging and construction applications. Agricultural by-products, namely vine pruning were combined with fungal mycelium to produce lightweight and structurally cohesive biocomposites through mycelial binding. The materials were characterized for density, compressive strength, water absorption, and biodegradability. Results indicate that the composites achieved densities between 206,84 - 386,67 kg/m³, compressive strengths up to 0.6 MPa, and rapid biodegradability under composting conditions. Morphological (SEM) and FTIR analyses confirmed effective substrate colonization and bonding. These findings demonstrate the potential of agricultural residue–basidiomycete biocomposites as sustainable, low-carbon alternatives to EPS for protective packaging and as eco-friendly insulating panels in construction. Future work will focus on upscaling production and assessing antimicrobial and fire-retardant treatments to enhance performance. This study contributes to circular bioeconomy strategies by valorizing low-cost lignocellulosic residues into high-value green materials.

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ID330 | New microplate setup for assessing the toxicity of water disinfection by-products using bioluminescent Aliivibrio fischeri

Author: Bruno Costa

CEMMPRE, Department of Life Sciences, University of Coimbra, 3000-456, Coimbra, Portugal

E-mail: brunoalexandrecosta76@gmail.com

Other authors: 1. Rui Martins; 2. Paula Vasconcelos Morais

1. CERES, Department of Chemical Engineering, University of Coimbra, 3030-790, Coimbra, Portugal;

2. CEMMPRE, Department of Life Sciences, University of Coimbra, 3000-456, Coimbra, Portugal

Background: Disinfection by-products (DBPs) are formed during drinking water disinfection and are an emerging concern for aquatic ecosystems due to their widespread occurrence and potential toxicity. Research on DBPs toxicity and environmental risks has only addressed a small fraction of known DBPs, with most still lacking empirical toxicity data, particularly on bioindicators such as the marine photobacterium Aliivibrio fischeri.

Methods: Ten selected DBPs (chloroform, TCM; monochloroacetic acid, MCAA; dichloroacetic acid, DCAA; trichloroacetic acid, TCAA; bromoacetonitrile, BAN; dibromoacetonitrile, DBAN; dichloroacetaldehyde, DCAL; dibromoacetaldehyde, DBAL; N-nitrosodimethylamine, NDMA; 2-chlorophenylacetonitrile, 2-CPAN) were tested in A. fischeri UCCCB 273 (= ATCC 774 = DSM 507 = JCM 18803) by bioluminescence assay using a new microplate setup developed in-house. DBPs were tested over broad concentration ranges, and inhibition effects were recorded at 5, 15, and 30 minutes. IC50 and IC20 values were calculated from derived dose-response models, and environmental risk quotients (RQs) were estimated using these values, environmental median occurrence data and predicted no-effect concentrations. Cumulative mixture risks (RQC) for the included DBPs were assessed using a concentration addition model.

Results: All DBPs exhibited dose-dependent acute toxicity to A. fischeri. BAN (IC50;20 = 1-70; 0.66 mg/L), DBAN (IC50;20 = 6.30; 2.00 mg/L), and 2nCPAN (IC50;20 = 16.03; 3.14 mg/L) were the most toxic after 30 minutes exposure. Most DBPs posed low environmental risks (RQ < 0.1), but BAN (RQ50;20 = 0.35; 0.91), DCAA (RQ50;20 = 0.07; 0.16), DBAL (RQ50;20 = 0.06; 0.15), and DBAN (RQ50;20 = 0.03; 0.10) presented moderate risks (0.1 < RQ < 1.0). Cumulative mixture assessment revealed moderate to high environmental risk (RQC50;20 = 0.58; 1.44) when considering additive effects.

Conclusions: The tested DBPs exhibited dose-dependent toxicity to A. fischeri, with BAN, DBAN, and 2-CPAN being the most toxic. BAN, DCAA, DBAL, and DBAN showed moderate environmental risks, and the cumulative DBPs mixture presented high risk, highlighting concerning additive effects. These results note the importance of considering both individual and combined DBP toxicity to assess their risks and provide essential data for effective management and regulatory decisions for addressing DBPs effects in aquatic environments.

ID343 | Pinus pinaster In Vitro Shoot Volatiles from Genotypes with Varying Susceptibility to Pine Wilt Disease

Author: Jorge M. S. Faria

1 INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal; 2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Lisbon, Portugal.

E-mail: jorge.faria@iniav.pt

Other authors: Gonçalo Pereira

INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal

Background: The pinewood nematode (Bursaphelenchus xylophilus, PWN) is the causal agent of Pine Wilt Disease (PWD), a major threat to coniferous forests worldwide. Pinus pinaster (maritime pine) displays variable susceptibility to PWN, yet the biochemical mechanisms underlying resistance remain poorly understood. Understanding these mechanisms benefits the development of sustainable control strategies.

Methods: To investigate potential resistance metabolomic markers, we established in vitro shoot cultures of P. pinaster genotypes differing in susceptibility to PWD. Microshoots were inoculated with PWN and monitored over a one-month period. Progression of PWD symptom stages was detailed. Volatile organic compounds (VOCs) emitted during infection were collected using headspace extraction and analyzed by thermal desorption–gas chromatography–mass spectrometry (TD–GC/MS). Non-inoculated (control) samples were also analyzed to assess baseline VOC profiles.

Results: Both genotypes emitted comparable proportions of α- and β-pinene. However, the genotype with lower susceptibility displayed higher levels of limonene. Upon PWN infection, the highly susceptible genotype exhibited PWD symptoms earlier, while the less susceptible genotype demonstrated delayed disease progression. Additionally, the susceptible genotype exhibited a progressive increase in the proportions of α-pinene, β-pinene, and limonene, corresponding with symptom severity. In contrast, the less susceptible genotype showed a decrease in these compounds over the course of disease progression.

Conclusions: These results suggest that VOC emissions may play a role in P. pinaster defense responses, potentially serving as biochemical markers of resistance. The use of in vitro culture systems provided a reproducible platform for studying host-pathogen interactions in a controlled environment. VOC profiling emerges as a promising tool for identifying early resistance indicators, offering valuable insights for breeding and management strategies aimed at enhancing resilience to PWN.

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ID344 | In Vitro Tissue Culture Systems to Study Sustainable Management Alternatives for Plant Parasitic Nematodes

Author: Jorge M.S. Faria

1 INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal; 2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Lisbon, Portugal.

E-mail: jorge.faria@iniav.pt

Other authors: Gonçalo Pereira

INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal

Background: Diseases caused by plant parasitic nematodes (PPNs) pose a significant threat to agriculture and forestry. Traditional management strategies mostly rely on chemical nematicides to control PPNs. However, growing environmental and human health concerns have led to a banning of many conventional pesticides. Replacement formulations often show reduced efficacy. As consumer demand for low-impact, environmentally sustainable agriculture increases, the development of alternative pest control methods has become increasingly urgent.

Methods: Conventional screening methods for novel nematicides typically involve direct contact bioassays, which fail to assess phytotoxicity or account for compound biotransformation within the plant. In vivo assays using greenhouse or field conditions offer a more realistic evaluation but are time-consuming and resource intensive.

To address these limitations, in vitro plant-nematode co-culture systems have been developed as a biotechnological screening platform. These systems allow for the simultaneous assessment of nematicidal activity and potential host toxicity in a controlled environment. Co-culture approaches include transgenic root systems co-cultured with soil-dwelling PPNs, and in vitro pine shoot cultures co-cultured with Bursaphelenchus xylophilus, the pinewood nematode (PWN). These systems are maintained under defined nutritional and environmental conditions, with reproducible and easily manipulated biological parameters.

Results: Validation experiments using naturally derived nematicidal compounds confirmed the utility of these in vitro co-culture systems. They effectively differentiated between nematicidal activity and host toxicity, providing a more holistic and accurate screening tool compared to traditional assays. Current research is leveraging these platforms to perform rapid functional validation of parasitism regulator genes (effectors) through gene silencing or overexpression in nematodes. This enables the identification of promising molecular targets for future control strategies.

Conclusions: In vitro plant-nematode co-culture systems represent an innovative, rapid, and sustainable approach for screening novel nematicidal compounds and exploring the functional relevance of molecular targets. These tools offer a practical alternative to in vivo testing and hold significant promise for advancing environmentally responsible pest management strategies, especially in forestry.

ID345 | Tuning the substrate specificity of galactose oxidase by ancestral sequence reconstruction

Author: Paulo Durão

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780–157 Oeiras, Portugal

E-mail: pdurao@itqb.unl.pt

Other authors: Tomás Frazão, Tiago Lopes, Vânia Brissos, Lígia O. Martins

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780–157 Oeiras, Portugal

Background: The bioconversion of 5- and 6-carbon sugars is central to lignocellulose biorefineries for the hydrolysis of feedstocks into commodity chemicals. This involves the oxidation of alcohol groups to industrially relevant aldehydes, carboxylic acids, and ketones. Galactose oxidases (GalOx) are promiscuous enzymes that oxidize D-galactose (D-Gal), and D-Gal-containing polysaccharides, but also primary alcohols, while using O2 as a clean and readily available oxidant, aligning with the principles of green chemistry. GalOxs belongs to the Auxiliary Activity (AA) family 5 of the Carbohydrate-active Enzyme (CAZy) database, specifically within the AA5_2 subfamily of copper radical oxidases. However, despite their biotechnological potential, only a handful of AA5_2 enzymes have been biochemically characterized, leaving fundamental gaps in our understanding of their substrate specificity, catalytic mechanisms, and evolutionary diversity.

Methods: We have taken advantage of the existence of the CAZy database and used an ancestral sequence reconstruction (ASR) pipeline to infer extinct molecular sequences from the internal nodes of a phylogenetic tree built with bacterial and eukaryotic AA5_2 enzymes. A computational analysis - solubility, folding propensity, hydrophobic clusters, among others - of the ancestors was performed.

Results: A phylogenetic relationship among copper radical oxidases was established, inferred the ancestor lineage for a specific bacterial GalOx, and selected the most promising ancestors to be “resurrected” after computational analysis. Experimental characterization of the enzymes will ensue.

Conclusions: ASR is a powerful tool for advancing our understanding of GalOx substrate scope, enabling us to pinpoint catalytic features that can be engineered and enhanced for biotechnological applications. Overall, we intend to create a toolbox of enzymes with tailored functionalities, enabling more efficient and sustainable industrial processes.

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ID346 | Dodecan-1-ol as an Alternative to Conventional Nematicides for Sustainable Crop Soil Management

Author: Adélia Varela

INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal;

GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Lisbon, Portugal.

E-mail: adelia.varela@iniav.pt

Other authors: Jorge M.S. Faria1,2, Ricardo Machado1, Paula Fareleira1,2

1INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon, Portugal;

2GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Lisbon, Portugal.

Background: Plant-parasitic nematodes (PPN) are a persistent challenge in agriculture, causing root damage that leads to significant yield losses. Conventional PPN management has largely relied on synthetic nematicides, many of which negatively impact soil ecosystems by harming beneficial microorganisms and reducing overall soil functions. Due to rising concerns over environmental safety and human health, numerous chemical formulations have been banned or restricted. In this context, dodecan-1-ol, a naturally derived fatty alcohol, shows a higher nematicidal strength than conventional nematicides with lower predicted risks to soil biodiversity and ecosystem. This study aimed to evaluate its effects on soil microbial activity, under in vitro conditions.

Methods: Bioassays were performed on undisturbed soil collected in Oeiras, Portugal. Soil samples (35 g) were added with dodecan-1-ol, carvacrol (an approved biopesticidal compound), and the conventional pesticides oxamyl or emamectin benzoate. The activities of the soil enzymes dehydrogenase, alkaline phosphatase, β-glucosidase and aryl-sulfatase, were determined by spectrophotometric assays at 1 and 7 days after compound addition.

Results: For dodecan-1-ol, dehydrogenase activity significantly increased at 24 hours but reversed to control levels after 7 days. A similar response was observed for β-glucosidase activity, while phosphatase and aryl-sulfatase levels remained comparable to the control at both time-points. Carvacrol and oxamyl also induced initial stimulatory effects on dehydrogenase activity, followed by a return to control levels by 7 days. However, exposure to carvacrol seemed to induce more lasting effects on soil enzyme activities.

Conclusions: Our findings suggest that dodecan-1-ol is not detrimental to soil microbiota, only transiently boosting overall microbial metabolic activity (dehydrogenase) and carbon cycling potential (β-glucosidase). The neutral impact on phosphatase and arylsulfatase indicates that it might not disrupt phosphorus and sulfur cycling processes. The transient nature of the stimulatory effects, without long-term negative impacts on the measured enzymes, points to dodecan-1-ol as a potential sustainable nematicidal alternative.

ID374 | Microalgal B-EPS from *Neospongiococcum gelatinosum*: structural features and functional potential for sustainable applications

Author: Muhammad Haris

1-Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal;

2-CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal;

3-CICECO- Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: muhammad.harris@staff.uma.pt

Other authors: Juan Luis Gómez Pinchetti4, Artur Ferreira3, Antera Martel Quintana4, Nereida Cordeiro1,2

4 BEA- Spanish Bank of Algae, Institute of Oceanography and Global Change (IOCAG), University of Las Palmas de Gran Canaria, 35214 Las Palmas de Gran Canaria, Spain;

3 CICECO- Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal;

4 BEA- Spanish Bank of Algae, Institute of Oceanography and Global Change (IOCAG), University of Las Palmas de Gran Canaria, 35214 Las Palmas de Gran Canaria, Spain;

1 Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal (and) 2 CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal

Extracellular polymeric substances (EPS) emerge as sustainable and multifunctional biopolymers with growing relevance in different application areas. Bonded extracellular polymeric substances (B-EPS), also referred to as capsular polysaccharides (CPS), represent a structurally distinct fraction of EPS that remains tightly associated with the cell surface, offering unique properties for advanced applications. Exploring new microalgal species and their EPS is crucial to broaden the structural and functional diversity of these biopolymers, paving the way, for example, to innovative nanoencapsulation systems. Here, a B-EPS isolated from Neospongiococcum gelatinosum (BEA 0744B) was structurally and functionally characterised. Elemental and spectroscopic analyses revealed a polysaccharidic backbone containing minor protein-like moieties and showed the absence of sulfation, an uncommon feature among microalgal EPS. The B-EPS displayed moderate surface charge and high thermal stability, supporting its robustness for industrial use. Functionally, the B-EPS showed excellent water- and oil-holding capacities, strong solubility in water and polar solvents, and high foaming ability and stability. Emulsification tests demonstrated the formation of stable emulsions outperforming conventional surfactants, while rheological analysis confirmed a shear-thinning, pseudoplastic behaviour ideal for thickening applications. Together, these findings position the B-EPS from N. gelatinosum as a structurally robust biopolymer of microalgal origin, supporting sustainable and carbon-negative bioeconomy approaches. Its versatility, combining solubility, emulsifying capacity and rheological behaviour, highlights its suitability for clean-label functional foods and related applications. Moreover, these properties provide a strong basis for EPS-based nanoencapsulation, offering innovative strategies to overcome sensory limitations of microalgae-derived foods and to advance circular bioeconomy solutions.

ID379 | Bioremediation Potential of Gut-Associated Bacteria Isolated from Hediste diversicolor Exposed to BPA and TBBPA

Author: Valéria Giménez

1 Center for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: valeriacgimenez@ua.pt

Other authors: Etelvina Figueira1, Jhon A. Suescun-Sepúlveda2, Martha E. Trujillo2, Adília Pires1

1 Center for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

2 University of Salamanca, Dpto de Microbiología y Genética, Campus Unamuno, 37007 Salamanca, Spain

Background: Hediste diversicolor is a benthic polychaete commonly found in estuarine environments, where its bioturbation activity contributes to sediment mixing and affects the biogeochemical cycle of nutrients and contaminants. Previous studies have shown that the gut microbiota of other marine benthic polychaete species can degrade environmental pollutants, including microplastics such as polystyrene. Therefore, the gut microbiota of H. diversicolor may also contribute to the degradation of persistent organic pollutants, such as plastic additives (PAs), including Bisphenol A (BPA) and Tetrabromobisphenol A (TBBPA). These PAs are widely used in plastics and electronics and are recognized for their environmental persistence, bioaccumulation, and endocrine-disrupting effects in aquatic environments. However, microbial degradation of these contaminants, particularly by gut-associated bacteria from marine invertebrates, remains poorly understood.

Methods: H. diversicolor organisms were collected from seven sites along the Ria de Aveiro lagoon (Western Portugal), an anthropogenically impacted coastal system. Gut tissues were aseptically dissected to isolate cultivable intestinal bacteria. A total of 39 pure colonies were screened for their ability to degrade BPA and TBBPA. Each isolate was incubated in basal salt medium (BSM) supplemented with 10 mg/L of either BPA or TBBPA and maintained for 30 days at 26 °C under agitation (180 rpm). Bacterial growth was monitored by measuring optical density at 600 nm after 15 and 30 days of exposure.

Results: Among the 39 isolates, five did not grow, thirty-one exhibited slight growth, and three showed significant growth in BPA and TBBPA as sole carbon sources, suggesting a potential capacity for biotransformation or degradation. Based on 16S rRNA gene sequence analysis, the three isolates were identified in the genera Exiguobacterium, Shewanella, and Vibrio.

Conclusions: These findings indicate that H. diversicolor harbors gut-associated bacteria capable of tolerating and potentially degrading plastic additives, such as BPA and TBBPA, possibly through specific enzymatic pathways. This study highlights the potential of marine invertebrate-associated microbiota as a valuable source of bacteria for biotechnological applications in bioremediation. Further work is ongoing to sequence the bacterial full genomes to understand the metabolic pathways involved in BPA and TBBPA degradation.

ID381 | Influence of mulches on ericoid mycorrhizal colonization in blueberry roots: field trials in Fataca, Portugal.

Author: Pablo Pereira

Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV) 2780-157 Oeiras, Portugal;

E-mail: pablo.pereira@iniav.pt

Other authors: Filipe Pedra1, Paula Fareleira 1,2 Corina Carranca 1,2

1 Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV) Oeiras, Portugal;

2 GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal

Background: Berries have gained popularity over the last decade among consumers, especially the blueberry. Blueberries belongs to the Ericaceae family, prized for their sweet edible fruits, rich in vitamin C, minerals, and a number of antioxidants. Ericoid mycorrhizal fungi (ErMF) play a crucial role in the nutrition and growth of blueberry (Vaccinium virgatum Aston cv. Centra Blue.), particularly under field conditions. Understanding how mulches influence ErMF colonization can help optimize sustainable blueberry cultivation in Mediterranean environments.

Methods: This study investigated how five different mulches affected ErMF colonization in blueberry roots in a field experiment conducted in Fataca, Odemira (Southern Portugal), over two consecutive years (2024–2025). The treatments included: A: pine bark (control); B: Kraft® cellulose biofilm (0.80 m width, beige, COTESI); C: geotextile fabric, a polypropylene (PP) derived material (1.60 m width, black, 50×70,97 g mn²); D: polyethylene (PE) plastic film (1.50 m width, black, 40 µm thick); E: KRITIFIL® biofilm (PBAT-Poly(butylene adipate-co-terephthalate)-based material, 0.80 m width, black, 14 µm thick). Root samples were collected annually and analyzed to quantify ericoid mycorrhizal colonization.

Results: Overall, ErMF percentual colonization increased in 2025 compared to 2024 in all treatments, reflecting a progressive establishment over time. Among treatments, B and E exhibited the most pronounced increases in colonization, with +4.91% and +8.38%, respectively, compared to the control (A). Conclusions: Soil cover type influences ericoid mycorrhizal colonization in blueberry roots. Specific mulching materials, such as Kraft® cellulose and KRITIFIL® biofilms can enhance symbiotic interactions and support sustainable cultivation practices in Mediterranean environments, while also offering the advantage of being biodegradable in soil.

ID382 | From Seedling to Maturity: Mycorrhizal Changes in Maize" Field Observations in Golegã, Portugal.

Author: Pablo Pereira

Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV) 2780-157 Oeiras, Portugal;

E-mail: pablo.pereira@iniav.pt

Other authors: Paula Fareleira

Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV) 2780-157 Oeiras, Portugal; Green-It Bioresources for Sustainability,ITQB NOVA, 2780-157 Oeiras, Portugal

Background: Arbuscular mycorrhizal fungi (AMF) establish symbiotic associations with maize (Zea mays L.), enhancing nutrient uptake and plant resilience. However, the dynamics of root colonization and the predominance of fungal structures vary across development. Understanding these shifts is essential to optimize AMF benefits in sustainable agriculture.

Methods: A field trial was conducted in Golegã, Portugal, to assess AMF colonization across maize phenological stages. Root samples were collected from vegetative emergence (VE), through vegetative stages (Vn) and tasseling (VT), until the reproductive stage (R). Samples were cleared, stained, and microscopically analyzed to determine the degree of colonization and to identify predominant AMF structures (internal/external hyphae, arbuscules, vesicles) at each stage.

Results: AMF colonization increased progressively from early to late vegetative stages, ranging from 15% at VE to 72% by V10. Colonization peaked at VT with 80% of root length colonized, followed by a marked decline at R, reaching only 10%. Structural dynamics also followed maize phenology: VE to V7 was dominated by internal and external hyphae, reflecting initial fungal establishment; from V8 to VT, arbuscules prevailed, indicating maximum functional interaction and nutrient exchange; in R, vesicles became predominant, suggesting a shift towards fungal storage structures.

Conclusions: Our findings demonstrate that maize phenology strongly influences both the intensity and structural composition of AMF colonization. The increase in colonization during vegetative growth, its peak at VT, and the sharp decline during reproduction, reflect a dynamic symbiotic strategy adapting to host developmental demands. The predominance of hyphae in early stages, arbuscules during vegetative phases, and vesicles in reproductive stages underscores the functional plasticity of AMF. These results highlight the importance of considering crop growth stages when assessing AMF functionality and their integration into sustainable maize production systems.

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ID388 | Metabolic and Regulatory Responses of *Pseudomonas* under Oxygen-Limited Conditions

Author: Luís Carlos Moreira Costa

CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

E-mail: id11211@alunos.uminho.pt

Other authors: Salomé Duarte 1,2, Sónia Barbosa 1,2, João C. Sequeira 1,2, Michael Ho 3, Christian Vicera 4, M. Alcina Pereira 1,2, Giin-Yu Amy TAN 4, Po-Heng LEE 3, Andreia Salvador 1,2, Ana J. Cavaleiro 1,2

1 CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal;

2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal;

3 Department of Civil Engineering, Imperial College London, London, UK;

4 Department of Architecture and Civil Engineering, City University of Hong Kong, Hong Kong SAR, China

Background: Pseudomonas species are frequently detected in anaerobic wastewater treatment systems, and their presence has been associated with the anaerobic biodegradation of lipids into methane. Their ability to thrive in oxygen-limited environments, combined with their capacity to degrade recalcitrant compounds, suggest a potential ecological role in these processes, particularly considering that strict anaerobic conditions are not always maintained in practice. Gaining a better understanding of their behavior and function is therefore essential for guiding future improvements in anaerobic digestion processes. This study aims to detect changes in gene expression by Pseudomonas species under low O2 concentrations.

Methods: Pseudomonas sp. strain I1, isolated from a micro-aerated bioreactor fed with fatty acids, was grown in sealed batch bottles using mineral medium and acetate (10 mM) as the only carbon source. The O2 levels in the headspace were adjusted to 0% (anaerobic control), 0.1%, 0.2%, 0.5%, 1%, 2%, and 21% (aerobic control). The experiment lasted around six hours. During this time, O2 and acetate concentrations were measured at 0, 3, and 6 h by chromatography. RNA was then extracted and sequenced. Gene expression analysis was performed using the MOSCA pipeline, comparing the expression levels under each condition to those at 21% O2.

Results: Significant gene expression changes were observed in oxidative stress response, TCA cycle, electron transport chain (ETC), and flagellar assembly. Under low-O2 conditions, oxidative stress genes were downregulated, with thioredoxin peroxidase achieving a 105-fold decreased at 0.2% On.Data suggest that acetate is assimilated via the ACS pathway to acetyl-CoA. Under 0.5–2% On, idh was repressed while aceA was upregulated, indicating a metabolic shift from the TCA cycle to the glyoxylate shunt. Under low O2 metabolism likely shifts to gluconeogenesis for storage compounds.

Pseudomonas spp. employ five oxygen reductases in the ETC. High-affinity types for oxygen (cbb3) and the bc1 complex were strongly upregulated under low On, while low-affinity reductases showed low expression. Flagellar assembly genes were downregulated below 0.2% On, suggesting reduced motility as energy shifts to stress survival.

Conclusions: In response to low On concentrations, Pseudomonas spp. downregulate genes involved in oxidative stress responses and motility. A metabolic shift from TCA to the glyoxylate shunt was observed.

ID390 | Cell atlas of the honey bee hindgut

Author: Claudia Campos

GIMM, R. da Quinta Grande, n6, 2780-156 Oeiras, Portugal

E-mail: ana.campos@gimm.pt

Other authors: Ana Barradas, Waldan Kwong

GIMM, R. da Quinta Grande, n6, 2780-156 Oeiras, Portugal

Background: The honeybee gut plays a crucial role in digestion and immunity, with gut microbiota influencing host physiology. However, the cellular diversity of the bee gut and its modulation by microbiota remains underexplored. This study aims to identify the cell types in the bee gut and determine how they are influenced by the presence or absence of microbiota.

Methods: We used newly emerged adult bees, 7-day-old adult bees without microbiota, and 7-day-old bees inoculated with microbiota. The ileum and rectum were dissected separately, as they harbor distinct microbial communities. Single-nucleus RNA sequencing (snRNA-seq) and Seurat analysis of the obtained sequence was employed to identify cell clusters, and bulk RNA sequencing provided additional insights into gene expression differences across the groups.

Results: We identified multiple cell types in the adult bee gut and transcriptional differences were prominent between newly emerged bees and 7-day-old adults, with the former showing cell types still undergoing differentiation. We identified key marker genes for these cell types , allowing us to infer on their function, and determined their spatial localization in situ. Our observations also indicate that bees harboring a conventional microbiota display widespread expression of multiple antimicrobial peptides (AMPs) across all the cell clusters within the hindgut, without a distinguishable enrichment in any specific cluster.

Conclusions: Our findings demonstrate the dynamic development of bee gut cell types, with microbiota presence influencing immune-related pathways. Newly emerged bees show distinct undifferentiated cell types, providing insights into early gut development. We also showed that the presence of microbiota drives an immune response in all cell types, showing a systemic response of the host towards the microbiota. Further analysis of these marker genes will help elucidate the functional roles of these cell types and their contributions to gut health and host-microbiota interactions.

ID407 | *Pseudomonas thivervalensis* NFX104: an effective novel biocontrol agent against tomato gray mold disease

Author: Sara Tedesco

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

E-mail: sara.tedesco@ibet.pt

Other authors: Filipa Silva1,2, Margarida Pimenta1,2, Frédéric Bustos Gaspar 1,2, Marta Nunes da Silva3, Maria Teresa Barreto Crespo1,2, Francisco Nascimento1,2

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

Background: Botrytis cinerea, the causal agent of gray mold, infects over 1,400 plant species and causes annual losses of up to USD 100 billion. Chemical fungicides remain the primary control method, but fungal resistance, environmental risks, and regulatory restrictions limit their current use. Bacterial control agents represent sustainable alternatives to the use of chemical fungicides. In this work, we reveal the biological control properties of Pseudomonas thivervalensis NFX104 against gray mold disease.

Methods: Strain NFX104 was isolated from agricultural soil in Portugal and its genome completely sequenced. Secondary metabolite biosynthetic gene clusters were predicted using antiSMASH. Antifungal activity was assessed in vitro through dual plate assays and co-cultures with B. cinerea conidia under different bacterial cell densities and nutrient conditions. Moreover, bacterial disease suppressive activities were tested in planta (tomato detached leaf assays and pot assays). The NFX104 performance was benchmarked against the commercial biofungicide Bacillus amyloliquefaciens QST 713 (Serenade®).

Results: Genomic analysis identified NFX104 as P. thivervalensis (98.95% ANI) and revealed biosynthetic gene clusters for 2,4-diacetylphloroglucinol, hydrogen cyanide, and lanthipeptides. Strain NFX104 inhibited

B. cinerea mycelial growth by >35% and strongly suppressed conidial germination (>70%). On detached leaves and full tomato plants, NFX104 reduced disease incidence by 42% and 27%, respectively, matching Serenade®'s efficacy in both cases.

Conclusions: This study identifies P. thivervalensis NFX104 as a promising candidate against the gray mold disease agent, B. cinerea. Its consistent inhibition of conidial germination, moderate suppression of mycelial growth, and reduction in disease incidence highlights its potential for future agricultural applications.

ID410 | Preliminary study of the effects of biopreparations in microorganisms

Author: Daniela de Vasconcelos Teixeira Aguiar da Costa

1. Agrarian School of Viseu, Polytechnic Institute of Viseu, 3500-600 Viseu, Portugal. 2. Centre for the Study of Natural Resources, Environment and Society (CERNAS), 3500-600 Viseu, Portugal.

E-mail: daniela@esav.ipv.pt

Other authors: Joana Simões1, António Pinto 1,2,3; Cristina Amaro da Costa 1,2

1 Agrarian School of Viseu, Polytechnic Institute of Viseu, Portugal.

2 Centre for the Study of Natural Resources, Environment and Society (CERNAS), Viseu, Portugal.

3 Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, 5000-Vila Real; Portugal.

Background: Agroecological systems are important in responding to current societal challenges, namely the growing demand for sustainable and organic food. Agroecology, as a science, seeks answers to these current challenges at various stages of crop technical itineraries. It is therefore vital that research finds more natural alternatives with less environmental and health impacts, such as biopreparations. This study aims to analyse in vitro the microbial activity of seven biopreparations produced and used by farmers in Portugal to understand their effect and to systematise a set of clearer indications. The seven biopreparation protocols were based on information gathered from the literature and farmers' testimonials: garlic extract, purslane vinegar, prickly pear vinegar, fermented orange juice, horsetail decoction, nettle infusion, and garlic and chilli biopreparation.

Methods: The study of the effects of the biopreparations in microbial populations aimed to evaluate the inhibition of microbial activity. For this, it was carried out by impregnated discs with each biopreparation in vitro through the measure of the inhibition halo.

Results: The fermented orange juice demonstrated to have the highest inhibition potential of the fungus and bacteria activity in this study. The horsetail decoction was the one with the poorest responses and inhibitions.

Conclusions: This study came to bring to light the potentiality of these seven biopreparations for microbial activity inhibition. For the control of bacteria, garlic extract, fermented orange juice, purslane vinegar and pricky pear vinegar are the best to do it; on other hand, for the control of fungus, garlic and chili biopreparation, horsetail decoction, fermented orange juice and pricky pear vinegar were the best. Whereas these results, it is essential to continue to study these biopreparations and other to seek for more natural solutions in crop protection for microbial activity and other uses.

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ID412 |Bacterial population variability in wastewater treatment and thpotential persistence of specific lineages

Author: Célia Manaia

Universidade Católica Portuguesa, Porto, Portugal

E-mail: cmanaia@ucp.pt

Other authors: Diana Ribeiro1, Afonso Ruivo1, Isabel Santos1, Cristina Mena1, Ivone Vaz-Moreira1, Maria João Rosa2, Margarida Campinas2, Elsa Mesquita2, Catarina Silva2, Cláudio Costa3, Gabriela Faria3

1, Universidade Católica Portuguesa, Porto;

2, LNEC - Laboratório Nacional de Engenharia Civil, Lisboa, Portugal

3 Tratave – Tratamento de águas residuais do Vale do Ave, Portugal

Wastewater treatment plants (WWTPs) are essential barriers to attenuate the emission of contaminants, such as antibiotic-resistant bacteria (ARB) and resistance genes (ARGs), which monitoring is becoming a requirement (Directive (EU) 2024/3019). Despite the urgent need to improve wastewater treatment to remove ARB&ARGs, the complexity of microbial community dynamics during treatment remains poorly understood. This work discusses the bacterial community variations in full-scale real-world systems: i) between inlet and outlet of a WWTP with three full-scale activated sludge treatment lines, ii) due to ozonation, and iii) impact on the receiving river. Analyses included total coliforms and E. coli counts, quantitative PCR of selected genes (e.g. 16S rRNA, IS26, blaOXA-58, blaVEB), 16S rRNA gene metabarcoding, isolates identification by Maldi-TOF, and genome-based inference of ARB dissemination.

Activated sludge treatment led to important variations on bacterial community composition, being genera affiliated to the phylum Candidatus Saccharibacteria, followed by those affiliated to the class Betaproteobacteria those that most frequently increased after treatment. Members of the genera Acinetobacter, Aeromonas and Aliarcobacter were among the most abundant in the inlet and persisted among the most abundant in the outlet. Other genera such as Bacteroides, Enterococcus, Parabacteroides, Streptococcus or Trichococcus decreased with the treatment. Ozonation was associated with the increase of members of the genera Acidovorax, Flavobacterium and Romboutsia and post-storage reactivation with the increase of Cloacibacterium and Flavobacterium and decrease of Romboutsia. Considering a group of 12 ARGs and MGEs, abundance decrease was observed after the activated sludge treatment and no impact was observed in the receptor river. The abundance (copies/mL) of the genes sul1 and blaOXA-58 increased after storage of the ozonated effluent up to 0.9 log-units.

Presumptive coliforms isolated (~400) on CCA (Chromogenic Coliform Agar) or on CCA supplemented with cefotaxime (4 mg/L) were identified as Escherichia coli, Klebsiella pneumoniae, Citrobacter spp., and other and 16.4% (72/439) were multidrug resistant. The whole genome sequence of Klebsiella pneumoniae and Citrobacter freundii recovered from the inlet, outlet, after ozonation and river suggest clonal dissemination, for example a K. pneumoniae ST29 with plasmids ColRNAI and incFIB(K) from the WWTP to the river.

ID418 | Investigating the Biofilm Matrix for Biofouling Assessment through a Multidisciplinary Approach

Author: Sofia R. Cruz

LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

E-mail: up201706271@fe.up.pt

Other authors: Laura Cerqueira, Ana Pereira, Darla Goeres

The Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA

Background: Biofouling is the undesired growth and accumulation of biofilms in industrial units, causing economic and public health consequences1. Despite the ubiquity of microbes, industrial design often prioritizes mechanical and chemical parameters over bioaccumulation. In cooling systems, biofouling can cause up to 50% reductions in heat transfer and 25% efficiency loss, largely due to corrosion, blockages, and excessive biocide use2,3. Pathogens embedded within biofilms can persist in cooling systems and be released into aerosols during tower operation, creating serious health risks. The persistence of biofilms is largely due to the extracellular polymeric substance (EPS) matrix, which serves as a scaffold and chemical shield, ensuring greater tolerance to standard biocides. However, this matrix remains under-characterized due to limited standardization of analytical methods. This research focuses on optimizing multiplex staining protocols to characterize the EPS matrix of biofilms, providing a methodological basis for future studies on how shear flow and biocide treatment impact matrix structure.

Methods: Klebsiella pneumoniae ATCC 43816 biofilms were cultivated on stainless steel coupons in microtiter plates at 37°C under 120 rpm agitation and sampled at 7 and 14 days. Analytical methods included optical coherence tomography (OCT) for structural assessment, rheology for mechanical properties, and confocal laser scanning microscopy (CLSM) with EPS-targeted stains for proteins, polysaccharides, and extracellular DNA.

Results: Early OCT results showed an average biofilm thickness of (205 ± 52) µm and a porosity of 42%. Rheology indicated a dominant elastic modulus, suggesting a gel-like mechanical behavior of the biofilms. CLSM imaging identified SYPRO Ruby as a promising stain for visualizing matrix proteins, and additional stains are being evaluated for broader matrix component targeting.

Conclusions: This work advances the development of standardized techniques for EPS matrix analysis by integrating biological, structural, and mechanical characterization. The complementary methods provide a holistic understanding of biofilms, establishing a foundation to guide industry in selecting the most effective anti-biofouling strategies.

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ID445 | Effects of Light Quality on Phycobiliproteins Production by LEGE-CC Cyanobacterial Strains

Author: Mariana Reimão

1 CIIMAR/CIIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto;

2 ESS, Polytechnic of Porto (ESS|P.Porto); 3 NGC, Kod-Bio; 4 FCUP – Faculty of Sciences, University of Porto

E-mail: mariana.reimao.silva@gmail.com

Other authors: Daniela Santos 1,4,5; Liliana Almeida 1,2,5; Nádia Eusébio 3; Vítor Vasconcelos 1,4; Marisa Freitas 2,1

1 CIIMAR/CIIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto;

2 ESS, Polytechnic of Porto (ESS|P.Porto);

3 NGC, Kod-Bio;

4 FCUP – Faculty of Sciences, University of Porto;

5 ICBAS – School oof Medicine and Biomedical Sciences, University of Porto

Background: Cyanobacteria are a group of photosynthetic microorganisms known for their high adaptability and resistance to various environmental conditions. These microorganisms are currently widely studied for their biotechnological potential and high commercial value. Numerous studies have emphasized the impact of abiotic factors on pigment production by cyanobacteria, particularly the quality of light. The aim of this study was to evaluate the influence of light quality on phycobiliproteins (PBPs) production by marine and freshwater cyanobacteria strains.

Methods: The cyanobacterial strains were obtained from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) and cultivated in Z8 medium. Strains were exposed to different light colours (white, red, blue and green) in a multi-cultivator for 10 days. Biomass was collected by centrifugation and freeze-dried. Aqueous extracts were prepared through cell rupture by freeze-thaw cycles (-20ºC and 25ºC, respectively). The PBPs content was measured spectrophotometrically, according to Bennett and Bogorad (1973)1.

Results: In general, the results demonstrate that most strains exhibited higher pigment yields under white light, with PBPs content ranging from 10 µg/mL to 350 µg/mL. Regarding coloured lights, red light was the most effective in enhancing PBPs production, with high yields across several strains. The LEGE 16657 strain responded positively to all lights in terms of PBP production, with the exception of blue light, which only increased total PBP production in the LEGE 11425 strain.

Conclusions: Overall, this study suggests that light quality is a critical parameter to be controlled in cyanobacteria cultivation for maximising PBPs production. Results also highlight the strain-specific nature of chromatic acclimation, even within the same genus, and underscores the importance of characterising spectral responses at the strain level when optimising pigment production.

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ID447 | Designing SynCom Reveals Soil and Compartment as Key Drivers

Author: Clarisse Brígido

1 MED Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, Universidade de Évora, Largo dos Colegiais 2, 7004-516, Évora, Portugal

2 Santarém Polytechnic University, School of Agriculture, Quinta do Galinheiro - S. Pedro, 2001-904 Santarém, Portugal

E-mail: ccb@uevora.pt

Background: Root-associated bacterial communities are vital for plant health, but the mechanisms guiding their assembly remain unclear. Quorum sensing (QS), a bacterial communication process, may regulate these dynamics, particularly during legume-rhizobium symbiosis, offering opportunities for targeted microbiome manipulation.

Methods: Clover (Trifolium subterraneum) plants were grown in three contrasting soils (Valverde, Fazendas de Almeirim, Mitra). Independent-culture and culture-independent approaches were combined to characterize microbiota composition across compartments (rhizosphere, rhizoplane, endosphere) and at two developmental stages (15 and 30 days).

Results: Community composition varied significantly with soil origin and compartment. Proteobacteria dominated the rhizoplane and endosphere, reflecting strong root-driven selection, while the rhizosphere exhibited greater phylum-level diversity enriched in Acidobacteriota and Chloroflexi. Valverde soils supported the highest overall diversity, whereas communities from Fazendas de Almeirim and Mitra were more strongly dominated by Proteobacteria. Plant developmental stage (15 vs. 30 days) exerted little influence on phylum-level composition. Approximately 300 bacterial isolates were obtained from the rhizoplane, almost all taxonomically identified by 16S rRNA sequencing. This collection, spanning more than 50 genera, together with the identified core microbiome mainly composed of Rhizobiales, Burkholderiales, Flavobacteriales, Solirubrobacteriales, and Bacillales, is being used to build the SynCom.

Conclusions: Soil origin and root compartment are primary drivers of clover microbiota assembly, while plant age has a minor role. The rhizoplane culture collection enables the design of a representative SynCom that will be used within the SensiRoot project to dissect how QS regulates microbiota dynamics during the establishment of Trifolium subterraneum-Rhizobium leguminosarum symbiosis.

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ID458 | Stepwise Acclimation of Bacteria for COD Removal from Industrial Wastewater

Author: Ana Paula Chung

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.

E-mail: ana.chung@uc.pt

Other authors: Eva Domingues2, Alexandra Fortunato3, Joana Bidarra3, and Paula Morais1

1 University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.

2 University of Coimbra, CERES, Department of Chemical Engineering, Rua Silvio Lima, Polo II, 3030-790 Coimbra, Portugal.

3 SRAMPORT, Mechanical Transmissions, Ltd, Rua António Sérgio 15, 3025-041 Coimbra, Portugal.

Industrial wastewater often contains high concentrations of organic pollutants, including oils, grease, and detergents, resulting in chemical oxygen demand (COD) values that frequently exceed regulatory discharge limits. Biological treatment through bioaugmentation with bacteria capable of degrading complex and recalcitrant organic matter offers a promising alternative for COD removal when conventional methods are insufficient. This study aimed to assess the effectiveness of three lipid- and surfactant-degrading bacterial strains (Diaphorobacter sp., Pseudomonas sp., and Collimonas sp.), subjected to sequential acclimation cycles, for COD reduction in wastewater from a bicycle component manufacturing industry.

A sequential, three-cycle adaptation was performed by exposing the selected strains to industrial wastewater. In the first cycle, the strains were inoculated into wastewater diluted 1:1 with either R2B medium or a 0.02% KHnPOn solution. Subsequent cycles used undiluted wastewater, with or without 0.02% KHnPOn. Each cycle included assays in non-sterile and filter-sterilized wastewater, incubated at 25°C and 150 rpm for 4 days. COD was determined according to the standard method 5220D. After the final adaptation cycle, the microbial community structure was profiled using Illumina-based 16S rRNA gene sequencing to evaluate the persistence of inoculated strains and community shifts.

The stepwise acclimation strategy using the selected bacterial strains resulted in consistent COD reductions across all adaptation cycles compared to the initial values of 1880-2000 mg/L in the industrial wastewater. By the third cycle, COD values in all treatments were below the permissible limit of 1000 mg/l, except Diaphorobacter sp. in sterilized wastewater supplemented with phosphate. Overall, Collimonas sp. showed the most consistent performance across all conditions, reaching a maximum of 68 % of COD removal.

Metagenomic profiling of the third adaptation cycle showed that bioaugmentation reshaped the microbial community, with Collimonas sp. and Pseudomonas sp. establishing stable populations that correlate with the observed COD removal efficiencies. While Diaphorobacter sp. was not detected at the genus level, related taxa were present, suggesting functional contribution to pollutant degradation.

These results demonstrate that sequential acclimation supports the persistence of bioaugmented strains and enhances the biological treatment of complex industrial wastewater.

ID459 | Heavy metal biosorption by *Ochrobactrum tritici* 5bvl-1: role of cell viability and EPS

Author: Ana Paula Chung

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.

E-mail: ana.chung@uc.pt

Other authors: Luciano Chá Chá and Paula Morais

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.

Heavy metals are naturally occurring elements that are widely used in industrial and technological applications, and in biological systems, where they can act as enzymatic cofactors. However, their high toxicity at low concentrations and high solubility pose a significant threat to public health and ecosystems. Developing effective, low-cost and sustainable methods for their removal is therefore a priority. This study aimed to evaluate the efficiency of bacterial biosorbents produced from Ochrobactrum tritici 5bvl-1 in the biosorption of heavy metals, in particular Fe³n ions, under different environmental conditions.

Four types of bacterial biosorbents were produced using R2AB culture medium and HS medium to promote the production of extracellular polymeric substances (EPS). To obtain inactive biomass, part of the cultures was autoclaved. This resulted in four biosorbent types: Live (L), Live with Polymer (LP), Dead (D) and Dead with Polymer (DP). Batch biosorption assays were carried out, varying parameters such as pH and initial metal concentration. Metal removal was quantified using colorimetric assays and ICP-MS. Morphological and chemical characterization was performed by SEM-EDS and FTIR-ATR, and the data were analyzed with kinetic and isotherm models.

Biosorbents composed of dead biomass (D and DP) showed higher biosorption capacities (qe) compared to those with live biomass (L and LP). Additionally, EPS- enriched biosorbents (LP and DP) presented lower qe values when compared to their counterparts without EPS stimulation (L and D). Optimal biosorption was obtained at pH 2.5-3.0 and initial Fe³n concentrations above 1.5 mM, with most Fe³n removal occurring within the first 60 minutes. The process followed a pseudo-second-order kinetic model and was best described by the Langmuir isotherm, with biosorbent D exhibiting the highest maximum biosorption capacity (18.71 mg/g). In multi-metal systems, the biosorbents demonstrated higher affinity for Fe³n ions, followed by Cu²n and Ni²n. FTIR-ATR and SEM-EDS analyses confirmed the binding of Fe³n to functional groups on the biosorbent surface.

These findings highlight the potential of biosorbent D, from O. tritici 5bvl-1, as a sustainable and effective alternative for heavy metal removal. Its effectiveness across different conditions reinforces its applicability for bioremediation strategies in contaminated environments.

ID469 | Advancing Crop Resilience and Soil Health through Agroecological Practices

Author: Sofia I.A. Pereira

1Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327 4169-005 Porto, Portugal

E-mail: sapereira@ucp.pt

Other authors: Ana S.S. Sousa1, Mariana Godinho1, Manuel Oliveira2, Rui Soares3, Paula M.L. Castro1
1Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327 4169-005 Porto, Portugal;
2ADVID - Associação para o Desenvolvimento da Viticultura Duriense | CoLAB Vines & Wines, Vila Real, Portugal; 3Real Companhia Velha, Portugal

Background: The transition toward sustainable agriculture calls for innovative strategies that not only enhance crop productivity but also safeguard soil fertility, biodiversity, and long-term ecosystem health. Agroecological (AE) practices, including crop diversification, conservation tillage, and integrated pest management, play a pivotal role in fostering resilient and productive agricultural systems that balance agricultural needs with environmental responsibility. Within this framework, biofertilizers provide a promising strategy, enhancing nutrient efficiency, growth, and yields while reducing reliance on synthetic inputs. Similarly, cover crops improve soil fertility, water retention, and biodiversity, strengthening overall system sustainability.

Methods: Within this framework, biofertilizers and cover crops have been applied across various crops, including vineyards, maize, and tomato, within several EU-funded projects. The PRIMA project ReCROP investigated biotechnological tools and sustainable farming practices to enhance

crop resilience and soil biodiversity in mediterranean farming systems. The project Sun2Fork explores solar-driven technologies and microbial inoculants in soil less systems to boost tomato salinity tolerance. More recently, the VinAE project is establishing pilot vineyards in 5 major wineproducing countries to assess the effects of AE practices on grapevine performance, must quality, water use efficiency, and microbial and faunal diversity. In Portugal, fieldwork at Quinta dos Aciprestes (RCV) is testing hydrogels (alone and in combination with cover crops) together with microbial inoculants.

Conclusion: Together, these projects demonstrate how integrating microbial technologies with sustainable farming practices can drive climate-smart agriculture, improve soil functionality, and strengthen the long-term resilience and security of food production.

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ID473 | Exploring Bacteria-Microalgae Interactions to Enhance *Nannochloropsis* oceanica Productivity

Author: Manuel António Fernandes Murtinho

Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005 -139 Faro, Portugal

E-mail: mamurtinho@ualg.pt

Other authors: Tamára Santos1, Veronica Rossetto1, Inês Maia1, Hugo Pereira2, João Varela1,2

1 Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005 -139 Faro, Portugal; 2 GreenColab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, 8005-139, Portugal

Background: Microalgae produce valuable bioactive compounds, including carotenoids, vitamins, proteins, and lipids, with applications in food, feed, cosmetics, biofuels, fertilisers, and pharmaceuticals. The genus Nannochloropsis is particularly relevant due to its high lipid productivity, fast growth rates, and efficient biomass accumulation, and is widely used in aquaculture for animal feed and rotifer rearing. Recent studies have highlighted the role of bacteria in shaping microalgal performance, particularly in terms of productivity and biomass quality, although these interactions remain insufficiently understood. This project investigates how bacterial communities associated with Nannochloropsis oceanica can influence microalgal growth and biomass quality.

Methods: A collection of 43 bacterial strains isolated from the industrial production of N. oceanica cultures was identified using molecular approaches, including DNA extractions and 16S rDNA sequencing. Co-cultivation assays were performed between selected bacterial isolates and N. oceanica to evaluate their effects. Growth dynamics were monitored, while biochemical analysis (fatty acid profile, lipid, and protein content) provided insights into the impact of these interactions on N. oceanica biomass quality.

Results: The identification of the bacterial isolates at the species or genus level was achieved. In the co-cultivation trials, bacteria belonging to the genera Marinobacter, Pseudoalteromonas and Corynebacterium showed positive effects on the growth of N. oceanica, while isolates from Alcaligenes, Brevibacterium and Oceanobacillus had neutral effects. Out of the 43 bacterial isolates tested, only one exhibited a negative impact on the microalga. These findings support the hypothesis that bacterial interactions influence both the growth and biochemical composition of N. oceanica biomass. While certain isolates may have neutral or detrimental effects, others not only stimulate algal growth but may also enhance lipid or protein accumulation. Moreover, beneficial outcomes could depend on combinations of bacterial strains, reflecting the complexity of microbial consortia in both natural and industrial environments.

Conclusions: This study contributes to a better understanding of bacterial-microalga interactions and may provide new tools to optimize the production of N. oceanica.

ID476 | Bioinoculants as a strategy to mitigate water deficiency in maize growth

Author: Mariana Godinho

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

E-mail: msgodinho@ucp.pt

Other authors: Paula M. L. Castro1, Sofia I. A. Pereira1

1Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

Background: Intensive use of inorganic fertilizers to meet growing food demand has become a major threat to soil health and long-term agricultural productivity. At same time, drought remains a global challenge due to its severe impact on crop yields. Annual crops of economic relevance (e.g. maize) are particularly vulnerable. Plant growth-promoting bacteria enhance maize tolerance to drought stress by improving nutrient uptake and water retention, offering an eco-friendly farming solution. This study aimed to evaluate the effect of bioinoculation on maize growth under two different water regimes.

Methods: Bacterial strains from the CBQF, ESB-UCP collection were pre-selected for their plant growth-promoting traits, namely siderophore production and P solubilization. Further characterization included indole-3-acetic acid production, osmotic stress tolerance, extracellular enzyme activity, and mutual biocompatibility. Six strains, Bacillus pumilus 1Z1 (B1), Priestia aryabhattai LS 1-2 (B2), Bacillus pumilus ST2-22 (B3), Bacillus amyloliquefaciens LR 1-9 (B4), Pseudomonas graminis LS 3-5 (B5) and Pseudomonas fluorescens S3X (B6) were inoculated in pots containing agricultural soil, where maize was grown under well-watered (20% Volumetric water content, VWC) and moderate water-deficient conditions (12% VWC).

Results: Overall, water deficiency reduced fresh and dry shoot biomass. Under moderate water deficiency, bioinoculation enhanced dry shoot biomass compared to non-inoculated plants, with increases of 35, 13 and 12% in plants inoculated with strains B1, B5, and B6, respectively.

Conclusions: Bacterial inoculation alleviated the negative effects of moderate drought stress on maize growth, highlighting the potential of bioinoculants as a sustainable strategy to maintain crop productivity under water-limited conditions.

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ID477 | FLEXIZYME: development of tailor-made enzymes for fatty amines production

Author: Javier Buchó

Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE) Carrer d'Albert Einstein, 1, 46980 Paterna, Valencia, Spain

E-mail: javier.bucho@itene.com

Other authors: Annabel Serpico; Maria Carmen Tronch; Rosa Domenech

Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE) Carrer d'Albert Einstein, 1, 46980 Paterna, Valencia, Spain

Background: FLEXIZYME centres on the enzymatic transformation of waste-derived substrates into fatty amines (FA). Initially, fatty acids are extracted from lipid-rich residues and converted into fatty aldehydes through carboxylic acid reductase (CAR) catalysis. These aldehydes then undergo amination reactions mediated by transaminases (TAs) to form primary FA. Waste streams such as coconut, palm, and high-oleic sunflower oils serve as fatty acid sources, while lentils and beans provide amino acid. A major focus is on the discovery, engineering, and optimisation of novel TA enzymes capable of operating efficiently under industrial conditions. Candidate TAs have been identified via in silico mining, literature screening, and enzyme libraries available within FLEXIZYME's consortium. TAs are currently being evaluated through comprehensive characterisation studies, including substrate specificity profiling, amino donor specificity, solvent tolerance testing, kinetic modelling, and thermostability assessments.

Methods: Analytical methods (LC-MS/MS) have been optimised for FAs detection and deployed to determine conversion yields of various products (C12-C18) for the different TAs. Preliminary results suggest that, amongst the screened TAs (15 TAs), 2 are promising candidates for medium and long chain length fatty amines production. A Design of Experiment is currently ongoing to determine and optimise critical parameters for optimal yields such as amino donor concentration, enzyme load, time, temperature, etc.

Results: Screening efforts have identified promising TA candidates with high activity towards hydrophobic fatty aldehydes of various chain length (C12-C18), demonstrating the capacity to achieve conversion into FAs. The objective is to achieve conversion rates exceeding 80% under optimised conditions.

Conclusions: FLEXIZYME aims to demonstrate that transaminase-driven biocatalysis offers a sustainable and economically viable pathway for FA production from renewable waste. By enhancing discovery and optimisation of robust TAs, and leveraging advanced digital tools for smart process management, the project paves the way for the industrial adoption of green chemistry solutions. This work significantly contributes to the development of circular bioeconomy models, offering industries a high-performing, environmentally responsible alternative to conventional petrochemical methods.

ID480 | Microbial dynamics in a changing ocean

Author: Joana Barcelos e Ramos

University of the Azores, IITAA, Group of Climate, Meteorology and Global Change

E-mail: joana.b.ramos@uac.pt

Other authors: inês de castro1, susana chaves ribeiro1, francisco coelho2, antónio louvado2, newton gomes2, eduardo brito azevedo1

1 University of the Azores, IITAA, Group of Climate, Meteorology and Global Change;

2 Department of Biology and Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal

Background: Interactions between marine microbes are crucial to maintaining the equilibrium between carbon sinks and recycling, with implications for climate change. Still, their role in microbe's responses to the changing ocean is poorly understood.

Methods: This study examined the impact of shifts in carbonate chemistry, driven by elevated COn and altered alkalinity, on the composition and diversity of microbial communities. To link mechanistic understanding with ecological relevance, the research examined both simplified and natural communities. In particular, it assessed the response of a community composed of Gephyrocapsa huxleyi (G. huxleyi) and two bacterial species, Idiomarina abyssalis and Brachybacterium atlanticum, under increasing COn levels. Results: While species growth was influenced by co-existence, the effect of COn on G. huxleyi's cellular composition was only amplified in the presence of Idiomarina abyssalis. In natural communities exposed to rising COn concentrations, similar, but not identical, patterns emerged in community composition.

Conclusions: The lessons learned with global change experiments hinted at, but did not entirely predict, the small effect on phytoplankton abundance and the most sensitive bacterial groups under ocean alkalinity enhancement (OAE). Although necessary, strategies such as OAE need to be further tested to ensure that they do not cause more harm than benefit.

ID481 | Plant Growth-Promoting Bacteria Recovered from Winery Wastes

Author: Mariana Godinho

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

E-mail: msgodinho@ucp.pt

Other authors: Paula M. L. Castro1, Fátima Gonçalves2, Luísa Moura3,4, Rui Pinto3, Isabel Mourão4,5, Luís Miguel Brito4,5, Sofia I. A. Pereira1

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

2 Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade de Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal;

3 CISAS – Center for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Álvares, Viana do Castelo 4900–347, Portugal;

4 Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, R. D. Mendo Afonso, 147, Refóios, Ponte de Lima 4990-706, Portugal;

5 CIMO, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus Santa Apolónia, Bragança 5300-253, Portugal

Background: Wine industry generates by-products including grape stalks and washing sludge which are often burned, thus becoming an economically and environmentally unsustainable practice. A promising alternative is to reuse these wastes through composting. Isolating plant growth-promoting bacteria (PGPB) from composted wine residues represents a valuable strategy, as both PGPB and compost may enhance plant growth and soil health. This study aimed to isolate and identify bacteria from different composting piles and assess their ability to produce plant growth-promoting (PGP) substances.

Methods: Several bacterial isolates were isolated from composting piles using the serial dilution method and grown in specific culture media (e.g. King B). Seventy-four isolates were characterized according to Gram staining, cytochrome oxidase and catalase activities. In addition, their capacity to produce PGP substances (e.g. P solubilization, N2 fixation) was evaluated. Based on their ability to produce PGP substances, 23 isolates were screened for indol-3-acetic acid (IAA) production and identified using 16S rRNA sequencing. Results: The 16S rRNA sequencing revealed that nearly 40% of the isolates were identified as Bacillus, followed by 26% as Pseudomonas and 13% as Cytobacilllus. Isolates exhibiting the highest IAA production belonged primarily to the genera Bacillus, Brevibacillus and Agrobacterium, highlighting their potential as key PGPB. Additionally, results showed that nearly 11% of the isolates presented multiple PGP traits, including P solubilization, N2 fixation, and siderophores production. Only 7 isolates were able to synthesize multiple extracellular enzymes.

Conclusions: Composting of sludge and wine stalks from wine industry enabled the isolation and characterization of several bacterial strains with potential as plant growth promoters.

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ID484 | Allelopathic activity of cyanobacteria on green microalgae and other cyanobacterial strains

Author: Ana Rebelo Vieira

1-CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental), Terminal de Cruzeiros do Porto de Leixões, 4450-208, Matosinhos

E-mail: avieira@ciimar.up.pt

Other authors: Pedro Nuno Leão 1

1-CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental), Terminal de Cruzeiros do Porto de Leixões, 4450-208, Matosinhos

Cyanobacteria are photosynthetic bacteria widely distributed in terrestrial and aquatic environments and are recognized as rich sources of natural products (NPs) with potential therapeutic and agrochemical applications. However, the ecological or physiological roles of most metabolites remain poorly understood. One key interaction is allelopathy, where organisms release biochemicals that influence the growth, survival, and development of others. Cyanobacteria produce allelochemicals that provide competitive advantages, shaping community dynamics. Understanding these interactions can improve knowledge of phytoplankton ecology and support the discovery of novel allelochemicals for the biological control of harmful algal blooms. Despite reports of potentially allelopathic genera, few cyanobacterial allelochemicals have been identified.

To uncover new allelochemicals and assess their biological activity, we established a microscale screening method (adapted from Leão et al., 2009) to test over 50 strains from our in-house LEGE-CC culture collection. Culture media from these strains were assayed against green microalgae and bloom-forming cyanobacteria (Anabaena and Microcystis). Up to three strains exhibiting clear allelopathic effects will be selected for large-scale cultivation, followed by compound isolation and structural elucidation using chromatography, mass spectrometry, and NMR. The purified metabolites will then be tested in ecologically and pharmacologically relevant assays.

So far, four strains have shown allelopathic activity, notably Brasilonema sp. LEGE 16502 and Hapalosiphon sp. LEGE 17501, which strongly inhibit Microcystis aeruginosa LEGE 12461, a bloom isolate from a pond in Brazil. We hypothesize that this effect is mediated by allelochemicals, which we aim to identify and isolate. In summary, this project leverages the LEGE-CC collection to explore cyanobacterial allelopathic interactions and to identify bioactive compounds, with potential applications for the sustainable control of harmful cyanobacterial blooms.

ID489 | Timescale variations and circulation of bacteria in a treated wastewater-based aquaponics system

Author: Sílvia Ferreira

Universidade Católica Portuguesa, Porto, Portugal

E-mail: s-sfsferreira@ucp.pt

Other authors: Inês Leão 1, Verónica Camelo 1, G.P. Di Sansebastiano 2, Olga Nunes 3, Célia M. Manaia 1

1- Universidade Católica Portuguesa, Porto, Portugal;

2- Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A.), University of Salento, Campus Ecotekne, 73100 Lecce, Italy;

3- LEPABE- Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Background: Aquaponics is a sustainable farming system integrating plant and fish production in recirculating water. Water quality is a major safety issue, and the reuse of treated wastewater is an important and challenging innovation. Although it may mitigate water scarcity, especially in arid regions, it can carry potential health hazards. This study evaluated microbial abundance, community composition, and an antibiotic resistance proxy to infer potential microbiological risks associated with treated wastewater. Methods: Two 3-month cycles of a full-scale aquaponic system producing lettuce and tilapia were monitored at the start (T0) and end (T2). Systems S2&S3 used treated wastewater, while S1 used tap water. Samples from water, fish (gut, gills), lettuce roots and biofilter rocks were collected and analysed. Bacteria were desorbed by water sonication and concentrated by filtration prior to cultivation or DNA extraction. Bacterial communities were examined based on 16S rRNA metabarcoding, isolates by MALDI-TOF (n=177), and the genes 16S rRNA and class I integron integrase (intI1) were quantified by realtime PCR. Results: Bacterial abundance in water (16S rRNA gene) slightly increased in S1, remaining stable in S2&S3. The same pattern was observed in tilapia gut, while in gills, bacterial abundance decreased in S2&S3. The antibiotic resistance proxy intI1, detected in tilapia and lettuce at T0, was below the limit of quantification at T2 in roots and gut. However, in gills intI1 increased from <LOQ at T0 to ~3.0 log-units/g at T2 in S2&S3, being below detection in S1. This gene was observed to accumulate in biofilter, suggesting its recirculation in water. The bacterial community composition suggested the influence of treated wastewater with higher relative abundance of Plesiomonas (26% vs 3.4% in S1) and Shewanella (1.3% vs 0.01%) in S2&S3 water compared to S1. The bacterial community composition of lettuce roots was also different in S2&S3 versus S1, contrasting with the stable tilapia communities. Biofilters, receiving fish debris, presented a bacterial community close to that of fish. Bacteria isolates (e.g. Aeromonas jandaei, A. veronii, Mycobacterium fortuitum and Plesiomonas shigelloides) include potential pathogens or probiotic organisms. Conclusions: Aquaponic systems fed with treated wastewater remained stable over time, although, despite the high quality of treated wastewater, intI1 increased in fish gills and biofilters after 3 months.

ID490 | New pSEVA plasmids for heterologous expression of cyanobacterial secondary metabolites

Author: Diana Sousa

1 Cyanobacterial Natural Products Team, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal; 2 ICBAS – School of Medicine and Biomedical Sciences of the University of Porto, Porto, Portugal

E-mail: dianar2sousa@gmail.com

Other authors: Adriana Rego1, Eunice Ferreira1, Raquel Castelo-Branco1, Cloé Cantor1,3, Konrad Viehrig1, Paulo Oliveira1,4 and Pedro Leão1

1 Cyanobacterial Natural Products Team, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal;

3 Faculty of Pharmacy of Nancy/National Higher School of Agronomy and Food Industries, Nancy, France;

4 Biology Department, FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Background: Cyanobacteria are prolific producers of structurally diverse natural products (NPs). Capturing and heterologously expressing biosynthetic gene clusters (BGCs) from uncultured cyanobacteria can enable access to novel chemical diversity in these organisms.

Methods: Metagenomics was used to recover cyanobacterial metagenome-assembled genomes and uncover their biosynthetic potential. Long-amplicon PCR was used to capture a BGC from eDNA for heterologous expression. Homology cloning techniques were used to assemble new plasmids for heterologous expression in multiple hosts.

Results: Here, 86 metagenome-assembled genomes were retrieved from 20 environmental samples collected by the lab. Bioinformatics analysis revealed 1354 biosynthetic regions of interest. E. coli was chosen as host for the heterologous expression of a metagenome-derived microviridin BGC due to its high growth rates and vast genetic manipulation toolbox available. Expression on the referred host failed to produce the expected final metabolite evidencing either sub-optimal expression or lack of function of cyanobacterial enzymes. Switching expression to a cyanobacterial model strain host, such as Anabaena sp. PCC 7120 (Anabaena), would require the assembly of the BGC into a new vector. Hence in this work, new self-replicative plasmids for the heterologous expression of cyanobacterial BGCs in both E. coli and cyanobacteria model hosts were developed. The backbone of pSEVA251 was used. Heterologous expression of cyanobacterial BGCs using pSEVA251 as backbone was validated, in both E. coli and Anabaena, through the expression of a RiPP secondary metabolite under the regulation of the tetracycline inducible promoter PtetO. For the final plasmid constructs, the multiple cloning site (MCS) was excised. On the MCS location, the inducible promoters PnirA, Prha and PtetO were assembled. To facilitate host exchange, a restriction sequence (RS) was added downstream of the promoters to allow swapping both promoter and antibiotic resistance. GFP expression experiments in E. coli and Anabaena, underscored the need for a ribosomal binding site downstream of the RS. Currently, the final set of nine new self-replicative plasmids is being assembled.

Conclusions: Metagenomics results further evidence the huge biosynthetic potential of uncultured cyanobacteria. The newly developed plasmids for heterologous expression may assist the exploitation of the chemical diversity encoded in these microbes.

ID492 | Ibuprofen as a non-antibiotic modulator of Escherichia Coli phenotype and conjugation profile

Author: Maria Gomes

Universidade Católica Portuguesa, Porto, Portugal

E-mail: s-mmfgomes@ucp.pt

Other authors: Stefano Andrea Ferri 1, Manuela Antonelli 2, Célia M. Manaia 1

1- Universidade Católica Portuguesa, Porto, Portugal;

2- Politecnico di Milano, Milano, Italy

Background: Antibiotic resistant bacteria are a threat to public health. Although noticed mostly in clinical settings, these bacteria can occur in the environment, and it has been hypothesized that pollution may trigger their spread. This study used a model strain, the clinical isolate Escherichia coli CL218 (carrying blaKPC integrated in an incN plasmid and an incF plasmid), and a model non-antibiotic pollutant, ibuprofen, to test such hypothesis.

Methods: Two approaches were used: 1) The repeated subculturing (30 times) of isolate E. coli CL218 under exposure to ibuprofen (0; 6.25; 50 mg/L) on mineral medium supplemented with glucose. Alterations after successive subculturing were assessed based on nutritional profile (API 50CH, bioMérieux), antibiotic susceptibility, conjugation

frequency and profile, among other. 2) A full factorial design of experiments (2 levels, 3 factors) to evaluate individual and interactive effects of temperature (28ºC and 40ºC), ibuprofen (0; 6.25 and 50 mg/L), and dimethyl sulfoxide (DMSO, 0, 10% v/v) on conjugation frequency (with E. coli J53) and transconjugants genotype.

Results: Ibuprofen exposure did not lead to evident phenotype or genotype alterations, being additional phenotypic characterization still ongoing. Conjugation assays consistently showed that the plasmid harboring blaKPC, incN, was successfully transferred in all assays, while incF rarely was detected in the recipient strain. The only exceptions were observed at 40°C without ibuprofen/DMSO. As expected, DMSO, that contributes to permeabilize the cell membrane, enhanced conjugation frequency. In contrast, ibuprofen alone was observed to reduce the conjugation frequency, except at 40°C and 50 mg/L. An interaction effect between ibuprofen and DMSO was observed only at 40°C, suggesting a temperature-dependent modulation of compound interactions. Conclusions: Overall, these results suggest that while ibuprofen does not induce noticeable phenotypic or genotypic changes in E. coli, it can modulate plasmid conjugation in a temperature and condition-dependent manner, warranting further investigation into its role in horizontal gene transfer.

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ID503 | Antibacterial activity and inhibition of biofilm formation by selenium nanoparticles against bacteria isolated from diseased fish in Nigerian aquaculture farms

Author: Marta Tacão

CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: martat@ua.pt

Other authors: Marta Alves1,2, Francisca Pereira2, Rafael Tavares1,2, J.N. Ogbulie3, E.O. Ajima3, Victor Galhano1,2, Bruno Nunes1,2

1 CESAM, University of Aveiro, Portugal;

2 Department of Biology, University of Aveiro, Portugal; 3 Institute of Environmental Health and Environmental Justice, Federal University of Technology, Owerri, Nigeria

Background: Aquaculture is expanding in low- and middle-income countries, where bacterial diseases constrain fish health and productivity. In Nigeria, one of Africa's leading tilapia and catfish producers, outbreaks cause major losses, aggravated by multidrug-resistant bacteria and the potential presence of antibiotic residues in fish, affecting both target and non-target species. This study evaluated the antibacterial and antibiofilm efficacy of selenium nanoparticles (SeNPs) against bacteria from diseased fish as an antibiotic alternative.

Methods: Sixteen bacterial isolates were obtained from diseased tilapia and catfish from Nigerian farms. Isolates were identified by 16S rRNA gene sequencing, and antibiotic susceptibility testing was performed using disk diffusion against 3–5 antibiotic classes. Antibacterial activity of chemically synthesized SeNPs was evaluated by microdilution (≤100 µg of total Se /mL) to determine minimal inhibitory (MIC) and bactericidal concentrations (MBC). Culture collection strains were included. Biofilm formation was quantified using crystal violet microtiter assay. Medium-to-strong producers were tested in antibiofilm assays with 100 µg Se/mL.

Results: Isolates were affiliated to Aeromonas (2), Proteus (2), Pseudomonas (2), Bacillus (4), and Klebsiella (6). Except for Bacillus strains, all isolates were resistant to at least 1 antibiotic, and 4 were classified as multiresistant (3 Klebsiella, 1 Proteus). SeNPs inhibited growth of 2 Bacillus isolates and Vibrio vulnificus ATCC 27562 (MIC = 50 µg Se /mL) and Staphylococcus aureus ATCC 6598 (MIC = 12.5 µg Se

/mL), while showing bactericidal activity against Vibrio harveyi DSM 19623 (MIC = 25 µg Se /mL; MBC = 100 µg Se /mL). For the remaining, MIC exceeded 100 µg Se /mL. Biofilm assays revealed 4 moderate producers (3 Klebsiella sp., 1 Proteus sp.), with significant but limited biofilm inhibition by SeNPs ($p < 0.05$, 12.1–13.0%). Regarding the 2 strong biofilm producers, S. aureus ATCC 6598 biofilm was significantly reduced by 46.7 ± 5.7% ($p < 0.05$); in contrast, Escherichia coli ATCC biofilm significantly increased ($p < 0.05$, 57.8 ± 19.6%).

Conclusions: SeNPs exhibited antibacterial activity against diseased-fish isolates and culture collection strains. At the tested concentration, SeNPs significantly reduced biofilm formation in strong and moderate producers, including multiresistant strains, indicating potential for treating persistent infections in fish farms.

ID509 | Impact of Sludge Retention Time on Microbial Communities, ARGs, and MGEs in in CAS systems

Author: Sara Ribeirinho Soares

1LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

2ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

E-mail: saramariasoares@hotmail.com

Other authors: Sofia Cabral3; Vasco Braga4; Vítor J.P. Vilar5,2; Célia M. Manaia6; Olga C. Nunes1,2

1LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

2ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

3Luságua Serviços Ambientais, S.A., Avenida Marechal Gomes da Costa, 1800-255 Lisboa, Portugal

4Efacec Engenharia e Sistemas S.A., Rua Engenheiro Frederico Ulrich, 4470-605 Maia, Portugal

5LSRE-LCM – Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

6Catholic University of Portugal, CBQF - Centre for Biotechnology and Fine Chemistry – Associate Laboratory, Superior School of Biotechnology, Rua de Diogo Botelho 1327, 4169-005 Porto, Portugal

Background: Wastewater treatment plants are key barriers against antimicrobial resistance dissemination but can also create opportunities for antibiotic-resistant bacteria (ARB), resistance genes (ARGs) and mobile genetic elements (MGEs) to persist and interact. Conventional activated sludge (CAS) is the most common biological treatment technology, and solids retention time (SRT) is a major factor shaping microbial community composition and treatment performance.

Methods: Two parallel lab-scale CAS reactors were operated for 12 weeks under identical conditions, differing only in SRT (7 vs 30 days). Monitoring included physicochemical parameters (COD, ammonium, nitrate, total nitrogen), cultivable bacterial indicators (heterotrophs, Enterobacteriaceae, E. coli), and absolute and relative abundances of 16S rRNA, uidA, intI1, qnrS, IS26_1, IncP oriT, Tn5403 and ISPps in influent, effluent and sludge. Microbial community profiling (16S rRNA sequencing), functional predictions, and co-occurrence network analyses were used to evaluate structural and genetic responses to SRT. Results: The 30-day SRT reactor achieved higher ammonium and total nitrogen removal, though with nitrate accumulation. Enterobacteriaceae removal was improved, enhancing effluent microbiological quality. Likewise, the relative abundance of MGEs was also lower in the 30-day SRT treated effluent compared to 7-day SRT treated effluent. The 30-day SRT sludge had lower absolute 16S rRNA, intI1 and qnrS gene copy numbers than the 7-day SRT sludge. However, 30-day SRT sludge showed higher relative abundance of intI1, uidA, qnrS and all the analysed MGEs than the 7-day SRT sludge.

Taxonomic analysis showed enrichment of Burkholderiaceae and Anaerolineaceae in 30-day SRT system effluent, while 7-day SRT favoured Mycobacteriaceae. Functional predictions indicated broader metabolic potential under 30-day SRT, especially for carbohydrate metabolism, nitrogen cycling and xenobiotic degradation. Networks showed dense co-occurrence between ARGs and MGEs supporting the role of MGEs for antibiotic resistance dissemination.

Conclusions: Extended SRT improved nutrient removal and effluent safety but favoured the relative abundance of ARGs and MGEs in sludge. SRT optimisation must be combined with sludge treatment strategies to maximise efficiency while mitigating resistance dissemination risks.

ID513 | Microbial-Enhanced Phytoremediation of Cadmium and Ketoprofen in Estuarine Sediments: Microcosm and Mesocosm Validation

Author: Rafaela Perdigão

CIIMAR /CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

E-mail: rafaela.mendes@ciimar.up.pt

Other authors: Julio Merchán1,2, Margarida Pereira1,3, Joana P. Fernandes1,3, Loreto García1,3, C. Marisa R. Almeida1,3 and Ana P. Mucha1,3

1 CIIMAR /CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

2 Joint Master Programme in Marine Biotechnology, EU-CONEXUS;

3 FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Estuaries are vulnerable ecosystems where pollutants like pharmaceuticals and metals can accumulate and persist. A sustainable strategy to mitigate these contaminants involves combining bioremediation and phytoremediation, particularly using autochthonous microorganisms. This study aimed to optimize and validate an autochthonous microbial consortium for the removal of cadmium (Cd) and the pharmaceutical ketoprofen (KPF) in phytoremediation experiments, first in microcosms and then in mesocosms. The consortium, composed of four bacterial strains (3 Pseudomonas sp. and 1 Hydrogenophaga sp.), showed individual degradation capacities of ~50–90% of 1 mg/L KPF in minimal salts medium supplemented with sodium acetate over two weeks. In 7-day microcosm experiments with Juncus maritimus plants and rhizosediments from the Douro river estuary, treatments included exposure to Cd (3 mg/L), KPF (0.6 mg/L), both pollutants, alongside abiotic and adsorption controls. Bioaugmentation significantly enhanced KPF removal under mixed contamination (70% vs. 30% in natural attenuation), while the J. maritimus rhizosediment system effectively removed Cd from elutriates (80–95%), regardless of bioaugmentation. Changes in microbial communities, as well as KPF concentrations in plants and rhizosediments, are currently being assessed. A 3-month mesocosm experiment is underway to validate the pollutant removal capacity of the same consortium under more realistic conditions, using J. maritimus and 4.5 kg of rhizosediments from the Douro river estuary. Tidal conditions are simulated by flooding the mesocosm containers daily for 6 hours with artificial brackish water (10% NaCl) enriched with nutrients. Treatments include Cd (1.3 µg/g sediment; added once), KPF (0.1 µg/g sediment; added weekly), and their combination, with and without bioaugmentation (applied biweekly). Control containers without contaminants were also included to represent natural conditions. Samples are being collected to monitor Cd and KPF concentrations in water, sediment, and plants, as well as to assess microbial community composition in sediments. Our findings will contribute to evaluating the feasibility of integrating bioaugmentation with phytoremediation to address mixed contamination by metals (Cd) and pharmaceuticals (KPF) in estuarine environments.

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ID516 | Effect of biodiverse cover crop mixtures on soil microbiological indicators in a maize cropping system

Author: Adélia Varela

1 Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P.), Oeiras, Portugal; 2 GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal

E-mail: adelia.varela@iniav.pt

Other authors: Anton Denysov 1, Pablo Pereira 1,2, Ricardo Soares 1, Isabel Videira e Castro 1,2, Ana Barradas 3, Ana Paula Nunes 4, Maria do Céu Godinho 5, Paula Fareleira 1,2

1 Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P.), Oeiras, Portugal;

2 GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal;

3 Fertiprado – Sementes e Nutrientes Lda., Vaiamonte, Portugal;

4 COTHN - Centro Operativo e Tecnológico Hortofrutícola Nacional – Centro de Competências, Alcobaça, Portugal; 5 Instituto Politécnico de Santarém - Escola Superior Agrária, Santarém, Portugal.

Background: Cover crops have been regarded as a valuable approach for restoring soil health in intensive agriculture, but effects may depend on species composition. This study assessed how two biodiverse cover crop mixtures influence the soil microbiological component, including culturable bacteria and fungi, beneficial groups of soil microorganisms, and soil enzymatic activities, compared with a control without cover crops.

Methods: A field trial in central Portugal (Golegã) assessed soil microbial traits over two consecutive maize growing seasons. Two cover-crop mixtures were included during the autumn-winter period: CCSJB (legumes and grasses, including clovers inoculated with rhizobia) and CCBr (similar mixture also containing brassicas). A control plot was maintained without cover crops. Soil samples were collected at the end of the cover-crops cycle to determine culturable bacteria and fungi, fungal diversity, phosphate-solubilizing bacteria, rhizobial abundance, and activities of key enzymes involved in C-, P-, and S-cycling (dehydrogenase, β-glucosidase, alkaline phosphatase, arylsulfatase). Maize roots were harvested at the middle of the growing season to assess endomycorrhizal colonization.

Results: Both mixtures improved soil biological quality versus the control. CCSJB promoted a richer microbial community, increasing fungal diversity, rhizobial abundance, and maize–mycorrhizal associations. CCBr, although ineffective for rhizobia or mycorrhizae, markedly stimulated soil enzyme activity (particularly dehydrogenase and arylsulfatase), indicating an intensification of microbial metabolism and nutrient cycling, likely driven by bioactive compounds released by Brassica roots, such as glucosinolates and their breakdown products. The control plot displayed the lowest microbial activity and functional diversity.

Conclusions: Cover crops clearly improved soil biological functioning, with distinct outcomes: CCSJB reinforced symbiotic networks (rhizobia and mycorrhizae), while CCBr boosted soil biochemical processes via enzyme stimulation. These complementary effects suggest a strategic choice of cover crops mixtures to target either symbiotic support or soil biochemical capacity.

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ID525 | Engineering Oral Fish Vaccines: Enhancing Mucosal Immunity by Employing SporoVaccines and Molecular Adjuvants

Author: Bárbara Guedes2

2 CIIMAR, Interdisciplinary Center of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

E-mail: bguedes@ciimar.up.pt

Other authors: Gabriela Gonçalves1,2, Rafaela A. Santos2, Aires Oliva-Teles1,2, Ana Couto1,2, Cláudia R. Serra1,2
1 Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

2 CIIMAR, Interdisciplinary Center of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

The aquaculture sector has grown noticeably over the past few decades. In 2022, for the first time, global aquaculture production of aquatic species surpassed capture fisheries, marking a significant milestone and highlighting the industry's fundamental role in addressing the increasing global demand for food. Nonetheless, bacterial diseases still represent a major challenge to sustainable aquaculture, causing significant animal losses and contributing to the misuse of antibiotics. As antibiotic resistance continues to escalate, identifying effective alternatives is essential for protecting the health of humans, animals, and the environment. Vaccination is considered the most sustainable and efficient strategy for disease prevention, offering a suitable approach to reduce the industry's dependence on antibiotics. Of the several immunisation techniques employed in the aquaculture sector, oral vaccination stands out for its simplicity, cost-effectiveness, and suitability for mass administration, specifically to larvae and juvenile fish.

Bacillus subtilis spores, known for their remarkable resistance, have emerged as promising vehicles for delivering heterologous antigens to the harsh environment of the gastrointestinal tract. Spore-based vaccines(SporoVaccines) have previously demonstrated their potential1. This research aims to further enhance SporoVaccines effectiveness by incorporating molecular adjuvants that target immune receptors and amplify immune responses. Although commonly employed in injectable vaccines, adjuvants remain largely underexplored for oral immunization. Researching molecular adjuvants for oral delivery systems presents a powerful opportunity to improve oral vaccination performance.

Focusing on two major fish pathogens, Aeromonas salmonicida and Streptococcus iniae, this project will employ SporoVaccines in combination with molecular adjuvants to enhance immunity and reduce disease impact. European seabass(Dicentrarchus labrax) and rainbow trout(Oncorhynchus mykiss) will be employed as biological models, with the goal of adapting and employing our findings to other economically relevant fish species and pathogens. The outcomes of this research are expected to contribute to a sustainable, and antibiotic-free aquaculture industry.

[1] Goncalves,G., Santos,R. A., Coutinho,F., Pedrosa,N., Curado,M., Machado,M., Costas,B., Serrano,M., Carvalho,A. P.,Díaz-Rosales,P., Oliva-Teles,A., Couto,A., Serra,C. R. Frontiers in Immunology,2022,13,10123

ID529 | Saline and hypersaline microorganisms: hidden resources for sustainable biosurfactant production

Author: Joana Gomes

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

E-mail: joana.gomes@ceb.uminho.pt

Other authors: Cátia Santos-Pereira1; Joana Sousa1; Ana Pereira1; Susana Chaves2; Yang He3,4; Marta Simões3,4; André Antunes5; Sara Silvério1,6; Lígia Rodrigues1,6

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal;

2 Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal;

3 State Key Laboratory of Lunar and Planetary Sciences, Macau University of Science and Technology (MUST), Macau SAR, China;

4 China National Space Administration (CNSA), Macau Center for Space Exploration and Science, Macau SAR, China;

5 Institute of Science and Environment, University of Saint Joseph, Macau SAR, China;

6 Associate Laboratory in Biotechnology and Bioengineering and Microelectromechanical Systems (LABBELS), University of Minho, Braga, Portugal

Background: Surfactants are surface-active molecules with amphipathic properties that attract increasing interest due to their several applications across a wide range of areas. However, most commercially available surfactants are petroleum-based and can have significant environmental impacts. With rising awareness of sustainability issues, the demand for greener alternatives is increasing. Biosurfactants, produced by microorganisms, are emerging as alternative substitutes for these pollutant compounds. Extreme environments, like those found in saline and hypersaline sites, are expected to enclose a unique microbial diversity, making them interesting sources of novel compounds, including biosurfactants.

Methods: To explore the biosurfactant potential of saline and hypersaline environments, a combination of culture-dependent and culture-independent strategies was employed. Samples of water, soil and salt were collected from selected sites in Portugal, Spain and Cape Verde. In the culture-dependent approach, samples were spread onto sterile agar plates, containing Modified Growth Medium with different % (w/v) NaCl or original sample water supplemented with nutrients, for microorganism isolation at 37 °C. For metagenomic analyses, DNA was extracted from water and soil using commercial kits and metagenomic libraries were constructed with CopyControl Fosmid Library Production Kit. After, both the microorganism collection and metagenomics libraries were screened for biosurfactant production with Blood Agar and Victoria Pure Blue BO tests using a JANUS® Automated Workstation.

Results: Using culture-dependent strategies, we obtained a collection of 450 microorganisms representing diverse taxa from the sampled environments. Through function-based metagenomics, 4 metagenomic libraries were constructed, yielding a total of 2.250 clones. With Blood Agar and Victoria Pure Blue BO tests we could identify 52 microorganisms and 723 clones with potential for biosurfactant production.

Conclusions: This study clearly demonstrates the remarkable microbial richness found in saline and hypersaline environments of Portugal, Spain and Cape Verde. By integrating both culture-dependent and culture-independent strategies we could provide valuable genetic resources for exploring

microbial diversity and find promising biosurfactant producers. These findings highlight the value of exploring extreme environments for novel biotechnological resources.

ID535 | Soil microbiological responses following mineral fertilizer replacement with manure and slurry in apple orchards

Author: Anton Denysov

Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV, I.P.) Oeiras, Portugal

E-mail: anton.denysov@iniav.pt

Other authors: Pablo Pereira1,2, Adélia Varela1,2, Ricardo Soares1, Marta Gonçalves3, Maria do Carmo Martins4, Miguel Leão de Sousa3, Paula Fareleira1,2

1Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV, I.P.) Oeiras, Portugal;

2GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal

3Instituto Nacional de Investigação Agrária e Veterinária, I.P., (INIAV, I.P.) Alcobaça, Portugal

4COTHN - Centro Operativo e Tecnológico Hortofrutícola Nacional – Centro de Competências, Alcobaça, Portugal;

Background: Sustainable fertilization strategies are increasingly needed in intensive orchard systems to reduce reliance on synthetic inputs, enhance soil health and promote carbon sequestration. Replacing part of mineral fertilizers with organic amendments may support tree nutrition and soil biological quality, though effects can vary with the material. This study evaluated how integrating local livestock effluents from the Oeste region into apple orchard fertilization affects key soil microbial and biochemical indicators.

Methods: A field trial was conducted in two commercial orchards of PGI Maçã de Alcobaça: FR (sandy soil) and CT (clayed soil). Four fertilization treatments were tested: M1 (100% mineral), M2 (75% mineral + 25% bovine manure at budbreak), M3 (75% mineral + 25% pig slurry at budbreak), and M4 (50% mineral + 25% bovine manure at budbreak + 25% pig slurry after harvest). Soil samples were collected after one fertilization cycle and again eight months later, following budbreak applications, to assess culturable bacteria and fungi, phosphate-solubilizing bacteria, fungal diversity, and enzyme activities (dehydrogenase, alkaline phosphatase, β-glucosidase, arylsulfatase). Apple roots were also sampled and examined for endomycorrhizal colonization. All data were integrated into a Soil Quality Index (SQI).

Results: In the first sampling, both orchards showed higher SQI under M2 compared to M1, while M3 reduced SQI. Combining manure and slurry (M4) neutralized slurry's negative effect. In the second sampling, patterns persisted in CT, but in FR the trend reversed for M2, with SQI declining, whereas M3 was slightly lower and M4 increased relative to M1.

Conclusions: Manure can improve soil biological functioning, but effects depend on soil type and time. In sandy FR, early gains under manure were not sustained, likely due to faster organic matter turnover, nutrient leaching, or other factors, whereas benefits persisted in clayey CT. Slurry alone tended to reduce SQI, while combining it with manure buffered negative effects. These findings highlight that manure-based fertilization can enhance soil health, but site-specific management and monitoring are essential.

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ID542 | Understanding the mechanisms of inactivation of ultraviolet LEDs to develop effective water disinfection systems

Author: João Filipe Soares Sério

1 iBET, Instituto de Biologia Experimental e Tecnológica, Portugal; 2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Portugal.

E-mail: joao.f.s.serio@gmail.com

Other authors: Carolina Santos1,2, Maria Eduarda Martins1, Ana Paula Marques2, Vânia Pobre2, Cecília Arraiano2, Carolina Feliciano2, Mónica Serrano2, Adriano Henriques2, João Goulão2,3 Crespo, Maria Teresa Barreto Crespo1,2, Vanessa Jorge Pereira1,2

1 iBET, Instituto de Biologia Experimental e Tecnológica, Portugal;

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Portugal;

3 LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal.

As the population expands and the impacts of climate change unfold, innovation in water treatment processes will be critical for the industry to meet emerging challenges and ensure the availability of safe, sustainable water supplies. Ultraviolet (UV) light-emitting diodes (LEDs) are emerging as a greener alternative to conventional mercury-based UV systems that offer various advantages, such as wavelength tunability, higher energy efficiency, and environmental safety.

This study evaluated the inactivation mechanisms of LEDs emitting at different wavelengths - 255nm, 260nm, 265nm, 270nm, and 280nm - against environmental strains of Escherichia coli and Enterococcus faecium, isolated from real surface water matrices.

LEDs emitting light across multiple wavelengths proved to be extremely effective to inactivate both target bacteria, achieving greater than 5-log reduction for Escherichia coli and 6-log reduction for Enterococcus faecium using a small UV fluence of 6 mJ/cm2. For both organisms, no significant reactivation was observed under dark and light conditions. The average concentration of cyclobutane pyrimidine dimers produced after exposure to the different wavelengths was similar among target bacteria as well as after the reactivation experiments. Fluorescence microscopy revealed differences in DNA's morphology of both organisms after exposure to LEDs, especially at high UV fluences. Moreover, E. coli showed wavelength-specific differentially expressed transcripts, suggesting different UV responses. The LEDs emitting at 265nm originated the highest number of specific transcripts in comparison with the other wavelengths tested. Many of the genes up-regulated after LED exposure are associated with DNA damage and repair responses, including mechanisms such as translesion synthesis that facilitate recovery from DNA lesions.

Gaining a deep understanding of the mechanisms of inactivation of LEDs will lead to the development of alternative and more effective water disinfection systems. This study shows the efficacy of UV-C LEDs for water disinfection across a range of wavelengths, particularly at 265nm, highlighting the important contribution of DNA damage to bacterial inactivation, and emphasizing their applicability for the water industry.

ID544 | Soil Microbiome Modulates the Fitness and Success of Popillia japonica populations

Author: Jorge Frias

Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Açores, Portugal;

E-mail: jorge.mv.frias@uac.pt

Other authors: Duarte Toubarro1, Hugo Monteiro1, Mário Teixeira1 and Nelson Simões1

1 Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Açores, Portugal;

The soil microbiome is known to affect soil-dwelling organisms, such as the immature stage of Popillia japonica. In a previous experiment, we showed that the gut microbiomes of P. japonica larvae differed between high- and low-density populations. This observation allowed us to postulate that soil and microbiomes from high-density areas should modulate the expression of more favourable genes, in contrast to soil from low-density areas. In this study, we conducted a thorough transcriptomic analysis to identify the molecules and pathways linked to variations in insect fitness at a high-density station (S. Jorge Island) and a low-density station (São Miguel Island) in the Azores archipelago.

Insects from the low-density station presented several downregulated enriched molecular functions linked to enzymatic activity and immune response. On the upregulated side, they presented enriched molecular functions and biological processes linked to tissue damage and detoxification processes. Immuno-physiological assays validated insect transcriptomic analysis. Insects from the high-density site presented a significantly faster phenoloxidase response than those from the low-density site. Despite both larval populations inhibiting gram-negative bacterial growth, we observed slight differences in their antimicrobial capacity. This research provides a deeper understanding of how PJ populations respond at different density stations, thus elucidating the factors that underlie the adaptability, survival, and proliferation of this invasive pest species.

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ID547 | Integrating Livestock Effluents into Apple Orchard Fertilization: Effects on Tree Physiology and Yield

Author: Anton Denysov

National Institute for Agrarian and Veterinary Research (INIAV), I.P., Oeiras, Portugal

E-mail: anton.denysov@iniav.pt

Other authors: Marta Gonçalves[1], Susana Ferreira[1,2], Margarida Rodrigues[1], Francisco Martinho[1], Verónica Amado[1], Sidónio Rodrigues[1], Pedro Bulcão[1], Paula Fareleira[3,4], Miguel Leão de Sousa[1,4]

1 National Institute for Agrarian and Veterinary Research (INIAV), I.P., Alcobaça, Portugal;

2 Instituto de Desarrollo Regional, Universidad de Castilla-La Mancha (UCLM), Albacete, Spain;

3 National Institute for Agrarian and Veterinary Research (INIAV), I.P., Oeiras, Portugal;

4 GREEN-IT – Bioresources for Sustainability, Oeiras, Portugal

Background: Livestock effluents represent a valuable source of nutrients and organic carbon capable of improving soil organic matter, structure, and water retention, while stimulate microbial biomass and activity. Their integration into orchard management can reduce dependency on synthetic fertilizers, enhance soil microbial diversity, and contribute to climate-smart agriculture, reducing environmental impacts associated with intensive livestock farming.

Methods: A three-year field trial (2023–2025) was conducted in three commercial orchards of Malus domestica Borkh. 'Royal Gala', with different soil textures and planting densities: FR (sandy soil, 2924 trees han¹), CT (clay soil, 3367 trees han¹), and CG (loamy-sandy soil, 5208 trees han¹). Four fertilization strategies were compared: (i) 100% mineral fertilization (T1), (ii) 75% mineral + 25% bovine manure (T2), (iii) 75% mineral + 25% pig slurry (T3), and (iv) 50% mineral + 25% bovine manure + 25% pig slurry (T4). In T2–T4, 12.5% of slurry was also applied during the post-harvest across the alleyways. Net photosynthesis, SLA, chlorophyll content index, leaf reflectance, chlorophyll a fluorescence and yield were monitored, to assess tree responses to partial substitution of mineral fertilizers.

Results: During 2024 photosynthesis was highest under T3 in FR (14.70±0.24 µmol COn mn² sn¹) and CT (14.40±0.26 µmol COn mn² sn¹) and T2 performed best in CG (13.40±0.34 µmol COn mn² sn¹). CCI peaked under T1 in FR (46.66±1.84) and CG (34.79±2.85), and under T4 in CT (37.57±1.66). FR (T1) and CT (T4) trees showed increased leaf thickness. Yields differed by site, with T3 reaching 52.67±2.74 t han¹ in FR, T1 83.96±7.28 t han¹ in CT, and T4 20.05±2.18 t han¹ in CG. Generally, no significant differences were found between yields by location, with a positive trend for T3 compared to T2.

Conclusions: Integrating livestock effluents into fertilization strategies did not negatively affect physiological performance or yield, confirming their potential as sustainable nutrient sources. These practices are expected to influence soil microbial networks by increasing organic C inputs, improving habitat conditions, and promoting functional diversity. Microbiological analyses will provide insight into microbial-mediated nutrient cycling.

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ID548 | Green Biocides: Innovative Biotechnological Solutions for Cultural Heritage Protection

Author: Cátia Salvador

1. Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000 -671 Évora, Portugal.

2. HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, 7000-809 Évora, Portugal.

E-mail: cscs@uevora.pt

Other authors: Cátia Salvador1,2*, Patrícia Gatinho 2,5, M. Rosário Martins2,3, A. Teresa Caldeira1,2,4

1. Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000 -671 Évora, Portugal.

2. HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, 7000-809 Évora, Portugal.

3. Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, 7000-671 Évora, Portugal.

4. City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, Institute for Research and Advanced Training, University of Évora, 7000-809 Évora, Portugal.

*5. Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain. *Corresponding Author: cscs@uevora.pt*

Background: Biodeterioration poses a significant risk to cultural heritage, requiring sustainable and safe mitigation strategies. Conventional chemical biocides often exhibit high toxicity, limited material compatibility, and microbial resistance. Biotechnological biocides, such as killer toxins naturally produced by yeasts, offer a promising, eco-friendly alternative. The ART3mis Project (2022.07303.PTDC) focuses on developing novel, cost-effective, and green biocidal solutions to preserve cultural heritage while safeguarding human health and the environment.

Methods: Yeast strains from the genera Saccharomyces, Kluyveromyces, and Torulaspora were molecularly identified and tested against biodeteriogenic bacteria and fungi isolated from heritage materials. Lyophilised culture extracts and protein fractions were evaluated for antimicrobial activity, with high molecular weight compounds (>30 kDa) suspected to be responsible for bioactivity. Toxicity was assessed using Artemia franciscana assays, and results were compared with commercial biocides.

Results: Selective, strain-dependent antimicrobial activity was observed, particularly from S. cerevisiae 1 and 5, K. lactis 9, and K. marxianus 13. Protein fractions confirmed that killer toxins were the primary bioactive compounds. Most yeast extracts exhibited negligible toxicity, even at 10 mg/mL, whereas commercial biocides caused 100% brine shrimp lethality at concentrations 160–380 times lower. These findings demonstrate the potential of yeast-derived killer toxins to inhibit biodeteriogenic microorganisms effectively, with low environmental toxicity.

Conclusions: This study highlights the feasibility of developing sustainable, eco-friendly, and low-toxicity biocides based on yeast killer toxins. Such biotechnological solutions represent a safer and cost-effective alternative to conventional chemical treatments, providing significant advancements in cultural heritage preservation while minimising environmental and human health risks.

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ID549 | Hybrid leaching using bio-based molecules for selective copper and gold removal from printed circuit boards

Author: Beatriz Rito

1 University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Calcada Martim de Freitas, 3000-456 Coimbra, Portugal

E-mail: Beatriz-Rito@hotmail.com

Other authors: Pedro Farias1, Manorma Sharma2, Licínio Ferreira2 and Paula V. Morais1

1 University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Life Sciences, Calcada Martim de Freitas, 3000-456 Coimbra, Portugal;

2 University of Coimbra, CERES, Department of Chemical Engineering, Coimbra 3030-790, Portugal

Background: Printed circuit board waste streams (WPCB) are growing at a worrying rate due to the pace of electrical and electronic equipment innovation and replacement. Several technologies have been developed to recycle WPCB, such as chemical approaches using alkaline leaching or microbial-driven strategies using naturally occurring microorganisms and their metabolic products to recover valuable metals. Therefore, this work aimed to develop a hybrid leaching process that includes a chemical step followed by a biological treatment through the application of biological leaching molecules, which can selectively recover copper (Cu) and gold (Au), respectively, from Au-rich PCBs.

Methods: A group of bacterial strains was characterized both bioinformatically and experimentally for their ability to produce metals-mobilizing molecules into the extracellular environment with optimization of growth conditions. The metabolic products obtained (spent media) were characterized by HPLC. Au-rich PCBs were milled and subjected to chemical leaching using a mixture of ammonia-based solutions for Cu selective removal. Subsequently, for Au extraction, the residue was further incubated in a cascade mode with biological leaching spent media produced by the most promising bacterial strain, with periodic spent media renewal. The residues were analyzed by XRF.

Results: The strain M67T8 exhibited a pH decrease to 2.8 after 24 hours of incubation when supplemented with glucose. The HPLC analysis of the spent media revealed that the strain produces a mixture of organic acids. The hybrid leaching process implementation demonstrated that Cu alkaline leaching exceeded 98% with high selectivity (~80%). The removal of this major element contributed to the increasing relative abundance of minor elements in the residue, as well as their bioavailability, facilitating the bioleaching. Consequently, the biological leaching achieved a gold removal of 74,43%.

Conclusions: The use of bacteria and their ability to produce highly active spent media is beneficial for hybrid leaching processes aimed at selectively removing copper and gold from printed circuit boards. Furthermore, it aligns with sustainable development goals by promoting eco-friendly electronic waste management.

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ID550 | Enrichment of ofloxacin-degrading microorganisms from activated sludge

Author: Joaquín Alberto Pereira Marrero

LSRE-LCM. ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

E-mail: up201604408@fe.up.pt

Other authors: Ana Sofia Oliveira1, Ana Margarida Gorito1, Adrián Manuel Tavares da Silva1, Olga Cristina Nunes2, Ana Rita Lado Teixeira Ribeiro1

1 LSRE-LCM. ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;

2 LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

Wastewater treatment plants (WWTPs) are often ineffective at removing pharmaceutical residues from wastewater, including antibiotics (ABs). Thus, these compounds are discharged in effluents, representing a major pathway into aquatic environments. Among ABs, fluoroquinolones (FQs) are organofluoride compounds of concern due to their extensive use, chemical stability, and toxicity. The difficulty of degrading FQs through conventional processes in WWTPs contributes to their pseudo-persistence, increasing the risk of ecological disruption and potential human health risks. Due to these concerns, FQs have been included in the European Watch Lists, the most recent Decision 2025/439/EU listing ofloxacin (OFL) for Union-wide monitoring. Moreover, as a chiral compound, OFL may exhibit enantioselective bioactivity, adding complexity to its environmental behavior and its role in the spread of antimicrobial resistance. This study aims to identify a bacterium or bacterial consortium capable of degrading OFL. To achieve this, activated sludge collected from the WWTP of a pharmaceutical company was used as inoculum of enrichment cultures using different nutrient sources (yeast extract + OFL, and OFL at 64 and 170 mg/L). The lowest tested OFL concentration was selected to match the highest EUCAST MIC where resistant isolates persist, while the highest tested concentration was chosen to provide 100 mg/L of organic carbon from OFL to microbiota. Microbial growth was monitored through optical density measurements, while OFL degradation was assessed by high-performance liquid chromatography with fluorescence detection, as well as fluoride release. This work is still in progress and its findings suggest advances in identifying and isolating OFL-degrading bacteria, as well as in understanding their responses to chiral compounds.

ID554 | Protein Extracts from Pristine Bacterial Strains: a Potential Tool Against Biodeterioration in Biodeterioration in Cultural Heritage

Author: Cátia Salvador

1. Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000-671 Évora, Portugal. 2. HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, 7000-809 Évora, Portugal.

E-mail: cscs@uevora.pt

Other authors: Patrícia Gatinho 1,2, Cátia Salvador 1,3(*), M. Rosário Martins 1,4, Ana Z. Miller 2,1, A. Teresa Caldeira 1,3,5

1 HERCULES Laboratory - Cultural Heritage Studies and Safeguard & IN2PAST Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Institute for Research and Advanced Training (IIFA), University of Évora, 7000-809, Évora, Portugal.

2 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain.

3 Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000-809, Évora, Portugal.

4 Department of Medical and Health Sciences, School of Health and Human Development, University of Évora, 7000-671, Évora, Portugal

5 City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, Institute for Advanced Studies and Research, University of Évora, 7000-809 Évora, Portugal.

Background: Biodeterioration caused by microbial colonisation poses a significant threat to the conservation of Cultural Heritage. Conventional biocidal treatments, although effective, often present environmental and toxicological risks. There is a growing interest in alternative, eco-friendly strategies such as the use of bioactive metabolites produced by bacteria from pristine environments, which may offer selective antimicrobial activity with lower environmental impact.

Methods: Bacterial strains from pristine environments, belonging to the genera Agrococcus, Brevibacillus, Evansella and Sulfitobacter, were cultured. Concentrated Protein Extracts (CPEs) were obtained via sequential ultrafiltration and screened for antimicrobial activity. Protein content and molecular weight profiles were analysed.

Results: Preliminary results suggest that certain CPEs exhibit inhibitory effects against biodeteriogenic microorganisms. Notable variability in antimicrobial activity was observed across different molecular weight fractions and among bacterial extracts. Further analysis is ongoing.

Conclusions: CPEs show potential as sustainable biocontrol agents. These early findings support further exploration of microbial diversity for the development of green alternatives in Cultural Heritage preservation.

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ID555 | Exploring the diversity and biological activities of wood-colonizing marine fungi

Author: Ana Cristina Esteves

CESAM – Center for Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal

E-mail: acesteves@ua.pt

Other authors: Alberto C. Abreu, Micael F. M. Gonçalves, Artur Alves

CESAM – Center for Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal

Lignicolous marine fungi are specialized in colonizing wood in marine environments, where their lignocellulolytic activity is crucial for nutrient cycling and organic matter decomposition. Despite their ecological role, these fungi remain understudied. We aim to assess the diversity and bioactive relevance of lignicolous marine fungi.

Driftwood and pillar barks were collected from coastal and estuarine habitats in Portugal. Additionally, pine and beech baits were submerged in a coastal marine (Viana do Castelo) and in an estuary (Ria de Aveiro) for 6 months. Representative isolates were selected for molecular sequencing and analysis of the internal transcribed spacer (ITS) region of ribosomal DNA. To confirm the taxonomic identification of potential novel taxa, we performed phylogenetic analysis and morphological studies. For bioactivity screening, we tested a range of extracellular enzymatic activities and evaluated the antimicrobial activity against bacteria and fungi. The impact of sea salts on bioactivity was evaluated.

We obtained 663 fungal isolates and identified 59 genera. The composition of lignicolous communities varied based on the type of wood colonized and on the time of submersion. Wood baits supported more marine obligate taxa, while pillar barks promoted stress-tolerant genera. Driftwood exhibited the highest genus richness but the lowest evenness, indicating a hotspot of fungal diversity. We show that there is a transition between pioneer and intermediate species/genera. Five novel species, one of which belong to a new genus were described. These new taxa revealed enzymatic activities of interest for biotechnological industries, as lipases and proteases. Sea salts inhibited the activities of gelatinases, pectin lyases and cellulases. Fusarium limianum sp. nov. showed antagonistic properties against strains of Staphylococcus aureus and Candida albicans, while Fluctus maritimus gen. et sp. nov. inhibited F. oxysporum and Aspergillus terreus.

These findings reveal the hidden taxa and bioactive potential of lignicolous fungi in transitional and coastal waters of Portugal. Uncovering this knowledge gaps will reveal their ecological relevance and enable future biotechnological applications.

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ID557 | Decoding Parchment Origins: An Integrated Molecular and Microbial Approach

Author: Maria João Penetra

HERCULES Laboratory -Cultural heritage, Studies and Safeguarding & IN2PAST – Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Évora University, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

E-mail: d53086@alunos.uevora.pt

Other authors: António Candeias1,2,3, Catarina Miguel1,3, Ana Teresa Caldeira1,2,3

1HERCULES Laboratory -Cultural heritage, Studies and Safeguarding & IN2PAST – Associate Laboratory for Research and Innovation in Heritage, Arts, Sustainability and Territory, Évora University, Palácio do Vimioso, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

2Chemistry Department, School of Sciences and Technology, Évora University, Rua Romão Ramalho 59, 7000- 671 Évora, Portugal

3City University of Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, University of Évora, Casa Cordovil, 7000-651 Évora, Portugal

Background: For centuries, parchment—crafted primarily from the skins of sheep, calves, and goats—has preserved cultural and intellectual heritage. Yet, determining the specific animal source is difficult, as conventional approaches, including DNA analysis, are often limited by variable preservation states and the intensive treatments used during production1-4.

Methods: In this study, we analyzed a collection of historical parchments with the aim of identifying molecular markers linked to animal origin. Mitochondrial DNA analysis and 16S rRNA metabarcoding were applied to characterize both host and microbial signatures. In parallel, non-invasive techniques—Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) and Raman Spectroscopy—were used to define molecular fingerprints of the parchment substrate.

Results: The findings suggest that the animal origin might influence the microbiological diversity present in the samples, as reflected by the different cluster formations using parchment samples with known origins.

Conclusions: By correlating microbial community profiles with substrate type and molecular features, this work demonstrates an integrated framework for parchment characterization, with implications for heritage conservation and the reconstruction of historical production practices.

ID558 | Production of antibacterial compounds by the marine-derived fungus *Penicillium adametzioides*

Author: Ana Cristina Esteves

CESAM – Center for Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal

E-mail: acesteves@ua.pt

Other authors: Rafael Cafede, Alberto C. Abreu, Jaqueline T. Bento, Artur Alves

CESAM – Center for Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal

Marine fungi are microorganisms with significant, but underexplored, biotechnological potential. At the same time, the growing crisis related to antibiotic bacterial resistance has driven the search for new sources of effective antimicrobial compounds. In this context, marine fungi have attracted growing interest, given their adaptation in harsh environments, which favors the activation of secondary metabolic pathways distinct from those observed in terrestrial fungi. This project aims to optimize the production, extraction, and fractionation of antimicrobial compounds by adjusting fungal growth conditions.

The marine strain Penicillium adametzioides CMG390 was cultivated in liquid medium under different pH conditions (5.6, 7.0, and 8.0). Fungal metabolites were extracted with water, 75% ethanol, and 75% methanol. The antibacterial activity of the extracts obtained was evaluated by diffusion assay against Kocuria rhizophila ATCC 9341. In addition, the extracts with the highest antimicrobial activity were subjected to partial fractionation by high-pressure liquid chromatography (HPLC), and the thermal stability of the bioactive compounds at 100 °C was evaluated.

The findings revealed that the extracts from cultures grown at a pH of 8.0 exhibited greater antibacterial activity. Notably the aqueous extracts showed consistent antimicrobial properties, regardless of the culture medium´s pH. Based on these data, two aqueous extracts produced at pH 5.6 and 8.0 were selected for partial fractionation by HPLC to isolate bioactive compounds. In the extract of P. adametzioides cultivated at pH 5.6, two fractions with antibacterial activity were identified, while the extract from cultivation at pH 8.0 revealed four distinct fractions with activity. Some of these fractions maintained their activity after exposure to heat treatment, indicating the presence of thermostable compounds.

This study shows that both the pH of the culture medium and the type of solvent used significantly influenced the production and extraction of compounds with antibacterial activity. Additionally, it contributes to advancing knowledge about marine fungi and highlights the high biotechnological potential that these species hold for developing new antimicrobial agents.

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ID584 | Insect Pathogen *Pseudomonas sp.* nov. Genome

Author: Daniel Ladeira

Centre of Biotechnology of Azores (CBA), Faculty of Science and Technology, University of Azores

E-mail: d4nil4deir4@gmail.com

Other authors: Jorge Frias, Hugo Monteiro, Mário Teixeira, Carla Cabral, Nelson Simões and Duarte Toubarro

Centre of Biotechnology of Azores (CBA), Faculty of Science and Technology, University of Azores

In a screen for Popillia japonica infestation, a few larvae were found showing symptoms of bacterial infection in S. Jorge Island. A bacteria strain was isolated and identified, based on 16s rRNA analysis, as belonging to Pseudomonas genus. To further characterize this isolate, genomic DNA extraction and Whole-genome sequencing were performed, revealing a genome size of ~6.4 Mb, with typical Pseudomonas features. Genome annotation predicted 5619 coding sequences, 1719 encoding transport proteins, 2007 associated with host-pathogen interactions, 644 encoding secretion proteins and 1339 encoding virulence factors. From these 151 are fully annotated and functionally categorized: secretion systems, adherence and biofilm formation factors, toxins and cytolysins, capsule LPS and immune evasion, iron acquisition and other micronutrients, regulatory proteins, and other invasion factors. Some virulence factors were associated with more than one functional category, resulting in a total functional count exceeding the number of unique factors. Furthermore, 10 genomic islands and 22 clusters were predicted, this last showing 24 biosynthetic categories. Altogether, these features provide new insights on the pathogenicity and host-pathogen mechanisms, enhancing our understanding on the impact this Pseudomonas strain has on its host.

ID589 | Solutions for Aquaculture: Exploring Antimicrobial Activities of Spirulina platensis and *Tetraselmis chuii* Against MDR Vibrio

Author: Inês Santos

University of the Azores, Faculty of Sciences and Technology, Department of Biology, Azores Biotechnology Center (CBA), Portugal

E-mail: Ijsantos122@gmail.com

Other authors: Duarte Seixas; Rodrigo Sampaio; Antonio Machado; Duarte Toubarro

University of the Azores, Faculty of Sciences and Technology, Department of Biology, Azores Biotechnology Center (CBA), Portugal

Antimicrobial resistance has emerged as a major global challenge, particularly in aquaculture, where multidrug-resistant (MDR) Vibrio species are responsible for severe infections in shrimp, mollusks, and fish, and also posing a considerable threat to human health. To address the urgent need for sustainable alternatives to conventional antibiotics, microalgae such as Spirulina platensis and Tetraselmis chuii are increasingly recognized as promising sources of bioactive compounds with antimicrobial potential, particularly against biofilm-associated pathogens.

In this study, crude and serially diluted ethanolic and methanolic extracts of S. platensis and T. chuii were assessed for their antibacterial activity against V. cholerae, V. alginolyticus, and V. parahaemolyticus. Antimicrobial potential was evaluated by determining the MIC and MBC using standard microdilution assays in 96-well plates. Extract concentrations ranged from 2 mg/mL to 0.0009 mg/mL, corresponding to dilutions up to 1:100.

Spirulina extracts did not show significant inhibition of V. parahaemolyticus, V. cholerae, or V. alginolyticus. In contrast, T. chuii extracts exhibit an inhibitory effect, particularly against V. parahaemolyticus. MIC and MBC determinations revealed strain-dependent susceptibility, with notable bacteriostatic activity observed at intermediate dilutions, suggesting a potential modulatory effect on bacterial growth rather than complete eradication.

These findings highlight the potential of T. chuii as a source of natural bioactive compounds capable of modulating Vibrio infections relevant to aquaculture systems. The observed species- and strain-dependent responses reinforce the need for further optimization of extraction protocols and bioactive fractionation to enhance efficacy. This study contributes to the growing evidence that microalgae represent a promising and eco-friendly platform for the development of alternative antimicrobial strategies aimed at reducing AMR risks in aquaculture and potentially safeguarding public health.

ID608 | SPATT Methology for Cyanotoxins Monitoring in Volcanic Lakes (Azores, Portugal)

Author: Alexandra Silva

Universidade dos Açores, Faculdade de Ciências e Tecnologia, 9500-321 Ponta Delgada, Portugal

E-mail: 2020107576@uac.pt

Other authors: Rita Cordeiro2, Rúben Luz2, Vítor Goncalves1,2, Amélia Fonseca1,2

1 Universidade dos Açores, Faculdade de Ciências e Tecnologia, 9500-321 Ponta Delgada, Portugal;

2 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIO-POLIS Program in Genomics, Biodiversity and Land Planning; UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, 9500-321 Ponta Delgada, Portugal

Background: The lakes of the Azores are important both for their diversity and natural landscape value, but they are subject to eutrophication and climate change, which influence bloom occurrences. Certain cyanobacteria produce cyanotoxins (MC, CYN, STX, ATX, NOD), posing risks to public, and environmental health. Monitoring relies on conventional methods (PCR, ELISA, HPLC), but these methods have limitations because they only analyze specific samples. This study introduced SPATT (Solid Phase Adsorption Toxin Testing), a passive method that allows sample collection over a defined period.

Methods: Four seasonal campaigns (spring, summer, fall, and winter) were conducted in 15 lakes of the Azores archipelago. Three sample types were collected: (1) water with a phytoplankton net, (2) 100 mL composite water for MC, CYN and NOD and 9 mL with 1 mL of ELISA kit preservative for STX and ATX and (3) SPATT samples.

Results: PCR results showed that genes associated with MC, ATX and STX synthesis were present in some lakes, such as Azul, Verde, Santiago and Funda, but not for CYN or NOD. ELISA showed a higher incidence of positives in SPATT than in water, with MC and CYN occasionally exceeding the World Health Organization (WHO) guideline values for recreational waters, particularly in lakes Azul, Furnas and Caiado. ATX and STX were detected in several lakes, although there are no WHO reference values currently established for these toxins. In HPLC, toxins were detected mainly in autumn/winter, with Caiado lake presenting the highest concentration of 2.161 µg/mL of MC-YR. PCR confirmed the presence of genes associated with cyanotoxin production, but this didn't always match ELISA and HPLC results.

Conclusions: These results indicate that cyanotoxins are present in several lakes, with microcystins most frequently detected, especially in autumn and winter, sometimes at levels posing health and environmental risks. SPATT allowed toxin detection over time, complementing conventional methods and improving bloom risk assessment. Despite its monitoring potential, further studies are needed to optimize SPATT for different environmental conditions and validate its use in freshwater.

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ID611 | Enantioselective antibiotic resistance: assessing fluoroquinolones stereochemistry as a driver of AMR evolution in wastewater bacteria

Author: Alexandre van Heerden

LSRE-LCM, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

E-mail: up202103731@up.pt

Other authors: Ana Sofia Oliveira1, Daniela Pereira1, Ana Margarida Gorito1, Olga Cristina Nunes1,2, Ana Rita Lado Teixeira Ribeiro1

1 LSRE-LCM, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;

2 LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Conventional wastewater treatment plants (WWTPs) are not designed to effectively remove pharmaceutically active compounds, particularly antibiotics, which pseudo-persist in environmental matrices. The prolonged presence of antibiotics in WWTPs creates a selective environment, where sub-inhibitory concentrations may amplify the evolution of resistant bacterial populations, thus accelerating the proliferation of multidrug-resistant bacteria. Consequently, WWTPs act as critical hotspots for antimicrobial resistance dissemination, posing significant risks to environmental and public health. Fluoroquinolones (FQs), widely used in human and veterinary medicine, pose significant environmental concerns. Many of them are chiral and their distinct enantiomeric forms interact differently with biological systems, resulting in varying degrees of persistence and toxicity. This study aimed to investigate the potential enantioselective antibacterial activity of two FQs used in human and veterinary medicine, ofloxacin (Oflo) and flumequine (Flu), respectively. To this end, bacterial biomass was collected from a municipal WWTP, and from upstream and downstream sites of the receiving river, filtered, and subsequently exposed to minimum inhibitory concentrations (MICs) of Oflo (0.3 mg/L) and Flu (2.5 mg/L), as well as to slightly higher concentrations of 0.5 mg/L and 4.0 mg/L, respectively. Bacterial isolation and subsequent purification of Escherichia coli was performed through selective cultivation, with taxonomic characterization of isolates being achieved through 16S rRNA gene sequencing. It was possible to isolate 93 pure bacterial strains, which were then submitted to an antimicrobial susceptibility testing following the Kirby-Bauer disk diffusion method to assess the resistance profiles against racemic mixtures of the target FQs. Results showed that 48 isolates were resistant to at least one of the target FQs, and 31 isolates were resistant to both. The resistant strains were further evaluated using the microdilution method to determine the specific MICs values against each enantiomerically pure form that were obtained by semi-preparative enantioselective liquid chromatography. This study will provide critical insights into the enantioselective antibacterial effects of FQs, addressing a key gap in understanding how stereochemistry influences antibiotic resistance development in wastewater bacteria.

ID612 | Adaptive laboratory evolution targeting caproic acid tolerance in a newly identified oleaginous Cyberlindnera jadinii

Author: Maria João Sousa

1 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal; 2 Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, 4710-057 Braga, Portugal.

E-mail: mjsousa@bio.uminho.pt

Other authors: Ticiana Fernandes 1,2, Pier Parpot3,4, Isabel Soares-Silva1,2, Teresa Rito1,2, Pedro Soares1,2, Ricardo Franco-Duarte1,2

1 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal;
2 Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, 4710-057 Braga, Portugal.
3 CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal;
4 CQUM, Centre of Chemistry, Chemistry Department, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

Background: Volatile Fatty Acids (VFAs), generated during anaerobic digestion of organic wastes, represent cost-effective substrates for producing added-value compounds while reducing the reliance on petrochemical routes. Cyberlindnera jadinii, noted for its phenotypic and genomic plasticity, exhibits robust growth and notable lipid accumulation (19.5–26.7% w/w) on VFAs [1]. However, inhibition by these acids remains a bottleneck for the development of a more efficient process.

Methods: Adaptive laboratory evolution (ALE) was applied to one C. jadinii strain to enhance tolerance to a defined VFAs mixture, with caproic acid concentrations gradually increased from 0.1 up to 1.0 g/L corresponding to real effluent-maximum levels [2], while maintaining other VFAs constant. Genomic DNA from evolved and parental strains was extracted and whole-genome analysis followed a pipeline established in our group [3].

Results: ALE generated five evolved isolates with improved phenotypic robustness and distinct dynamics, with three isolates exhibiting up to a tenfold increase in caproic acid tolerance in the VFAs mixture compared to the parental strain. Whole-genome sequencing revealed 37 mutated genes shared across all evolved strains, reflecting a multivariate adaptive response involving membrane stabilization, cell wall remodelling, autophagy regulation, cytoskeletal integrity, and detoxification. Top-performing isolates harboured additional mutations in mitochondrial electron transfer and vesicle trafficking, underpinning superior resistance.

Conclusions: This study reveals novel genetic determinants of VFAs tolerance and assimilation, establishing C. jadinii as an attractive organism for sustainable biowaste valorisation.

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ID626 | Alkaline extraction of phenolic compounds from Eucalyptus leaves: influence on antioxidant and antimicrobial activity

Author: Tiago Barros Afonso

Universidade Católica Portuguesa, CBQF Centro de Biotecnologia e Química Fina-Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

E-mail: tbafonso@ucp.pt

Other authors: Teresa Bonifácio-Lopes1, Eduardo Costa1, Tiago Macedo2, Joana Moreira2, Juliana Oliveira2, Manuela Pintado1

1 Universidade Católica Portuguesa, CBQF Centro de Biotecnologia e Química Fina-Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;

2 Centro de Nanotecnologia e Materiais Técnicos, Funcionais e Inteligentes – CeNTI, Rua Fernando Mesquita 2785, 4760-034 Vila Nova de Famalicão, Portugal

Background: The valorization of agro-industrial by-products is a strategic approach to reducing biomass waste while advancing circular bioeconomy practices. Eucalyptus leaves, an abundant residue from the forestry and pulp industries, are particularly rich in phenolic compounds with well-known antioxidant and antimicrobial activities. These bioactivities highlight the potential of eucalyptus extracts for sustainable applications in the cosmetic and textile industries. However, their efficient recovery requires optimized extraction methodologies capable of disrupting the complex plant matrix and maximizing compound release. In this work, we explored alkaline extraction, focusing on the influence of particle size and alkali concentration on the recovery of phenolic-rich extracts with bioactive potential.

Methods: Eucalyptus leaves were processed at two particle sizes (4 mm and 10 mm) and extracted using NaOH at two concentrations (0.1 M and 0.3 M). Extracts were analyzed for total phenolic and flavonoid content as well as antioxidant capacity using ORAC assay. Phenolic composition was characterized by LC-ESI-QqTOF-HRMS. Furthermore, the antimicrobial efficacy of the extracts was tested against different Gram-negative and Gram-positive bacteria as well as the yeast Malassezia furfur. Additionally, the biocompatibility of the extracts was evaluated on a human keratinocyte cell line (HaCaT).

Results: Smaller particle sizes (4 mm) combined with lower NaOH concentrations (0.1 M) yielded extracts with higher total phenolic and flavonoid content. In terms of antioxidant activity, no significant differences were observed between the different extracts. LC-ESI-QqTOF-HRMS analysis revealed the presence of phenolic acids (e.g., quinic and gallic acid) and flavonoids (e.g., quercetin-3-O-glucuronide) with compositional differences across conditions. All extracts displayed strong antimicrobial activity against Gram-positive bacteria and M. furfur, with MIC values below 1.25 mg/mL in most cases. However, cytotoxicity assays indicated reduced HaCaT viability at concentrations ≥ 500 µg/mL.

Conclusions: Alkaline extraction proved effective for recovering phenolic compounds from eucalyptus leaves, yielding extracts with strong antioxidant and antimicrobial activities. These findings support their potential for skin-related applications, including cosmetics and functional textiles, although further optimization of extraction conditions and safety thresholds is required.

ID631 | Effects of Ocean Alkalinity Enhancement on Marine Microbial Communities

Author: Inês de Castro

University of the Azores, Group of Climate, Meteorology and Global Change, IITAA- Institute of Agricultural and Environmental Research and Technology, 9700-042, Angra do Heroísmo, Azores, Portugal

E-mail: inesmcastro@ua.pt

Other authors: Susana C. Ribeiro 2, António Louvado 3, Mário Cachão 4, Newton Carlos Marcial Gomes 2, Eduardo Brito de Azevedo 1, Joana Barcelos e Ramos 1

1University of the Azores, Group of Climate, Meteorology and Global Change, IITAA- Institute of Agricultural and Environmental Research and Technology, 9700-042, Angra do Heroísmo, Azores, Portugal

2University of the Azores, Group of Food Science and Health, IITAA- Institute of Agricultural and Environmental Research and Technology, 9700-042, Angra do Heroísmo, Azores, Portugal

3Department of Biology and Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

4Instituto D. Luiz, University of Lisbon, Lisbon, Portugal; Department of Geology, Faculty of Sciences, University of Lisbon, 1749n016 Lisbon, Portugal.

Rising CO2 levels are driving interest in carbon removal strategies, including ocean alkalinity enhancement (OAE). By adding alkaline substances such as quicklime (CaO) or hydrated lime (Ca(OH)2), OAE can both increase CO2 uptake and counteract ocean acidification. While promising, the consequences for marine microbes remain poorly understood, despite their central role in ecosystem functioning.

To address this, we tested the effects of OAE on natural microbial communities collected from the North Atlantic (Azores). Two fractions were studied: the Total Community (TC), containing the full planktonic assemblage (<200 µm), and the Bacterioplankton Community (BC), enriched for bacteria by further filtration (<0.7 µm). We monitored plankton abundance, bacterial composition, and extracellular enzymatic activity (EEA) under OAE conditions.

OAE reduced phytoplankton growth, particularly diatoms, and altered bacterial community structure in a community-specific way. Members of Oceanospirillales consistently responded across both TC and BC. We also observed increased EEA, mainly within the bacterial community, suggesting shifts in organic matter processing.

These results indicate that OAE can influence microbial dynamics in the short term. Understanding whether such effects persist across different ecosystems will be critical for assessing the ecological safety of large-scale OAE.

ID635 | Bacterial cellulose hydrogels as multifunctional platforms forsustainable microalgae cultivation and harvesting

Author: Marisa Camacho Gonçalves Faria Pascoal

Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal

E-mail: marisa.faria@staff.uma.pt

Other authors: Diogo Sardinha 1, Ivana Mendonça 1,2,3, Filipa Rodrigues 1,2, Manfred Kaufmann 4,5, Vítor Vasconcelos 2,6, Nereida Cordeiro 1,2

1 Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal;

2 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal;

3 CICECO - Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal;

4 Marine Biology Station of Funchal, Faculty of Life Sciences, University of Madeira, 9000-107, Funchal, Portugal;

5 MARE - Marine and Environmental Sciences Centre / ARNET - Aquatic Research Network, ARDITI - Regional Agency for the Development of Research, Technology and Innovation, 9020-105 Funchal, Portugal;

6 Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

Background: Microalgae are a promising source of food, feed, and biomaterials, but their large-scale application is limited by the challenge of combining efficient cultivation with cost-effective harvesting. A simple, nature-inspired solution is to integrate both steps by embedding microalgae within renewable bacterial cellulose hydrogels (BCH).

Methods: BCHs were obtained from bacterial cellulose remnants by simple mechanical processing. In aerated cultures, BCH particles created a stable environment that supported the growth of Nannochloropsis gaditana and Chlorella sorokiniana while naturally promoting aggregation and facilitating recovery.

Results: Spectroscopic and microscopic analyses revealed strong cell-BCH adhesion, heteroaggregation, and aggregate morphologies consistent with enhanced culture stability. The system not only improved culture stability but also acted as a biodegradable bioflocculant, enabling efficient harvesting across different species. Optimization of process conditions yielded recovery efficiencies of nearly 99% for Nannochloropsis gaditana and over 80% for Chlorella sorokiniana. Furthermore, the protective effect of BCH preserved cell viability and integrity after harvesting.

Conclusions: This dual role, supporting growth and biomass recovery, reduces dependence on energy-intensive or chemical-based methods, aligning with the principles of the circular bioeconomy and green processing. The approach offers a scalable and environmentally friendly platform with potential applications in sustainable aquaculture, food biotechnology, and biomanufacturing.

ID637 | Ultrasound-Assisted Deep Eutectic Solvent Pretreatment of Spent Coffee Grounds for Sustainable Recovery of Bioactive Compounds: A Green Biorefinery Approach

Author: Shismany de Fátima Assunção Araújo

CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Campus de Gualtar, Portugal

E-mail: id10947@uminho.pt

Other authors: José Antônio Teixeira and Clarisse Nobre

LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

Background: The global coffee industry generates millions of tons of spent coffee grounds (SCG) annually, a recalcitrant lignocellulosic residue with significant potential for valorization owing to its high content of bioactive compounds. This study investigates a green delignification strategy based on the synergistic application of deep eutectic solvents (DES) and ultrasound (US) to enable the sustainable and selective extraction of lignin, phenolics, melanoidins, and oligosaccharides.

Methods: Two betaine-based DES (molar ratio 1:2, 20% water) were evaluated: betaine:lactic acid (BLA) and betaine:levulinic acid (BLEV). Pretreatments were applied to 2.0 g SCG (solid-to-liquid ratio 1:10) using an ultrasonic probe (40%amplitude,30min,4sON/2sOFF). A water-only ultrasound treatment served as control (CTRL).Structural modifications were analyzed by FTIR. The recovered lignin was evaluated for antioxidant activity through TPC, FRAP and ABTS assays.

Results: DES pretreatment enhanced lignin recovery: 0.216 ± 0.020g for BLA, 0.150 ± 0.025g for BLEV, vs 0.016 ± 0.006g for CTRL. FTIR confirmed partial delignification with reduced lignin-associated bands (1510 and 1455 cmn^{1}) and aliphatic C–H (2920/2850 cmn^{1}). The recovered lignin were evaluated for antioxidant activity: TPC was 18.10 ± 0.02mg GAE/g (BLA) and 16.89 ± 0.26mg GAE/g (BLEV) vs 35.84 ± 2.89mg GAE/g (CTRL); FRAP capacity was 102.0 ± 0.3µmol TE/g (BLA) and 97.5 ± 1.8µmol TE/g (BLEV) vs 172.8 ± 24.2µmol TE/g (CTRL). For ABTS, IC50 values were 2.218µg/mL (BLA), 1.880µg/mL (BLEV) and 1.05µg/mL (CTRL).

Conclusions: Ultrasound-assisted DES pretreatment proved effective for SCG delignification preserving antioxidant activity. Ongoing work addresses the recovery of phenolics from DES liquors, melanoidins and enzymatic saccharification of solids to release oligosaccharides as promising prebiotic candidates. This strategy represents a sustainable and selective route for SCG valorization within a green biorefinery framework.

ID642 | Antifouling Profile of Cyanobacteria-derived Nucleosides: From Efficacy to Environmental Safety

Author: Sandra Pereira

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal 2 Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal

E-mail: sandra.pereira@ciimar.up.pt

Other authors: Leonor Ferreira1,2, Isabel B. Oliveira1, Catarina Gonçalves1,2, Andreia Palmeira1,3, Maria Turkina4, Vitor Vasconcelos1,2, Alexandre Campos1, Mariana Reis1 and Joana R. Almeida1

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

2 Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal

3 Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

4 Department of Biomedical and Clinical Sciences, Faculty of Medicine and Clinical Sciences, Linköping University, Linköping, Sweden

Biocides are still widely employed in marine coatings to control the accumulation of organisms, known as marine biofouling, on submerged surfaces and vessels. Although effective, these biocides are environmentally harmful due to their broad toxicity against both target biofouling organisms and non-target marine life. Increasing regulatory restrictions on conventional biocidal antifouling (AF) agents have driven the search for sustainable alternatives, with cyanobacteria emerging as prolific producers of bioactive metabolites with high biotechnological potential. In this study, the marine cyanobacterium Leptothoe sp. LEGE 181152 was investigated through an AF bio-guided approach, leading to the isolation of two nucleosides – adenosine (1) and 2′-deoxyadenosine (2) – reported here for the first time as antifouling agents. Their AF profile was assessed using a combined strategy that included efficacy testing, mechanistic elucidation, and environmental risk evaluation. Both compounds inhibited mussel settlement (EC50 = 6.63 and 8.74 µM, respectively) without inducing mortality (LC50 > 200 µM). At these concentrations, both compounds also increased in vitro acetylcholinesterase activity. Docking studies confirmed their affinity to this enzyme. Proteomic analysis of exposed mussel plantigrades suggested EGF-like and vWFA proteins as possible candidate effectors for compound 2, while additional impacts were observed on proteins linked to ciliary motility and ATP metabolism. Moreover, compounds 1 and 2 inhibited the growth of the diatom Navicula sp. (~40% and 60%, respectively) but showed no significant effects on marine biofilm-forming bacteria. This apparent selectivity towards eukaryotic organisms may indicate target-specific AF action. Ecotoxicity assays with non-target species nauplii (Artemia salina, Amphibalanus amphitrite), together with predicted low bioaccumulation and persistence, further support their environmental safety. Altogether, these findings highlight adenosine and 2′-deoxyadenosine as novel, low-risk natural AF agents with strong potential for incorporation into marine coatings. This work reinforces the relevance of cyanobacteria as a source of sustainable antifouling solutions, advancing blue biotechnology towards environmentally responsible biofouling management.

ID646 | Alentejo's Essential Oils and β-Cyclodextrin Complexes: A Natural Strategy Against the Biodeterioration of Heritage Assets

Author: Maria Rosário Martins

1 HERCULES Lab. and IN2PAST, University of Évora, 7000-809 Évora, Portugal.

2 Department of Medical Sciences and Health, School of Health and Human Development, University of Évora, 7000-671 Évora, Portugal.

E-mail: mrm@uevora.pt

Other authors: Sílvia Macedo Arantes 1,3, António Candeias 1,4,5, Ana Teresa Caldeira1,4,5

1HERCULES Lab. and IN2PAST, University of Évora, 7000-809 Évora, Portugal.

3 Institute for Advanced Studies and Research, IIFA, University of Évora, 7000-809 Évora, Portugal.

4 Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000-671 Évora, Portugal.

5 City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, Institute for Advanced Studies and Research, University of Évora, 7000-809 Évora, Portugal.

Background: Biodeterioration is one of the major threats to the safeguarding of cultural heritage (CH). Microorganisms colonize artefacts and cause irreversible structural and aesthetic damage, compromising their conservation. Although synthetic biocides are widely used to control microbial colonization, their low biodegradability and high toxicity raise concerns regarding their safe use. Essential oils (EOs), recognized for their broad-spectrum antimicrobial properties and low toxicity, represent a promising and sustainable alternative. However, their high volatility and the limited knowledge of their biocompatibility with artefact matrices remain key challenges.

Methods: This study evaluated the antimicrobial properties of free EOs from indigenous aromatic plants of the Alentejo region, e.g. Origanum virens, Mentha spicata, and Calamintha nepeta, and their EO/β-cyclodextrin (β-CD) inclusion complexes. EOs were extracted by hydrodistillation in a Clevenger apparatus and chemically characterized by gas chromatography. Inclusion complexes were prepared using the β-CD coprecipitation method at different EO/β-CD ratios. Antimicrobial activity was assessed by solid-medium diffusion assays against biodeteriogenic microorganisms (Arthrobacter sp., Bacillus sp., Exophiala sp., and Rhodotorula sp.), previously isolated from heritage assets.

Results: EOs exhibited high antimicrobial potential, that could be correlated with their chemical profile. Nevertheless, a reduction in antimicrobial activity was observed for inclusion complexes. Encapsulation efficiency was higher than 50% for 30/70 EO/β-CD complexes. Differences in encapsulation efficiency among EOs indicated that their chemical profiles influence the affinity with β- CD and antimicrobial potential.

Conclusions: This study highlights the potential of EOs, namely O. vulgare EO, as antimicrobial agents for CH preservation. Although β-CD encapsulation may enhance EO stability, its impact on antimicrobial performance and compatibility with artefact matrices warrants further investigation to optimize their application in heritage conservation.

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ID675 | How does the genomic diversity of CAZymes in marine fungi reflect adaptation to different marine

Author: Artur Alves

CESAM, Departamento de Biologia, Universidade de Aveiro, 3810-913 Aveiro, Portugal

E-mail: artur.alves@ua.pt

Other authors: Nuno Reisinho, Micael Gonçalves

CESAM, Departamento de Biologia, Universidade de Aveiro, 3810-913 Aveiro, Portugal

Marine fungi are key drivers of nutrient cycling in ocean ecosystems through the secretion of carbohydrate-active enzymes (CAZymes). These enzymes, including glycoside hydrolases (GHs), glycosyltransferases (GTs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs), break down complex polysaccharides, supporting marine food webs and contributing to the global carbon cycle. Many of them also display salt tolerance and thermostability, properties that make them attractive for biotechnology.

In this study, we analyzed the CAZyme repertoires of 27 described marine fungi using data from MycoCosm, NCBI GenBank, and the dbCAN3 annotation platform. Across these species we identified 203 CAZyme families, with predicted repertoire sizes ranging from fewer than 300 to more than 700 genes. Comparative analyses revealed a conserved enzymatic core dominated by GHs and AAs, together with strong interspecific variation. Grouping marine isolates by substrate showed clear ecological patterns: marine fungi isolated from water and plant/wood sources harbored the largest and most diverse CAZyme repertoires, whereas deep-sediment and sponge isolates displayed markedly reduced profiles. Principal component analysis confirmed this ecological structuring, separating water and plant/wood fungi from deep-sediment and sponge groups, and distinguishing mud-associated isolates by their enrichment in CBMs (CBM1, CBM18 and CBM50, suggesting adaptation to chitin- and cellulose-rich detritus). Water and plant/wood isolates were additionally enriched in GH3, GH5, GH43 and PL1 families linked to the degradation of plant- and algae-derived polysaccharides (hemicelluloses, pectin), whereas deep-sediment fungi retained only small GH and PL repertoires, reflecting the lower availability of complex carbohydrates in those environments.

These findings provide the first broad survey of CAZyme diversity in described marine fungi, underscoring their central roles in marine carbon cycling and highlighting their potential as a source of robust enzymes for industrial and environmental applications.

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ID676 | The symbiotic sphere of Cyanobacteria: their microbiome composition and chemical interactions

Author: Adriana Rego

CIIMAR – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

E-mail: adrianairego@gmail.com

Other authors: Raquel Castelo Branco, Diana Sousa, André Regufe, Pedro N. Leão

CIIMAR – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

Background: Cyanobacteria are a diverse and widely distributed group of Gram-negative bacteria capable of oxygenic photosynthesis which are also known to be prolific producers of unique secondary metabolites. One of the most important physical adaptational mechanisms of cyanobacteria in natural conditions, is the formation of biofilms, usually composed of multi-cyanobacterial species encapsulated in extracellular polymeric substances and polysaccharidic mucilage. This polymeric matrix creates a favorable microenvironment for microbial communities to grow (also referred to as cyanosphere). Even in laboratorial conditions, cyanobacteria are usually maintained as non-axenic unicyanobacterial cultures, due to the impossibility of growing in axenicity, which highlights the intricate interactions between cyanobacteria and the cyanosphere microbiome. However, a lack of systematic analysis on the microbiome composition and chemical interactions occurring in cyanobacteria-dominated environmental biofilms and cyanobacteria growing in culture limits our understanding of this system functioning.

Methods: Here, we applied shotgun metagenomics sequencing for 20 environmental cyanobacterial biofilms, collected from marine, freshwater, soil, and hot spring environments. In addition, we used (meta) genomes of 110 cultured cyanobacteria strains from LEGEcc culture collection. Microbiome composition, including both taxonomical and functional analysis was performed, including the identification of a core microbiome and biosynthetic pathways for both primary and secondary metabolism.

Results: From a total of 97 cyanobacterial metagenome-assembled genomes (MAGs) and 110 cyanobacterial genomes, we identified a core microbiome of 32 genus, mostly distributed by Pseudomonadota, Bacteroidota and Actinomycetota phyla. From this predicted core microbiome, 11 high-quality MAGs were recovered and their functional analysis performed to uncover key metabolic pathways.

Conclusions: Our results highlight the existence of a core microbiome shared across the cyanobacteria strains assessed, from which main metabolic pathways will be depicted in detail.

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ID677 | Ideal conditions of activation of agarose beads with divinyl sulfone: improving the enzyme immobilization

Author: Diandra de Andrades

Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, 14040-901, SP, Brazil

E-mail: dcandrades@gmail.com

Other authors: 2 Pedro Abellanas-Perez, 3 Roberto Fernandez-Lafuente, 4 Maria de Lourdes Teixeira de Moraes Polizeli

2 Departamento de Biocatálisis, ICP-CSIC, C/Marie Curie 2, Campus UAM-CSIC, Cantoblanco, 28049 Madrid, Spain;

3 Departamento de Biocatálisis, ICP-CSIC, C/Marie Curie 2, Campus UAM-CSIC, Cantoblanco, 28049 Madrid, Spain;

4 Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, 14040-901, SP, Brazil

The demand for robust biocatalysts in industrial applications has stimulated the refinement of diverse enzyme engineering strategies. Immobilization has become particularly relevant, not only for enabling enzyme recovery and reuse but also for extending catalytic lifetime, enhancing stability, and in some cases modifying selectivity. Among the supports investigated, agarose modified with divinyl sulfone (DVS) has attracted attention due to its ability to form multipoint covalent bonds with proteins, thereby reinforcing structural rigidity and limiting deactivation pathways. The efficiency of this strategy, however, depends strongly on the activation protocol, as excessive alkalinity or prolonged reaction times may lead to undesired polymerization or hydrolytic side reactions, thereby reducing the number of accessible reactive groups. In this work, we explored the influence of activation parameters on the performance of DVS–agarose as an immobilization matrix. Different pH values, reaction times, and DVS concentrations were tested. To quantify the fraction of reactive vinyl sulfone groups, the supports were treated with ethylamine and analyzed by elemental composition. Bovine trypsin was employed as a model enzyme to assess immobilization efficiency and stabilization. Our data revealed that higher pH and extended reaction periods increased the incorporation of vinyl sulfone functionalities but lowered their effective reactivity, most likely due to polymerization events. The nitrogen-to-sulfur ratio of ethylamine-modified supports proved to be a reliable indicator of stabilization potential. Optimal results were obtained under activation with 360 mM DVS at pH 11.5 for 2 h, which provided the best balance between reactive group density and enzyme stabilization. Under these conditions, immobilized trypsin displayed markedly enhanced thermal resistance compared to preparations obtained under alternative protocols. Overall, the study underscores the importance of carefully tuning agarose activation parameters to maximize the potential of DVS chemistry for enzyme immobilization. The optimized methodology provides a framework for developing more stable and versatile biocatalysts, thereby supporting future advances in industrial biocatalysis.

ID678 | Arbuscular mycorrhizal fungal diversity colonizing the roots of *Picconia azorica*

Author: Sara Luna

University of Azores, Biotechnology Centre of Azores, School of Agricultural and Environmental Sciences, Angra do Heroísmo, Azores, Portugal

E-mail: sara.pb.luna@uac.pt

Background: Arbuscular mycorrhizal fungi (AMF) are obligate symbionts belonging to the phylum Glomeromycota that form mutualistic symbioses with about 90% of land plant species. They provide the host plant with mineral nutrients and water, in exchange for photosynthetic products. AMF community from the rhizosphere of the endemic Laurisilva tree, Picconia azorica, have been assessed by morphological analysis of AMF spores (Luna, 2017; Melo et al., 2018). However, due to host preference or environmental factors, spores may not reflect the in-planta composition of AMF communities. Therefore, we aimed to study the AMF community colonising the roots of P. azorica, from Terceira and São Miguel islands.

Methods: The communities of AMF present in the roots of the endemic plant P. azorica, from Terceira and São Miguel islands, were assessed by microscopic observation of morphological structures and by molecular methods, such as terminal restriction fragment length polymorphism (T-RFLP).

Results: We observed that root colonization was dominated by species belonging to Glomeraceae. Phylotype richness did not differ significantly between islands, however, it differed within each island. We also observed that soil variables influence AMF community and that some morphotypes identified in the soil were not found colonizing the roots (Luna, 2017).

Conclusions: Results suggest that AMF communities in native forest of Azores are determined by geographic and historical factors as well as soil chemical properties and environmental conditions. And the fact that P. azorica is highly dependent on AMF is in line with the showed potential of AMF application as promoters of plant establishment in conservation and restoration of native forests of the Azores archipelago. Nevertheless, further study of mycorrhizal relationships of this ecologically important and threatened endemic plant is needed.

ID681 | *Arthrobacter nicotinovorans* EAPAA enhances must attributes: evidence from Spanish and Portuguese case studies

Author: Ana Sofia da Silva Sousa

(1) Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005, Porto, Portugal;

(2) EBM, Estação Biológica de Mértola, Rua Prof. Batista da Graça nº1, 7750-329, Mértola, Portugal

E-mail: asssousa@ucp.pt

Other authors: Ángeles Prieto Fernández(3), Carmen Trasar-Cepeda(3), Paula Castro(1), Sofia Isabel Almeida Pereira(1)

(3) MBG-CSIC sede Santiago de Compostela, Avda. de Vigo s/n. Campus Vida E-15705 Santiago de Compostela, Spain;

(1)Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005, Porto, Portugal;

Background: Mediterranean agriculture faces severe challenges from soil degradation and water scarcity, intensified climate change, and unsustainable practices, like the overuse of chemicals. These pressures drive biodiversity loss, soil erosion, and declining crop productivity, threatening food security and farming's long-term viability. Enhancing resilience in agri-food systems requires the combination of sustainable agronomic practices and innovative biotechnological solutions. Among these, the use of plant-growth-promoting bacteria (PGPB) is a currently sought strategy to support crop performance. Despite their proven benefits under controlled conditions, it is essential to widen the assessment of PGPB robustness and stability in real-world situations.

Methods: Two case studies in Ribeiro (Spain) and Douro (Portugal) evaluated the effects of Arthrobacter nicotinovorans EAPAA inoculation on grapevine (Vitis vinifera L.), focusing on must quality and soil properties. The strain EAPAA has been shown to enhance P solubilization and N2 fixation. Field trials covered an average area of 300 m2 in Spain and 600 m2 in Portugal.

Results: Must composition analysis show that inoculation significantly affected sugars and organic acid levels in both sites. In Spain, inoculation increased glucose and fructose by 18% and 31%, respectively; tartaric and malic acid by 17.5% and 9.2%, respectively, and succinic acid by 29.8%-71.3%, alongside higher soil P available, labile C compounds, and biological activity. In Portugal, sugars increased by 33% and tartaric and malic acids by 50%, improvements that directly influence the quality of the wine.

Conclusion: This study demonstrates the adaptability of A. nicotinovorans EAPAA and its beneficial effects on both must quality and soil health.

Acknowledgments: This work was supported by the Agroecology Partnership Project VinAE–Vineyards network for co-creation and expansion of AgroEcological strategies to face viticulture challenges: a basis for cross-border living labs (AgroEco/0002/2024), exclusively financed by national funds through FCT and the Spanish AEI (Ref. PCI2025-163147, MICIU/AEI/10.13039/501100011033/UE). The authors thank CBQF scientific collaboration under the FCT project UIDB/50016/2020. A. Sousa thanks the FCT grant (2024.15499.PRT) for the financial support. S. Pereira thanks FCT Assistant Researcher contract (2023.15056.TENURE.047) through FCT-TENURE Program funded by the Recovery and Resilience Plan (PRR).

ID682 | Innovative PGPB-based fertilizers as a sustainable solution to promote crop resilience in degraded soils

Author: Ana Sofia da Silva Sousa

(1) Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005, Porto, Portugal; (2) EBM, Estação Biológica de Mértola, Rua Prof. Batista da Graça nº1, 7750-329, Mértola, Portugal;

E-mail: asssousa@ucp.pt

Other authors: Lur Epelde (3), Paulo Célio Alves (2,4,5), Paula M.L. Castro (1), Sofia I.A. Pereira (1)

(3) NEIKER - Basque Institute for Agricultural Research and Development, Derio, Spain;
(2) EBM, Estação Biológica de Mértola, Mértola, Portugal;
(4) BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal;
(5) CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Porto, Portugal;
(1) Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal;

Background: Biofertilizers, made of non-pathogenic and living microorganisms, such as plant growth-promoting bacteria (PGPB), support crop growth in degraded soils as eco-friendly alternatives to chemical fertilizers. They enhance nutrient availability, reduce pollution, and boost stress resilience. However, field performance and effectiveness remain inconsistent due to short shelf life, low bacterial viability, and unstable trait expression. This study aims to perform a comprehensive laboratory study by testing a novel biofertilizer carrier to ensure long-term efficiency, along with a proper selection of bacterial strains based on a greenhouse pot assay.

Methods: Fifty-three representative bacteria were isolated from soil samples collected in Alentejo for assessment of traits such as P solubilization, N2 fixation, osmotic stress tolerance, and production of lytic enzymes, siderophores, and extracellular polymeric substances (EPS). A greenhouse pot experiment using wheat is ongoing to test the real ability of each isolate to influence plant growth under water stress conditions. A PGPB-based formulation using a new carrier, EPS, a gel-like polymer that confers protection to microbial cells, is being developed, making use of the most promising isolates.

Results: Among the isolates, 30% showed the ability to solubilize P, 54.4% to fix N2, and 62.3%, 37.7%, and 18.9% to produce proteases, pectinases, and cellulases, respectively. 16S rRNA isolates sequencing revealed the presence of genera such as Bacillus, Pedobacter, and Streptomyces. Dried EPS was mixed with a concentrated bacterial suspension, and granules were produced. Granule's physical resistance and bacterial viability tests are being carried out.

Conclusions: This approach will support the development of a robust formulation with strong potential for success.

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ID686 | Prevalence of bacterial agents of bovine mastitis in dairy farms of Terceira Island, Azores, Portugal

Author: Fábio Pereira

4 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal; 5 IITAA, Universidade dos Açores

E-mail: fabiopereirami148@gmail.com

Other authors: Lénio Machado 1(*), Sandra Benevides 1(*), José Linhares 2(*), Carlos Pinto 2,3(*), Ricardo Bexiga 4 (*), Sara Melo Sousa 2(*), Ricardo Franco-Duarte 4(*), Lurdes Dapkevicius 2,5(**)

1 Laboratório Regional de Veterinária, Açores;

2 Faculdade de Ciências Agrárias e do Ambiente, Universidade dos Açores;

3 Centro de Biotecnologia dos Açores, Universidade dos Açores

Background: Besides its negative impact on animal health, well-being, and economic sustainability of milk production, bovine mastitis constitutes a potential reservoir of pathogens with impact on human health. Knowing which bacterial mastitis pathogens are present within a given geographical area is important to design control measures within the One Health approach. The objective of this work was to assess the prevalence of important mastitis pathogens in dairy farms in Terceira Island, as a basis for designing strategies to mitigate the impacts of this bovine disease.

Methods: Bulk tank milk from 185 of the 606 dairy farms in Terceira Island (Azores, Portugal) was sampled. The presence of Staphylococcus aureus, Streptococcus uberis, Streptococcus agalactiae, and Mycoplasma bovis by qPCR, using the VetMAX MastiType Micro4 qPCR Kit.

Results: M. bovis was not detected and Strep. agalactiae was detected in less than 4% of the samples. Staph. aureus and Strep. uberis were found in more than 60% of the samples.

Conclusions: Both environmental (Strep. uberis) and contagious (Staph. aureus) bacterial mastitis agents were frequently detected in the samples, highlighting the need for a complex, One Health based approach mastitis mitigation in Terceira Island.

ID689 | Microbial dynamics and their influence on greenhouse gas emissions in shallow lakes in Corvo Island (Azores, Portugal)

Author: César Andrade

1 IVAR – Instituto de Investigação em Vulcanologia e Avaliação de Riscos, Universidade dos Açores, Ponta Delgada, Portugal

2 FCT – Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal

E-mail: cesar.cc.andrade@azores.gov.pt

Other authors: José Virgílio Cruz 1,2, Fátima Viveiros 1,2, Letícia Ferreira 1,3, Pedro Raposeiro 2,4, Jorge Frias 5, Duarte Toubarro 2,5

3 CIVISA – Centro de Informação e Vigilância Sismovulcânica dos Açores, Universidade dos Açores – Rua da Mãe de Deus, 9500-801 – Ponta Delgada

4 CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos & Laboratório Associado InBio

5 CBA – Centro de Biotecnologia dos Açores, Universidade dos Açores, Ponta Delgada, Portugal

Surface water bodies are recognized as important natural sources of carbon-based greenhouse gases, with COn and CHn emissions strongly influenced by the trophic state of lake ecosystems. A study to investigate spatial and temporal variations on the diffusive flux of the CO2 and CH4 (ΦCO2) and (ΦCH4) fluxes in two volcanic shallow lakes on Corvo Island (Azores) was made, coupling geochemical and microbial tools. Water in both lakes is from the Na-Cl type, depicting alkaline pH values and low EC (63 to 90 µS cm-1). The total ΦCO2 calculated using the sGs method range between 2.5 t km-2 d-1 and 6.5 t km-2 d-1, values closer to the mean value for lakes in the Azores with depth lower than 5 m. Regarding total ΦCH4 values are much lower, ranging from 31 to 38 kg km-2 d-1 falling in the lower range of worldwide datasets. Those emissions depict a seasonal effect, as total GHG emissions (CO2 plus CH4) range from 0.66 to 1.1 t CO2-eq.d-1 in warmer conditions, about twice the values during colder periods.

Sediment samples were collected in summer and winter from both lakes using a gravity corer (20–40 cm depth), with the top layer discarded and subsamples stored at −80 °C until analysis. DNA was extracted with the DNeasy PowerSoil Pro Kit (QIAGEN) and quantified using Qubit™ assays. Full-length 16S rRNA genes were sequenced on the PacBio SMRT platform, and reads were processed into OTUs. Microbial diversity and community composition were assessed through alpha/beta diversity indices and multivariate statistical analyses (PERMANOVA, differential abundance) performed in R.

The microbial characterization also depicts seasonal variations, showing a close relation with the diffusive ΦCO2 and ΦCH4 fluxes. During winter, anaerobic and sulfate-reducing bacteria are abundant, when microbial respiration intensifies, while in summer an enrichment in fermentative bacteria, including Clostridiaceae and Enterobacteriaceae is observed. High CHn production in summer results from increased methanogenic activity, associated to the presence of methanogenic archaea, instead methane oxidation is more relevant in winter, showing higher abundances of methanotrophs, such as Methylomonadaceae and Methylococcaceae.

ID690 | Microbiological control of CO2 and CH4 emissions in maar crater lakes from São Miguel (Azores)

Author: Letícia Ferreira

(1) IVAR – Instituto de Investigação em Vulcanologia e Avaliação de Riscos, Universidade dos Açores, Ponta Delgada, Portugal; (2) CIVISA – Centro de Informação e Vigilância Sismovulcânica dos Açores, Universidade dos Açores, Ponta Delgada, Portugal

E-mail: leticia.r.ferreira@azores.gov.pt

Other authors: César Andrade (1,3), J. Virgílio Cruz (1,3), Fátima Viveiros (1,3), Duarte Toubarro (3,4), Jorge Frias (5), Pedro Raposeiro (3,5)

(1) IVAR – Instituto de Investigação em Vulcanologia e Avaliação de Riscos, Universidade dos Açores, Ponta Delgada, Portugal;

(2) CIVISA – Centro de Informação e Vigilância Sismovulcânica dos Açores, Universidade dos Açores, Ponta Delgada, Portugal;

(3) Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal;

(4) CBA – Centro de Biotecnologia dos Açores, Universidade dos Açores, Ponta Delgada, Portugal;

(5) CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos & Laboratório Associado InBio

A study was carried out in two crater monomitic lakes of São Miguel Island (Azores). The study had as objective to try to quantify the diffuse flux of CO2 (ΦCO2) and CH4 (ΦCH4) and the correlation with the bacteriological activity. The selected lakes were Congro and Santiago, with maximum depths of 22 m and 33 m, respectively.

The CO2 flux measurements were made using portable equipment, following the accumulation chamber method, while the ΦCH4 were determined using static floating chambers (SFC). The carbon dioxide measurements were conducted during March 2023 (winter) and June 2023 (summer), while methane were collected during Sepetember 2023 (summer) and December 2023 (winter). Sediment samples were collected using a UWITEC© gravity corer: the top 1–2 cm were discarded, and the subsequent 5 cm preserved in sterile plastic bags full-length 16S rRNA gene sequencing.

ΦCO2 values in both lakes were lower in summer, with a mean equal to 2.9 and 1.4 g m-2 d-1, in Congro and Santiago lakes, respectively, when the water column is stratified. Conversely, ΦCH4 showed higher emissions during the stratified period (mean value equal to 0.036 and 0.020 g m-2 d-1, respectively).

Bacterial families associated with COn production, through heterotrophic respiration, fermentation, and syntrophic metabolism, were identified in both lakes. The most abundant taxa Clostridiaceae, Enterobacteriaceae, Holophagaceae and Methylomonadaceae. Seasonal variation in the microbial community composition was evident, being enriched during winter in the most abundant taxa, such as Clostridiaceae and Enterobacteriaceae, indicating intensified fermentation. In contrast, Methylomonadaceae, responsible for methane oxidation and subsequent COn production, peaked during summer, in both lakes showing active methanotrophic activity during this period. Regarding CH4 production, methanogens showed a slightly higher abundance during summer at Congro Lake, such as Methanosarcinaceae. In Santiago Lake, Syntrophaceae and unclassified Syntrophales were more abundant during summer, supporting also higher CHn emissions compared to winter.

ID698 | Environmental transitions recorded by bacteriohopanepolyols: implications for lake biogeochemical cycles in the Azores

Author: Pedro M. Raposeiro

2 Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal

3 Biodiversity and Genetic Resources Research Centre (CIBIO), InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning, UNESCO Chair—Land within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, Ponta Delgada, Açores, Portugal

E-mail: pedro.mv.raposeiro@uac.pt

Other authors: Nora Richter1 Martin Souto 3*, Vitor Gonçalves 2,3, Miguel G. Matias 4

1Department of Surface Waters – Research & Management, Eawag, Dübendorf, Switzerland

2 Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal

3 Biodiversity and Genetic Resources Research Centre (CIBIO), InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning, UNESCO Chair—Land within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, Ponta Delgada, Açores, Portugal

4 Museo Nacional de Ciencias Naturales, Madrid, Spain

Background: Human impacts are leading to changes in lake ecosystems, often resulting in a shift in lake trophic state. However, it is unclear what the long-term implications of these changes are for biodiversity and ecosystem functions. Lipid biomarkers, particularly bacteriohopanepolyols (BHPs), have the potential to provide us with in-depth information about changes in bacterial communities and the impacts this might have on biogeochemical cycles in lakes. This study evaluates how shifts in lake trophic states influenced bacterial communities in Azorean lakes over the last 2,000 years and their implications for biogeochemical cycles.

Methods: We use modern surface sediments from 26 Azorean lakes to characterize the lipid distributions, specifically BHPs, across a trophic gradient (from oligotrophic to eutrophic lakes). We then apply these lipid biomarkers to two lake sediment records where major transitions in the lake or nearby lake ecosystems were previously identified due to past human activities: Lagoa Comprida and Lagoa do Caldeirão.

Results: Preliminary results show that lipids typically associated with methane-oxidizing bacteria, such as aminobacteriohopanetetrol and amiboacteriohopanepentol, are more abundant in eutrophic lakes with seasonally low oxygen in the bottom water. Bulk biogeochemical analyses from Lagoa Comprida and Lagoa do Caldeirão show a similar pattern with a depletion in δ13C near the top of the core and an enrichment in δ15N, suggesting a more depleted source of carbon likely associated with methane production and increased primary productivity, respectively. A more detailed analysis using lipid biomarkers will provide additional insights into these transitions.

Conclusions: Our results highlight the utility of BHPs as proxies for reconstructing bacterial community shifts and associated biogeochemical changes in response to long-term human-induced eutrophication in lake ecosystems.

This work was supported by national funds through FCT – Fundação para a Ciência e a Tecnologia, within the project UID/50027 – Rede de Investigação em Biodiversidade e Biologia Evolutiva; by the SNSF Ambizione Fellowship (216050); and by the project TrophPredict – Predicting Trophic Responses to Global Change across Space and Time (PID2022-137749NB-I00), funded by MCIN/AEI/10.13039/501100011033 and co-financed by the European Regional Development Fund (ERDF) – A way of making Europe.

ID701 | Shrimp Shells Green Extraction for Cosmetic application

Author: Andreia F. Peixoto

1 LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal

E-mail: andreia.peixoto@fc.up.pt

Other authors: Andreia Marinho1, Rita Favas2,3, Hugo Almeida2,3,4, Ana C. Silva2,3,5, Duarte Toubarro6

2 UCIBIO, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

3 Associate Laboratory i4HB Institute for Health and Bioeconomy, FFUP, 4050-313 Porto, Portugal

4 Mesosystem Investigação & Investimentos by Spinpark, Guimarães, Portugal.

5 FP-BHS,FP-I3ID, Fac. of Health Sciences, University Fernando Pessoa, 4249-004 Porto, Portugal

6 Centre of Biotecnology of Azores (CBA), Faculty of Science and technology, University of Azores

The valorisation of seafood processing waste is a cornerstone of the transition toward a circular bioeconomy. Shrimp shells (SS), commonly discarded as by-products, are rich in high-value biopolymers such as chitin and a powerful antioxidant such as astaxanthin.[1] In the scope of Shrimp4NanoCosmetic project this work explores the versatility of this marine biomass in developing integrated solutions for circular cosmetics. The Shrimp4NanoCosmetic project intends to develop a biorefinery process to recover value-added products from SS, including chitin/chitosan and astaxanthin to incorporate in new cosmetic products in collaboration with Mesosystem's company (https://mesosystem.com/).

Using environmentally friendly extraction methods astaxanthin and chitosan were obtained, in combination with novel biotechnological strategies through microbial processing. Mechanochemistry, ultrasound assistance, organic acids (citric and maleic acid), biobased deep eutectic solvents (e.g. ChCl:levulinic acid) and vegetable oils were selected as alternatives to conventional chemical treatment. Variations of the ratio of shrimp shells to solvent, extraction time and temperature variables were study. For astaxanthin extraction the highest concentration (42.915 ± 0.022 µg/ml) was obtained with a 1:10 ratio, extraction time of 30 min, and a temperature of 60ºC.High efficiency was also obtained using ball-milling and citric acid in the recovering of chitin/chitosan. Chitin/chitosan were characterized by FTIR and SEM and compared with commercial pure chitosan.

[1] N. V. Veríssimo, C. U. Mussagy, A. A. Oshiro, C. M. N. Mendonça, V. C. Santos-Ebinuma, A. Pessoa, R.P.S. Oliveira, J. F. B. Pereira, Green Chemistry, 23 (2021) 9377.

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ID702 | Shell Lifetime as a Key Factor in Astaxanthin Recovery from Shrimp Waste

Author: Rita Favas

1 UCIBIO, Laboratory of Pharmaceutical Technology, 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.

E-mail: rita.favas@hotmail.com

Other authors: Andreia Peixoto3, Hugo Almeida1,2,4, Domingos Ferreira1,2, Ana Catarina Silva1,2,5

1UCIBIO, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal.

2Associate Laboratory i4HB Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal.

3LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal.

4Mesosystem, Research & Investment by Spinpark, Guimarães, Portugal.

5FP-I3ID (Institute for Research, Innovation and Development), FP-BHS (Biomedical and Health Sciences Research Unit), Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal.

Background: Shrimp shells are a common by-product of the seafood industry and are often discarded as waste, generating environmental challenges. These residues contain valuable bioactive compounds such as astaxanthin, a potent antioxidant with high potential for nutraceuticals and pharmaceuticals applications. However, as a raw material, extraction efficiency may depend not only on the extraction conditions, but also on raw material freshness. Therefore, this study aimed to investigate how shrimp shell freshness influences astaxanthin yield during extraction.

Methods: Shrimp shells were collected, cleaned, and dried. The ultrasound-assisted extraction of astaxanthin from shrimp shells was conducted through a series of experiments involving four independent variables: shell freshness (fresh vs. stored shells), shells-to-solvent ratio (1:10 and 1:60), extraction time (10 and 30 minutes), and temperature (30°C and 60°C).

Results: Across all tested conditions, the extracts obtained from fresh shells contained considerably higher concentrations of astaxanthin. The highest yield (42,915 ± 0.022 µg/mL) was obtained using a 1:10 shells-to-solvent ratio, an extraction time of 30 minutes, and a temperature of 60°C.

Conclusions: This study demonstrated that shell freshness can influence extraction efficiency, possibly due to pigment degradation, oxidation, or structural changes in the shell. These findings highlight the importance of standardizing raw material storage and considering this variable when using optimization strategies to recover high-value compounds.

ABSTRACTS

TOPIC #2: Food Microbiology & Biotechnology

ORAL COMMUNICATIONS

ID243 | YEASTRACT+ Goes Quantitative: Enabling Advanced Systems Biology through Integration of Transcriptomic Data

Author: Luís Coutinho

Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal

E-mail: luis.coutinho@tecnico.ulisboa.pt

Other authors: 1 Joana Andrade; 2 Maria Teixeira; 3 Diogo Couceiro; 4 Tiago Val; 5 Leonardo Paulo; 6 Isabel Sá-Correia; 7 Pedro T. Monteiro; 8 Miguel Cacho Teixeira

1 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisboa 1049-001, Portugal;

2 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisboa 1049-001, Portugal;

3 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal and INESC-ID, Lisboa 1000-029, Portugal;

4 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal and INESC-ID, Lisboa 1000-029, Portugal;

5 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal and INESC-ID, Lisboa 1000-029, Portugal;

6 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal;

7 INESC-ID, Lisboa 1000-029, Portugal and Department of Computer Science, Instituto Superior Técnico, University of Lisbon, Lisboa 1049-001, Portugal;

8 Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, iBB - Institute for Bioengineering and Biosciences, Associate Laboratory Institute for Health and Bioeconomy - i4HB, Lisboa 1049-001, Portugal

Background: The YEAst Search for Transcriptional Regulators And Consensus Tracking (YEASTRACT+) database is reaching 20 years of existence as a cornerstone resource for the study and prediction of transcriptional regulation in yeasts [1,2]. It aggregates thousands of curated regulatory associations between transcription factors (TFs) and target genes (TGs), supported by extensive bibliographic evidence. Methods: YEASTRACT+'s traditional reliance on qualitative data and switch-based methods for integrating gene expression into genome-scale metabolic models (GSMMs) has constrained its utility in advanced systems biology and metabolic engineering applications. To overcome these limitations, we are undertaking a major upgrade of the platform, transitioning YEASTRACT+ into a quantitative resource.

Results: This upgrade involves the integration of high-resolution transcriptomic datasets, including the analysis of over 10 terabytes of RNA-seq data and a comprehensive review of more than 270 publications containing MicroArray hybridization data. In parallel, we have performed detailed curation and annotation of nearly 500 transcription factors and their regulatory interactions, alongside precise mapping of experimental conditions across Saccharomyces cerevisiae, non-Saccharomyces yeasts of biotechnological potential and pathogenic yeast species.

Conclusions: By embedding quantitative omics data into YEASTRACT+, we enable more accurate refinement of computational models and unlock new possibilities for precision strain engineering, dynamic metabolic flux control, and predictive modeling of complex phenotypes. These advances are set to accelerate Design–Build–Test–Learn (DBTL) cycles and expand the platform's

applicability to diverse domains including environmental biotechnology, industrial fermentation, and human health and therapeutics.

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ID350 | Glycoside 3-Oxidases as Biocatalysts for Rare Sugar Synthesis

Author: André Taborda

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa. Av. da República, 2780-157 Oeiras, Portugal

E-mail: ataborda@itqb.unl.pt

Other authors: Tomás Frazão1, M. Rita Ventura1, Laura Masgrau2, Patrícia Borges1, Lígia O. Martins1

1 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa. Av. da República, 2780-157 Oeiras, Portugal;

2 Department of Chemistry, Universitat Autònoma de Barcelona, 08193, Bellaterra, Spain

Background: Rare sugars, such as D-psicose and D-allose, have near-zero calories, minimal glycemic impact, and added health benefits, including antioxidant, neuroprotective, and antidiabetic effects. In contrast, synthetic and natural sweeteners like stevia, aspartame, and saccharin, though low in calories, face growing skepticism among health-conscious consumers. The market is thus shifting toward sweeteners that not only cut calories but also offer proven health advantages. This transition is driven by the rising global burden of diet-related diseases, predominantly obesity and type 2 diabetes. The World Health Organization (WHO) reports over 1 billion people affected by obesity, while the International Diabetes Federation (IDF) projects 643 million adults with diabetes by 2030.

Methods: We recently identified in soil bacteria a flavoenzyme, glycoside 3-oxidase (G3Ox), that catalyzes regioselective oxidation at the C3 position of D-glucose (D-Glc), a largely unexplored reaction [1]. To elucidate the mechanistic basis of its substrate specificity, we combined kinetic, structural, and computational analyses. In parallel, we established a directed evolution (DE) pipeline to improve catalytic efficiency toward D-Glc [2]. The optimized variant was then applied in a chemo-enzymatic process for the synthesis of D-allose.

Results: Our investigation showed that G3Ox exhibits a 50,000-fold higher catalytic efficiency for C-glycoside oxidation at the C3 position compared to free D-Glc. This results from poor stabilization of free D-Glc at the catalytic site, while hydrophobic loops stabilize C-glycosides [1]. A hit variant with 20-fold higher activity, further improved through DE [2], enabled efficient D-allose synthesis with a final yield of 81% (Patent PT119793).

Conclusions: This work advances the mechanistic understanding of G3Ox enzymes and demonstrates their potential in biocatalysis for food, nutraceutical, and medical biotechnologies. Acknowledgments: FCT (2022.02027.PTDC; PhD fellowship 2020.07928) and COST Action CA21162.

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ID365 | Aptamer-based strategies to control foodborne toxins

Author: Ricardo Oliveira

1INIAV – National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Vairão, Vila do Conde, 4485-655, Portugal

E-mail: ricardo.oliveira@iniav.pt

Other authors: Maria Margarida Barros 1,2; Ana Campos 1,3; Joana Castro 1,3; Daniela Araújo 1,3; Carina Almeida 1,3,4

2Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal;

3 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;

4 LEPABE, ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Background: Foodborne toxins pose a major threat to public health, causing gastrointestinal illness and systemic complications. Among toxin-producing bacteria, Staphylococcus aureus, enterotoxigenic Escherichia coli (ETEC), and Shiga toxin-producing E. coli (STEC) are of particular concern due to their clinical and epidemiological relevance. In Portugal, staphylococcal enterotoxins (SE) are the main cause of foodborne outbreaks (1). Aptamers, synthetic oligonucleotides with high affinity and specificity, are emerging as valuable tools for toxin detection and neutralization. This work compiles results from projects focused on SE and ETEC, and introduces a new project targeting Shiga toxins.

Methods: Aptamers were generated through iterative in vitro SELEX (Systematic Evolution of Ligands by Exponential Enrichment) methodologies that were developed and optimized by our research group. Magnetic-SELEX was applied to purified staphylococcal enterotoxin A (SEA) as well as cell-SELEX to whole ETEC cells (2, 3). Their performance was assessed through binding affinity, specificity, and in vivo inhibition assays. For the Shiga toxins project, SELEX rounds are being initiated, combining capture-SELEX with next-generation sequencing and bioinformatic analysis to accelerate candidate identification.

Results: The selected aptamers showed high affinity and specificity. Four SEA-binding aptamers (KD 13.36–156.72 nM) were integrated into a lateral flow biosensor capable of detecting 100 ng of SEA, the minimum human pathogenic dose (2). Four ETEC-targeting aptamers (KD 11.04–30.6 nM) displayed no toxicity in the Galleria mellonella model after 96 h, and their co-administration with ETEC increased survival by 33% and improved health index values compared to infection alone (3).

Conclusions: These results highlight the potential of aptamer-based strategies for detecting and controlling major foodborne toxigenic pathogens. The successes with SEA and ETEC demonstrate applicability in diagnostics and therapeutics, while the Shiga toxin project expands their scope toward comprehensive food safety solutions.

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ID616 | The role of food cultures and fermentates in the shelf life of soy-based burgers

Author: Beatriz Nunes Silva

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina e Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

E-mail: bnsilva@ucp.pt

Other authors: Joost de Mooij2, Verónique Zuliani3, Paula Teixeira1

1 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina e Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;

2 Novonesis, Giessener Strasse, Pohlheim, Germany;

3 Novonesis, Route d'Aulnay, Saint Germain les Arpajon, France

Background: Food cultures and fermentates represent alternative (bio)solutions to traditional preservation approaches. Aligned with current industry and consumer trends, this study aimed at the development of an innovative plant-based meat analogue, a soy-based hamburger, incorporating two biosolutions. These biosolutions were applied with the aim of modulating the microbiome of the product and hindering the growth of pathogenic microorganisms such as Listeria monocytogenes.

Methods: Three batches of soy-based burgers were produced: batch A contained a buffered vinegar (0.5%); batch B was produced with soy previously fermented with Leuconostoc carnosum; and batch C served as the control, with no preservatives. Each burger (~40 g) was inoculated (n=2) with a cocktail of seven L. monocytogenes strains and stored at 7 ºC and 10 ºC, according to the EURL Lm Technical Guidance Document to evaluate the competence of laboratories implementing challenge tests and durability studies related to Listeria monocytogenes in ready-to-eat foods (9 days + 19 days, respectively). At defined time points (days 0, 7, 13, 21 and 28), L. monocytogenes counts were determined on PALCAM agar (37 ºC, 24-48 h) according to ISO 11290-2:2017).

Results: At day 0, L. monocytogenes levels in batches A, B and C were 4.3, 3.4 and 4.5 log CFU/g, respectively. With the exception of batch B, all burgers showed an increase in contamination levels throughout storage, with batch C reaching the highest value (8.5 ± 0.0 log CFU/g) after only 13 days. The maximum contamination level in batch A was observed at day 21 (7.1 ± 0.8 log CFU/g). Batch B showed the most promising results, with L. monocytogenes counts remaining below the detection limit (2.8 log CFU/g) until day 13 and only reaching 4.4 ± 0.7 log CFU/g after 28 days, which was considerably lower compared to the maximum levels observed in the other formulations.

Conclusions: The tested biosolutions show potential to improve food safety and extend shelf life. Future studies should evaluate alternative formulations with a focus on their impact on sensory attributes and consumer acceptance.

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ID624 | Inactivation kinetics of inoculated pathogenic microorganisms in egg white and yolk using moderate pressure treatment

Author: Gabriela Matos

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

E-mail: gabrielamatos@ua.pt

Other authors: Jéssica Tavares, Bruno Carriço-Sá, Joana Costa, Isabel Ferreira, Jorge A. Saraiva

1,5 - LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;

2,3,4 - LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto 4050-313 Porto, Portugal;

Eggs are a commercially important food due to their nutritional and functional properties, but their perishability and association with Salmonella spp. outbreaks imply safety risks. Thermal processing extends the shelf-life of liquid eggs but compromises its functionality, necessitating innocuous preservation methods. Hyperbaric storage (HS) is a novel method using low pressures (25-100 MPa) to inhibit microbial populations while preserving food quality. Recently, this method has emerged as a promising novel non-thermal pasteurization method, due to its ability to achieve significant microbial inactivation, known as moderate pressure pasteurization (MPP), and using pressures up to 200 MPa.

Experiments at different MPP levels (125/150/175 MPa) at room temperature (18-23ºC) were carried out up to 4 days in liquid egg white (EW) and yolk (EY). Microbiological evaluation was assessed through counts of inoculated pathogenic microorganisms (≈7.5 log CFU/mL), namely Salmonella enterica and Staphylococcus aureus, for the established pressure/time conditions. The inactivation curves were mathematically described by linear and nonlinear kinetic models. EW and EY were also evaluated for its protein profile, viscosity and lipid oxidation, in the same conditions, and compared to thermal pasteurization and refrigeration.

Microbiological results showed that, for both microorganisms, considerable microbial reduction was achieved, in some cases exceeding 5.0 log CFU/mL. This inactivation effect was more pronounced as pressure increased, with the fastest reduction observed at 175 MPa. In EW, S. enterica inactivation by MPP could be explained by linear kinetic model while S. aureus was better fitted to Weibull model. In EY, no clear inactivation pattern was detected across the different microorganisms and MPP conditions. The Dp-values were higher for S. aureus, and overall, in EW a higher microbial inactivation rate was observed compared to EY. Electrophoretic pattern showed that no aggregates of EW/EY proteins were formed after pressure treatment and no impact in lipid oxidation was observed in EY, although viscosity increased with pressure in EW and EY.

Thus, this innovative nonthermal pressure pasteurization (HS), using moderate pressure, has great potential to assure microbial safety of liquid EW and EY, comparable to thermal pasteurization, while maintaining its quality properties.

ID632 | Raw Poultry Meat: hyperbaric pasteurisation/preservation at room temperature ensuring quality, safety and extended shelf-life

Author: Jéssica Tavares

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, 3810-193 Portugal

E-mail: jessica.tavares19@ua.pt

Other authors: Gabriela Matos 1, Carlos Pinto 1, Susana Casal 2, Paula Teixeira 3, Jorge Saraiva 1

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, 3810-193 Portugal

; 2 LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Porto, 4050-313, Portugal;

3 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, 4169-005, Portugal

Global poultry meat consumption is projected to grow 21 % by 2034. However, in 2023, 52 foodborne outbreaks were reported in the EU, underlining the food safety concerns associated with this product. Poultry's high perishability limits its shelf-life and make its preservation challenging. Hyperbaric storage (HS) showed recently to be a food preservation method that uses controlled pressure to hurdle microbial growth, even achieving pasteurisation levels, but with less impact on colour compared to high-pressure processing (HPP) of raw meat.

This work evaluated the impact of HS (100-125 MPa, up to 28 days, 20-25 ºC) as a preservation/pasteurisation method of vacuum-packed raw chicken breast, focusing on safety and quality, compared to refrigeration (RF) and HPP (400MPa/5 min). Pathogenic (Staphylococcus aureus and Salmonella Enteritidis) and endogenous microorganisms were evaluated (total aerobic mesophilic, Enterobacteriaceae and lactic acid bacteria), along with quality parameters –colour, drip loss (DL), water holding capacity (WHC) and lipid oxidation.

The results showed that 125 MPa was more effective to inactivate pathogenic/spoilage bacteria along storage compared to 100 MPa, while at RF microbial development exceeding the consumption limits was observed (namely for endogenous microorganisms). By the 7th at 125 MPa, a >5.0 log CFU/g reduction was observed for S. Enteritidis, reaching a pasteurisation-like standard status, while, for S. aureus, this level was only reached after 21 days. For endogenous microorganisms, the detection limits were generally reached by the 28th day at 125 MPa. HPP at 400 MPa was mainly effective in reducing the loads of endogenous microorganisms. Regardless of the preservation/pasteurisation methodology, the total colour differences were >3, meaning that these could be perceived by the human eye. However, HPP samples showed a clear cooked-like appearance, while a lower impact on the surface colour was observed on HS samples. DL and WHC by the 28th for HS were similar to RF samples, while primary lipid oxidation showed similar values at 125 MPa and RF. An increase in the secondary oxidation was observed at 100/125 MPa, while RF samples showed to have passed to tertiary lipid oxidation.

These results show that HS chicken breast has the potential to be marketed as a raw product up to 28 days under mild pressures at 20-25 °C, without microbial safety concerns and with less impact on colour and quality changes, unlike RF and HPP.

ID633 | Food Safety at Home: A Longitudinal Analysis of Refrigerators in Household Environments

Author: Ana Rita Barata

1 National Institute of Agricultural and Veterinary Research, I.P (INIAV), 4485-655 Vairão, Vila do Conde, Portugal

2 Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade de Trás-os-Montes e Alto Douro, UTAD, 5000-801 Vila Real, Portugal

E-mail: anarita.barata@iniav.pt

Other authors: Beatriz Ferreira 1 Patrícia Oliveira 1 Hugo Guedes 1 Maria José Saavedra 2 Gonçalo Almeida 1,3

3 Center for Animal Science Studies - Institute of Agricultural and Agro-Food Sciences and Technologies (CECA-ICETA), University of Porto, 4050-453 Porto, Portugal

Foodborne diseases remain a persistent global challenge, with domestic environments increasingly recognized as critical nodes of microbial transmission. Inadequate refrigeration, poor hygiene, and improper storage practices can compromise food safety, yet most available evidence derives from cross-sectional surveys rather than longitudinal assessments. A particular concern arises from bacterial pathogens capable of persisting in households: Listeria monocytogenes can proliferate at typical refrigeration temperatures, Campylobacter spp. can survive via biofilm formation despite being thermophilic, and Salmonella spp. remain among the most frequent causes of foodborne outbreaks worldwide, often linked to eggs and domestic cross-contamination.

This two-year longitudinal study investigated 40 household refrigerators in Porto, Portugal, combining temperature monitoring, microbiological analyses, and evaluation of household practices. Refrigerator performance was assessed using electronic data loggers, while surface samples were collected with neutralizing cloths. Microbial analyses followed ISO reference methods: total viable counts (ISO 4833), Enterobacteriaceae (ISO 21528-2), and Escherichia coli (ISO 16649-1). Real-time PCR was applied for detection of the pathogens Salmonella spp., Listeria monocytogenes, and Campylobacter spp.

In 2024, 40% of refrigerators operated above the Codex Alimentarius threshold of 5 ± 1°C, rising to 47% in 2025, creating favorable conditions for microbial persistence. Regarding total viable counts, 53% of refrigerators showed an increased microbial load from 2024 to 2025. For Enterobacteriaceae, 58% of refrigerators exhibited a similar increase over the same period. Escherichia coli, indicative of fecal contamination, was detected in 5% of refrigerators in 2024 and in 18% in 2025. No pathogens were detected in either year.

The findings reveal that a substantial proportion of household refrigerators fail to maintain safe temperatures and experience increased microbial loads over time, highlighting persistent risks of foodborne contamination. These results underscore the need for enhanced consumer awareness, improved storage practices. Importantly, our longitudinal approach demonstrates changes over time that cross-sectional studies cannot capture, emphasizing the value of continuous monitoring for effective food safety management.

ID645 | Synergistic combination of essential oil and organic acid: substitute for potassium sorbate in fruit preparation

Author: Kavita sorathiya

1Universidade Católica Portuguesa, CBQF- Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

E-mail: s-ksorathiya@ucp.pt

Other authors: Adma Melo1 , Cristina Rodrigues 2 , Luís Paulico 2 , Rita Barracosa3 , Daniela Correira3 , Maria Conceição Hogg1 and M. Manuela Pintado1

1Universidade Católica Portuguesa, CBQF- Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal.

2FRULACT - Headquarters & Frutech RDI Centre, Rua do Outeiro, 589, 4475-150 Maia, Portugal.

3MC Sonae, Rua João Mendonça, 529, 4464-501 Matosinhos, Portugal.

Background: Chemical preservatives, such as potassium sorbate (PS), are widely used in food preservation; however, they raise concerns due to potential health risks and increasing consumer demand for clean-label products. The replacement of synthetic antimicrobials with natural antimicrobial has emerged as a sustainable strategy to improve food quality and shelf life. Propionic acid (PA) and lemongrass essential oil (LEO) exhibit potential antimicrobial activity and their combination enhances inhibition of spoilage and pathogenic microorganisms. This study investigated PA and LEO combinations as natural preservatives to enhance microbiological safety and extend the shelf life of strawberry preparations for use in yogurts.

Method: Different treatments were investigated using the strawberry preparation with the addition of different concentrations of the combinations of LEO and PA ranging from ¼ to 2xMIC, and PS (0,1%) as the negative control, and standardized inoculum of bacteria (10^5 CFU/mL - Lactobacillus plantarum, Escherichia coli), yeast (10^3 CFU/mL - Candida intermedia, Pichia fermentans) and fungi (10^2 or 10^5 spores/mL - Penicillium glabrum, Aspergillus niger) for 28 days. All systems were kept at refrigeration temperature (5 ± 2°C) and microbial growth was evaluated on days 0, 1, 7, 14, 21 and 28.

Results: The combination of LEO and PA at 2× MIC exhibited greater antifungal activity than PS. The combination of ¼× MIC inhibited 10^2 spores/g but not at 10^5 spores/g, while the combinations of ½× MIC and MIC inhibited fungal growth at both inoculum levels (10^2 and 10^5 spores/g). No bacterial or yeast growth was detected at any time point across all tested concentrations of the LEO + PA combination.

Conclusions: The results showed that LEO and PA are effective natural alternatives to replace PS for fruit preparation. However, further studies are needed to understand the effect of this combination on the fruit preparation when incorporated into yogurt and sensory tests to evaluate the effect of these antimicrobials on the sensory attributes of the final product.

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ID652 | Fusarium venatum as a sustainable protein source for developing functional food ingredients

Author: Helena Araújo-Rodrigues

1 Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina –Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho, 1327, 4169-005 Porto, Portugal.

E-mail: hrodrigues@ucp.pt

Other authors: Daniela Resende1, Maria João Moreira1, Guillem Tomás2, Maria João Monteiro1, Ricardo Goméz-Garcia1, and Manuela E. Pintado1

1 Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina –Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho, 1327, 4169-005 Porto, Portugal.

2 AINIA - Biotecnología Industrial, Parque Tecnológico de Valencia; Avenida Benjamín Franklin, 5-11, 46980 Paterna, Valencia.

Background: Mycoproteins are emerging as a sustainable protein source due to their high nutritional quality, rapid growth, and potential to be produced from industrial by-products. Protein hydrolysis to obtain peptides below 3 kDa is widely applied to enhance the bioavailability, solubility, and digestibility of proteins, as well as to improve their functional properties[1]. This study assessed the nutritional profile of Fusarium venatum (FV) biomass and evaluated different enzymatic-assisted hydrolysis conditions to optimize protein extraction and antioxidant activity.

Methods: FV mycoproteins were produced through a pilot plant-scale controlled fermentation (BIOSTAT Cplus). Nutritional composition was characterized using AOAC protocols, enzymatic kits, ICP, GC-FID, and HPLC. For protein extraction and hydrolysis, an enzymatic-assisted approach was tested, combining cell wall degradation with cellulase (0, 0.5, or 1% w/w) for 3 h and sequential proteolysis with alcalase (0, 1, or 2% w/w) for 0, 3, and 6 h. Total protein content (Kjeldahl) and antioxidant activity (ABTS, ORAC, and FRAP) were determined.

Results: Total protein content of FV biomass was 32.35% dry weight (DW), dietary fiber 39.86%, fat 4.70%, and ash 7.75%. FV contained 12.76% DW of total glucans (5.24% β-glucans; 7.52% α-glucans, DW). Protein extraction yields ranged between 47 and 82%, with all treatments improving protein extraction. Cellulase (0.5–1%) enhanced protein extraction, particularly when combined with sequential proteolytic hydrolysis for 3–6 h. Antioxidant activity generally increased with cellulase and extended hydrolysis, although ORAC values favored shorter times and lower enzyme concentrations. This antioxidant activity could be attributed to low molecular weight peptides (<3 kDa), generated by proteolytic hydrolysis. Four conditions were selected, achieving 68–80% of protein yield. Among these, 0.5% cellulase without sequential hydrolysis and 1% cellulase with 6 h of hydrolysis yielded high protein recovery and strong antioxidant activity, independent of alcalase level. Cellulase plays a key role in FV cell wall degradation and protein recovery. At the same time, extended sequential hydrolysis generally improves protein recovery and can enhance antioxidant potential.

Conclusions: This study highlights the high nutritional profile of FV and supports the use of targeted enzymatic strategies to optimize the nutritional and functional properties of mycoprotein extracts.

ID663 | The future of Biorecognition Tools: Using Phage-derived Receptor-Binding Proteins for Targeted Detection of Listeria monocytogenes

Author: Filipa Oliveira

CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: fmrofilipa2001@gmail.com

Other authors: Renato Mota [1], Maria Daniela Silva [1], Ana Oliveira [1,2], Sílvio Santos [1,2]

[1] CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;

[2] LABBELS – Associate Laboratory, Braga, Guimarães, Portugal.

Background. Listeria monocytogenes (LM) is a foodborne pathogen of clinical concern, responsible for severe infections such as meningitis, septicemia, and abortion, particularly affecting vulnerable populations including pregnant women, neonates, the elderly, and immunocompromised individuals. Its capacity to persist under diverse environmental conditions, together with high hospitalization and fatality rates, has led public health authorities to recognize LM as a critical target for improved surveillance and control strategies. Conventional detection methods are often slow and labor-intensive, delaying appropriate treatment and contributing to unnecessary use of broad-spectrum antibiotics. Bacteriophages, through their receptor-binding proteins (RBPs), offer a promising basis for innovative diagnostic platforms by enabling rapid, specific, and reliable recognition of bacterial hosts.

Methods. To identify RBPs with diagnostic potential, genomic data from virulent phages and lysogenic Listeria strains were screened in silico using BLASTp and HHpred, complemented by domain annotation tools (InterProScan, Motif Search, Pfam). Candidate RBP genes were cloned as fusion constructs with a blue fluorescent protein mTagBFP2, heterologously expressed in Escherichia coli, and subsequently purified. The recombinant proteins were assessed for binding specificity and sensitivity by epifluorescence microscopy against clinically relevant Listeria isolates and non-Listeria foodborne bacteria.

Results. Two phage-derived RBPs demonstrated selective recognition of Listeria spp., with marked specificity for LM serovars 1/2a and 4b, which are most frequently associated with listeriosis outbreaks. Binding was not detected for other foodborne pathogens or unrelated bacterial species, highlighting their high selectivity.

Conclusions. These findings demonstrate that phage-derived RBPs can act as precise biorecognition elements for LM, providing a foundation for next-generation diagnostic tools enabling rapid and targeted pathogen detection. By enabling faster and more specific identification of high-risk strains, such RBPs could improve food safety monitoring and support innovative strategies to reduce the burden of listeriosis.

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ID668 | Biofilm Formation by Staphylococcus aureus as a Driver of Cross-Contamination Between Food Handlers and Ready-to-Eat Foods

Author: Joana Paiva

1 Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal; 2 Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

E-mail: jpaiva@utad.pt

Other authors: Kamila Soares 1,2, †, Sónia Saraiva 1,2,3, Cristina Saraiva 1,2,3

1 Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal;

2 Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 5000-801 Vila Real, Portugal

† These authors contributed equally to this work

Background: The persistence of Staphylococcus aureus in food environments is closely linked to its capacity to form biofilms, which facilitates survival under adverse conditions. In food service settings, such as university canteens, food handlers can act as a critical vehicle for cross-contamination of ready-to-eat (RTE) foods, where post-processing contamination cannot be eliminated by thermal treatment.

Materials and Methods: In total, 156 RTE food samples and 102 swabs from food handlers' hands were analyzed. Sample collection followed ISO 6887-1:2017 and ISO 18593:2018, while isolation and confirmation of S. aureus complied with ISO 6888-3:2003. Biofilm formation was tested through the microtiter plate assay at 24 h, with biomass quantified by crystal violet staining and normalized against the reference strain S. aureus ATCC® 6538. Classification into non, weak, moderate, or strong producers was based on optical density cut-off values (ODc).

Results: Among the 29 S. aureus isolates recovered, 44.83% were able to form biofilms, predominantly at weak (24.14%) and moderate (20.69%) levels; no strong producers were detected (Figure 1). When comparing sources, 55.6% of RTE food isolates and 40.0% of hand isolates displayed biofilm-forming capacity. Average biofilm biomass, normalized against the ATCC® 6538 reference strain, was 62.69 ± 14.64 for RTE food isolates and 69.71 ± 36.42 for hand isolates, with no significant difference observed ($p > 0.05$). Figure 1. Biofilm-forming ability of S. aureus isolates from RTE foods and food handlers' hands (%). Conclusions: Nearly half of the S. aureus isolates showed the ability to form biofilms, which may facilitate persistence in food service settings. Although no strong biofilm producers were found, the moderate capacity detected is of concern in the absence of rigorous hygiene practices, especially for RTE foods where thermal treatment is no longer applied. The results reinforce the importance of preventing cross-contamination through food handlers, surfaces, and utensils to reduce risks along the food chain.

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ID669 | Characterization of the bacteriocin(s) produced by Bacillus sp. isolated from the rumen of cows

Author: Nuno Miguel Lopes Paiva

University of the Azores, Faculty of Agricultural and Environmental Sciences, Angra do Heroísmo, Portugal

E-mail: nunop1991@gmail.com

Other authors: Susana Isabel Chaves Ribeiro, Alfredo Emílio Silveira de Borba, Célia Costa Gomes da Silva.
University of the Azores, Institute of Agricultural and Environmental Research and Technology - IITAA, Angra do Heroísmo, Portugal.

Background: The rumen microbiota is a rich source of bacteriocin-producing bacteria with potential to improve animal health and enhance sustainable ruminant production. Its complex microbial interactions provide a favorable environment for discovering antimicrobial peptides. This study aims to characterize a bacteriocin produced by a Bacillus sp. isolated from the rumen.

Methods: Ruminal fluid from cows was used for bacterial isolation. Purity and morphology were assessed via phase contrast microscopy. Antimicrobial activity was evaluated using the agar well diffusion assay. The neutralized cell-free supernatant (NCFS) was tested for sensitivity to proteolytic enzymes, temperature, pH, surfactants, and organic solvents. Growth kinetics (log CFU/mL) and bacteriocin production (AU/mL) were determined. DNA was extracted and sent for whole genome sequencing (WGS) to STABVIDA. DNA was extracted and submitted for whole genome sequencing (WGS) to STABVIDA. The sequencing was conducted on the Illumina NovaSeq platform using a 150 bp paired-end strategy. Post-sequencing quality control was performed with FastQC software and BAGEL4 was utilized to identify genes encoding bacteriocins.

Results: The NCFS inhibited S. Enteritidis and Typhimurium, B. spizizenii, E. coli, and P. aeruginosa. Antimicrobial activity was abolished after protease treatment. The activity remained stable in acidic (pH 2.0) and neutral conditions but declined in alkaline environments. Temperature testing showed reduced activity above 100°C, with residual activity after autoclaving. The antimicrobial effect was most affected by organic solvents like chloroform, but remained stable with surfactants. Bacteriocin production began after 4 hours of growth (80 AU/mL), peaking at 12 hours (320 AU/mL), with a direct correlation to bacterial growth. WGS analysis revealed genes encoding at least five antimicrobial compounds and confirmed the bacterium as a Bacillus sp.

Conclusions: A bacteriocin produced by a Bacillus sp. was successfully characterized. The bacteriocin exhibited broad-spectrum activity, thermal stability and effectiveness in acidic and neutral pH. These properties suggest promising potential for applications in animal production and food safety. Future work will focus on further identifying the Bacillus sp. and expanding the characterization of its bacteriocins.

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ID684 | Genomes on the move: Transposable elements in the regulation of fermentation-related genes in non-saccharomyces yeasts

Author: Fábio Pereira

1-CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal;

2-IITAA(Institute for Agricultural and Environmental Research andTechnologies), University of Azores, Angra do Heroismo Campus, Portugal; & both authors contributed equally to this work

E-mail: fabiopereirami148@gmail.com

Other authors: Daniel S. Oliveira3,&, Ticiana Fernandes1, Maria João Sousa1, Cristina Vieira4, Teresa Lima2, Ricardo Franco-Duarte1

3 Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14850;

4 Laboratoire de Biométrie et Biologie Évolutive, Universite Claude Bernard Lyon 1, CNRS, UMR5558, Villeurbanne 69100,France

Saccharomyces cerevisiae is the most important yeast used in wine production worldwide. In recent years, its performance has been affected by climate change, which influences both the composition of grape must and the environmental conditions of fermentation. This has had an impact on its role as a wine fermentation starter, increasing the importance of unconventional yeasts. These yeasts may positively impact aroma complexity, flavor profiles, mouthfeel, and color stability, while also contributing to lower alcohol content. Therefore, recent research has been conducted to assess the potential of non-Saccharomyces species in wine production. This research has demonstrated the role of these yeasts in improving wine fermentation and introducing new wines to the market.

Our previous work with 530 non-Saccharomyces yeasts has identified a specific set of six genes uniquely present in fermentative species and absent in non-fermentative ones, and an expanded set of 35 genes constituting the complete “fermentome”. Despite the well-established phylogenetic relationship of fermentation-related genes, the regulation of these genes in non-Saccharomyces species remains unknown.

Transposable elements (TEs) are mobile genetic elements that can change their location in the genome, thereby producing genetic diversity. They are well known for altering the gene regulation of nearby genes when inserted into promoter regions. In this study, we identified the presence of TEs in the regulatory regions of key genes for the fermentation process in 293 non-Saccharomyces species, suggesting a molecular mechanism by which these genes can be regulated. This pipeline allowed us to analyze 528 genomes, of which 227 showed the presence of TEs in their promoter regions. With regard to the six genes only present in fermentative yeasts, 65 occurrences of TEs were detected. In turn, with regard to 30 other genes not directly related, our pipeline led to the detection of 424 TEs. All these results are now being studied to understand their potential impact on wine fermentation.

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POSTERS

ID229 | Natural preservation strategies for fruit - based bakery fillings

Author: Paula Teixeira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, 4169-005, Portugal.

E-mail: pcteixeira@ucp.pt

Other authors: elisabetta tomé 1, María Teresa bento de Carvalho 1, pedro sousa 1, isabel olíveira 2, miguel azevedo 2

1 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, 4169-005, Portugal.

2 Decorgel - Productos Alimentares S.A, 4785-647, Portugal

The storage stability of an industrial banana-based filling commonly used in bakery applications was evaluated over 70 days at 22 °C and 62% relative humidity. Potassium sorbate (0.095%) was replaced with a commercial red fruit extract (0.5%) as a natural preservative. Microbiological characteristics, including total viable counts, molds and yeasts and spore-forming bacilli, were assessed throughout storage. Additionally, physicochemical and rheological properties were evaluated. The results suggest that the commercial red fruit extract is a promising “clean label” alternative to potassium sorbate, effectively maintaining the desired quality and safety of the banana-based filling during storage.

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ID238 | Population Evolutionary Dynamics Driving Listeria monocytogenes Persistence in Food Processing Environments

Author: Teresa Nogueira

1 National Institute for Agrarian and Veterinary Research (INIAV), I.P., Vila do Conde, Portugal.

2 Centre for Ecology, Evolution and Environmental Changes (cE3c) & Global Change and Sustainability Institute (CHANGE), University of Lisbon, Portugal. (*)

E-mail: teresa.nogueira@iniav.pt

Other authors: Célia Domingues 1,2, Gonçalo Almeida 1,3, Paula Teixeira 4

1 National Institute for Agrarian and Veterinary Research (INIAV), I.P., Vila do Conde, Portugal.

2 Centre for Ecology, Evolution and Environmental Changes (cE3c) & Global Change and Sustainability Institute (CHANGE), University of Lisbon, Portugal.

3 Centre for Study in Animal Science (CECA), Institute of Sciences, Technologies and Agroenvironment (ICETA), University of Porto, Portugal.

4 Associated Laboratory, Center for Biotechnology and Fine Chemistry (CBQF), School of Biotechnology, Catholic University of Portugal, Porto, Portugal.

Although listeriosis is a relatively rare disease, it remains one of the most serious foodborne infections in high-income countries, with high hospitalization and mortality rates. A major concern in food safety is the persistent presence of Listeria monocytogenes in food processing environments. Despite intensive cleaning and disinfection protocols, certain strains of are repeatedly isolated from the same sites over extended periods. This persistence has important implications for public health, regulatory compliance, and the food industry.

Here we explore the ecological and evolutionary mechanisms that contribute to L. monocytogenes persistence. Using a population dynamics framework, we analyse how natural selection driven by disinfectant use, genetic drift from periodic population bottlenecks during cleaning cycles, and recolonization from environmental reservoirs (e.g., non-food-contact surfaces and niches difficult to sanitize) can shape microbial communities. While much attention has been given to resistance and adaptation via natural selection, we emphasize the underrecognized role of genetic drift in resulting in the random fixation of traits contributing to long-term survival of clonal populations in niche environments.

Understanding the balance between selection and drift, and how recolonization sustains microbial diversity, is essential for designing more effective control strategies. Targeted interventions such as environmental monitoring, redesigned sanitation regimes, and spatially informed cleaning protocols have the potential to reduce the burden of persistent L. monocytogenes in food production and improve food safety outcomes.

Domingues, C. P. F., Almeida, G., Teixeira, P., & Nogueira, T. (2025). Persistence of Listeria monocytogenes in food processing environments: challenges and future directions. Academia Molecular Biology and Genomics, 2(2). https://doi.org/10.20935/AcadMolBioGen7715

ID264 | Nutritional value and contaminant levels in selected marine invertebrates from two Portuguese production areas

Author: Cristina Alves

Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.),, Av. Dr. Alfredo Magalhães Ramalho, 1495-165 Algés, Portugal

E-mail: joao.silva5993@gmail.com

Other authors: João Silva1, Helena Maria Lourenço1, Susana Gonçalves1, Sónia Pedro1, Carla Pires1, Paula Bacelar Nicolau2, Narcisa Maria Bandarra1

1 Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.),, Av. Dr. Alfredo Magalhães Ramalho, 1495-165 Algés, Portugal,

2 Universidade Aberta (UAb), Palácio Ceia, Rua da Escola Politécnica, n.º 141-147, 1269-001 Lisboa, Portugal

Marine invertebrates, such as bivalves and echinoderms, offer a high nutritional value, particularly as sources of essential vitamins and minerals. However, they can also accumulate contaminants from their environment, including human pathogenic bacteria, heavy metals and toxins. The aim of this work was to study the nutritional value and contaminants levels in oysters and sea urchins from two Portuguese bivalve production areas in order to evaluate its contribution to a balanced diet and its incorporation in novel products.

Moisture, fat and ash were determined according to AOAC methods and protein was based on Dumas method. Fatty acid profile was performed by gas chromatography (GC). Mercury, Cd and Pb were analysed by atomic absorption spectrometry (AAS) according to EPA method 7473:1998 and NP EN 14083:2003, respectively. Other contaminants were tested using reference methods.

Preliminary results seem promising and support the inclusion of these food staple in the national diet. Nevertheless, the calculation of dietary reference intakes is recommended, using average portion sizes.

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ID271 | Extraction and Purification of Insect Proteins: Optimization for Application in the Food Industry

Author: Pedro Moleiro

Universidade de Aveiro,3810-668, Portugal E-mail: pdm@ua.pt

Other authors: Mara Freire, Luís Branco, Marisa Santos

Universidade de Aveiro, Universidade NOVA de Lisboa, Thunder Foods

Background: The increasing global population has intensified the demand for sustainable protein sources. Insects are promising candidates due to their high protein content, nutrient richness, low environmental impact, and cost-effective production compared to traditional sources like poultry and cattle. However, consumer acceptance remains a challenge in Western countries. It has been suggested that protein extraction and formulation into new food products can enhance end-user acceptance. With this in mind, the optimization of the extraction and purification was carried out to make the consumption of insects more practical.

Methods: Design of Experiments (DOE) was applied to optimize operational parameters and maximize protein extraction yields using aqueous solutions with three insect species: Tenebrio molitor, Acheta domesticus, and Hermetia illucens. Insect flour was mixed with solvent, centrifuged, and the supernatant collected for analysis. Proteins were recovered via isoelectric precipitation, followed by centrifugation and freeze-drying to obtain purified protein powder. Scale-up assays were conducted to evaluate industrial feasibility. Protein content was quantified using the BCA method for solutions and weight measurements for precipitates. Protein profiles were analyzed by SDS-PAGE.

Results: Optimized parameters were defined (temperature of extraction, duration, and the concentration of the aqueous solution) and yielded extraction efficiencies of over 89% for T. molitor, 85% for A. domesticus, and 80% for H. illucens. The final protein powder contained 50–90% extracted proteins and is dependent of the liquid/solid ratio, demonstrating flexibility for producing solid or liquid food variants by adjusting the volume used.

Conclusions: These findings highlight the potential of integrating insect proteins into novel food products as a sustainable strategy to meet global protein demands. Ongoing scale-up tests aim to assess industrial feasibility and further promote their adoption in human diets.

ID278 | Elimination of Listeria monocytogenes in Smoked Salmon Using Two Pressure-Based Pasteurization Techniques with Minimal Impact

Author: Alireza Mousakhani Ganjeh

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;

2 LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

E-mail: Mousakhani@ua.pt

Other authors: Carlos A. Pinto(1), Susana Casal(2), Jorge A. Saraiva(1)

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;

2 LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

Background: Listeria monocytogenes is a foodborne pathogen capable of inducing severe illnesses in humans, with particularly heightened risks for pregnant women, neonates, and individuals with weakened immune defences. Consumption of food contaminated with L. monocytogenes can lead to listeriosis, a serious infectious disease. This bacterium is widely distributed in the environment and has been identified in ready-to-eat (RTE) foods. Smoked salmon is considered a high-value RTE product, yet it typically exhibits a limited shelf life, even when stored under refrigeration. In addition, the presence of L. monocytogenes in smoked salmon poses a significant public health risk. This is largely because listeriosis outbreaks in humans are often linked to the consumption of smoked salmon, which is typically eaten without any further cooking. Therefore, it is so of upmost importance to develop smoked salmon preservation techniques, particularly a pasteurization process able to considerably inactive L. monocytogenes in smoked salmon with no or very minor changes in properties, since other pasteurization methods, like temperature and high pressure processing (HPP), and others, cause colour changes that impede smoked salmon commercialization.

Methods: This study evaluates so the effects of two different pasteurization methods - moderate pressure pasteurization (MPP, 220 MPa for 3 days) and HPP for comparison (600 MPa for 5 min) - on the microbiological safety by quantifying L. monocytogenes and E. coli elimination and color changes of smoked salmon.

Results: The microbiological analyses revealed that MPP was more effective at inactivating L. monocytogenes (from 7.80 log CFU/mL to below detection limits) than HPP (from 7.80 log CFU/mL to 2.94 log CFU/mL), while both methods eliminated Escherichia coli to below detection limits. Color analyses indicated that HPP-treated salmon exhibited increased lightness, whiteness, and total color changes compared to MPP and fresh samples, showing that MPP keeps the color change at a minimal level and below the threshold that allows consumer perception of color changes, thus allowing commercialization. Preliminary results also indicated minimal texture changes in smoked salmon subjected to MPP. Conclusions: These findings highlight that MPP (a novel pasteurization method being proposed by our research group) allows smoked salmon pasteurization, assuring food safety and longer shelf life, with color preservation.

ID286 | Cellular fat from A. gossypii: chemical characterization and functional assessment in emulsified lipid preparations

Author: Eduardo Coelho

CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal; LABBELS – Associate Laboratory, Guimarães, Braga, Portugal

E-mail: e.coelho@ceb.uminho.pt

Other authors: Fernanda L. Lüdtke, Cristina M.R. Rocha, José A. Teixeira, Lucília Domingues

CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal; LABBELS – Associate Laboratory, Guimarães, Braga, Portugal

Lipids are main food components, responsible for nutritional value, organoleptic properties and quality. Their increasing demand intensified primary production, causing deforestation and environmental damage. Also, consumer awareness on nutritional value has shifted preferences towards healthier oil and fats. Oleaginous microorganisms enable the design of sustainable vertical production processes and the delivery of tailored fats. Also, novel lipid delivery systems, e.g. emulsions and lipid nanostructures, improve stability of functional ingredients and reduce overall lipid content, while preserving rheological quality. This work explores the combination of these two strategies, producing tailored fats using A. gossypii and characterizing their chemical and functional properties in emulsified lipid matrices.

Two different A.gossypii strains were used, a wild type (WT) and a strain overexpressing a native desaturase (A333) [1]. Fermentations were conducted in bioreactors for assessment of bioprocess efficiency and to produce biomass for lipid characterization and emulsification trials. Lipids were characterized for fatty acids and mono/di/triacylglycerols content and profiles. Emulsions were prepared by high intensity homogenization of biomass and their physicochemical properties and stability were evaluated.

Fermentations produced biomass with lipid accumulation up to almost 30% of dry cell weight. A. gossypii fat was majorly composed of TAG, with A333 strain yielding lower TAG and higher DAG content. WT fat was higher in C18:1, lowered by ~20% in A333 with a proportional increase in C18:2. Accordingly, WT showed a higher content of di-unsaturated and lower content of tri-unsaturated TAG when compared with A333. The distinct lipid composition of the two strains resulted in emulsions with different physicochemical properties and stability profiles.

Precision fermentation of A. gossypii enabled the production of tailored fats with different degrees of saturation. These were demonstrated in proof-of-concept matrices and represent a suitable alternative in the formulation of healthier, more sustainable food products.

[1] Silva et al. Microbial Cell Factories, 18(62), 2019.

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ID298 | Biotechnological Valorization of Carob Pulp: A Dual Enzymatic-Fungal Strategy for Producing Antioxidant-Rich Prebiotic Ingredients

Author: Sofia Mendes Amoedo

Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

E-mail: sofiaamoedo09@gmail.com

Other authors: Daniela Correia 2, José A. Teixeira 1,3, Clarisse Nobre 1,3

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal;

2 MC Shared Services SA, Lugar do Espido, Via Norte, Maia, Portugal;

3 Associate Laboratory (LABBELS), Braga, Guimarães, Portugal

Background: The growing demand for functional foods encourages sustainable bioprocesses using agro-industrial residues. Carob pulp, rich in sucrose, phenolics, and fiber, is a promising substrate for prebiotic enrichment. Prebiotics like fructo-oligosaccharides (FOS) selectively stimulate beneficial gut microbiota, supporting digestive and metabolic health. This study proposes an in situ strategy to convert natural carob sugars into FOS using enzyme blends or fungi that secrete transfructosylating enzymes. Aguamiel, a sucrose-rich by-product, was also evaluated as a co-substrate, supporting clean-label food development and circular economy principles.

Methods: Sucrose extraction from carob pulp was optimized via central composite design (variables: pulp-to-water ratio, temperature, and extraction time). FOS production was assessed by: (i) enzymatic synthesis using four commercial blends (Pectinex® Ultra SP-L, Viscozyme® L, Catazyme® 25 L, Novozym® 960); (ii) microbial fermentation with Aureobasidium pullulans CCY 27.1.94 and Aspergillus ibericus MUM 03.49. Aguamiel was tested as a co-substrate. Antioxidant activity (FRAP, ABTS), total phenolics, and flavonoids were analyzed. FOS digestion was simulated using the standardized INFOGEST in vitro protocol.

Results: Optimized extraction yielded 85.7 ± 1.0 g/L of sucrose. Novozym® 960 showed the best FOS production (0.42 ± 0.01 gFOS/ginitial sucrose; purity: 29.6%). Fungi produced FOS, A. ibericus reached the highest yield (0.347 ± 0.003 gFOS/ginitial sucrose). Aguamiel improved sustainability, reaching 0.422 ± 0.007 gFOS/ginitial sucrose. Enzymatic treatment increased phenolics, flavonoids, and antioxidant activity (FRAP: 117.5 ± 0.6 µmol TE/g DW; ABTS IC50: 4.26 µg/mL). Only 10% of FOS were hydrolyzed during digestion, indicating high gastrointestinal resistance.

Conclusions: Carob pulp is a viable, sustainable substrate for in situ FOS production with enhanced antioxidant properties. Both enzymatic and fungal strategies proved efficient without synthetic media. Novozym® 960 was the most effective catalyst. Ongoing work focuses on incorporating the prebiotic-enriched carob flour into extruded breakfast cereals, supporting innovation in sustainable, health-promoting foods.

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ID317 | Unlocking the Nutrient Potential of Fish Discards: Hydrolysis of Tuna Skin, Bones, and Viscera for Functional Ingredient Recovery

Author: Diogo Miguel

iBB—Institute for Bioengineering and Biosciences, Bioengineering Department, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

E-mail: diogo.g.miguel@tecnico.ulisboa.pt

Other authors: Leonardo Ramos, Diogo Marques, Marilia Mateus, Helena Pinheiro, Carla Motta, Maria Teresa Cesário
Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA IP, Lisboa, Portugal

Fishing and aquaculture are vital to global food security, providing 15% of animal protein for 3.2 billion people, namely in developing regions. However, significant amounts of fish discards are wasted yearly along the supply chain. Fish byproducts (e.g., skin, bones, viscera) contain protein which can be valorized through enzymatic hydrolysis.

Protein-rich by-products from skipjack tuna, namely skin, bone, and viscera (55.3 ± 1.6%, 58.2 ± 0.3%, and 47.6 ± 5.3% dry weight basis), were used to produce fish protein peptones (hydrolysates) via enzymatic hydrolysis. Process optimization evaluated different enzyme concentrations, combination of proteolytic enzymes and lipid removal from the skin prior to hydrolysis. Performance was assessed on clarified hydrolysates, after insoluble biomass separation, by quantifying protein recovery yield, protein degree of hydrolysis (OPA assay), and soluble protein content (Lowry method). Hydrolysates were chromatographically characterized for peptide molecular weight distribution (SEC) and amino acid profile (UPLC). A peptone obtained from defatted skin was assessed as nitrogen source for Corynebacterium glutamicum growth.

The best performance resulted from sequential hydrolysis with Alcalase® and Flavourzyme® both at 3.5% (w/w; enzyme formulation per protein in fish discard) for skin and bone and 5% for viscera. Optimized conditions were 60 min at 55 °C and pH 9.0 for Alcalase®, and 180 min at 50 °C and pH 7.0 for Flavourzyme®. Thus, the degree of hydrolysis reached 70.3±2.1% for defatted fish skin, 55.2±0.0% for fish bone, and 38.01±0.5% for viscera. Skin and viscera peptones showed essential amino acid profiles similar to those of FAO recommended human diets (bone-derived peptone analyses are ongoing). Molecular size distribution of viscera peptone showed 93% of the peptides below 6.5 kDa. Lower size range SEC is ongoing, and SEC characterization of skin and bone peptones. C. glutamicum was cultivated in shake-flasks on medium containing skin peptone as nitrogen source and growth optimization is under way.

This study demonstrates that enzymatic hydrolysis of fish industry byproducts can generate protein-rich ingredients for food, feed, and fermentation applications.

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ID349 | Development of Prebiotic Fruit Preparations via In-Situ Enzymatic Conversion: Toward Sugar Reduction in Dairy Products

Author: Daniela Alexandra Gonçalves

CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

E-mail: daniela.goncalves@ceb.uminho.pt

Other authors: José António Teixeira; Clarisse Nobre

CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal; LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

Background: In line with the World Health Organization (WHO) recommendations to limit sugar consumption to less than 25 g/day, the food industry is pursuing the development of functional food products that promote health while maintaining desirable sensory qualities. Fructo-oligosaccharides (FOS), non-digestible carbohydrates with proven prebiotic activity and mild sweet-taste, offer a promising sugar alternative. This study aimed to develop a low-sugar, prebiotic fruit preparation for incorporation into dairy products by enzymatically converting sucrose into FOS in-situ using a fructosyltransferase-containing enzymatic complex—Novozym® 960.

Methods: Catalysis conditions (pH, temperature, enzyme-to-substrate ratio) were optimized to maximize FOS yield. The preparation's prebiotic potential was assessed through simulated gastrointestinal digestion including fecal fermentation using microbiota from healthy donors. Microbial activity and fermentation profiles were compared to those of commercial FOS (Actilight® 951S and Frutalose® OFP). Sensory properties were evaluated using Temporal Check-All-That-Apply analysis with 100 consumers and 13 trained tasters.

Results: Under optimal conditions (47.4 °C, pH 5.4, 1:99 v/v enzyme-to-substrate), Novozym® 960 yielded 0.630±0.003 gFOS/g initial sucrose. An amount of 293.2±0.7 g/L of FOS were produced within 1.25 hours at lab scale, with comparable results at pilot scale. Over 90% of FOS remained intact post-digestion, supporting effective delivery to the colon. Microbiota fermentation showed that Novozym®-FOS exhibited growth-promoting effects on beneficial gut bacteria comparable to Actilight® and Frutalose®. Sensory evaluation showed a 93% acceptance rate, with panelists describing a pleasant natural strawberry aroma, creamy and smooth mouthfeel, and a balanced fruit-sweet flavor.

Conclusion: The prebiotic strawberry preparation achieved 56% (w/w) FOS content, reduced sucrose content by 85% and lower caloric value by 18%. The in-situ enzymatic process proved effective and scalable, maintaining both nutritional function and sensory quality. These findings suggest strong potential for industrial application in functional dairy products.

Reference: Gonçalves, D.A. et al., 2024. "Assessment of Enzymatic Complexes for the In Situ Production of Prebiotic Fructo-Oligosaccharides in a Strawberry Preparation for Dairy Industry Applications." Future Foods 9: 100387

ID370 | Limnospira platensis LEGE 231238: A Strain with High-Value Industrial Potential

Author: Flavio Oliveira

1 CIIMAR/CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Terminal de Cruzeiros de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal and 2 Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4069-007 Porto, Portugal

E-mail: oliveira_flavio@outlook.pt

Other authors: Guilherme Scotta Hentschke 1,†, Graciliana Lopes 1, Tiago Conde 3, Ana Moreira 3, Marisa Pinho 3, Mária do Rosário Domingues 3, Hugo Pereira 4, Sara Ferreira 4, Vitor Vasconcelos 1,2, Claudia Hoepfner 5,6, Daniel Guzmán 5

† Contributed equally to work

3 Centre for Environmental and Marine Studies, CESAM, Department of Chemistry, Santiago University Campus, University of Aveiro, 3810-193, Aveiro, Portugal;

4 GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas 8005-139, Faro, Portugal;

5 Faculty of Sciences and Technology, Center of Biotechnology, Universidad Mayor de San Simón, Cochabamba, Bolivia;

6 GEMA Center for Genomics, Ecology and Environment, Universidad Mayor, Santiago, Chile

Spirulina is a commercially valuable cyanobacteria widely used in the nutraceutical and biotechnology sectors. This study aims to identify new strain with enhanced potential for commercial applications through biochemical and taxonomic analysis.

The strain Limnospira platensis LEGE 231238, isolated from the saline Alalay Lake, Bolivia, was identified by taxonomical polyphasic approach (16S rRNA ML and BI phylogenies, p-distance, morphological and ecological characterization) and biochemically characterized.

Biochemical analysis of the dry biomass (% of dry weight) revealed 45.40 ± 0.54 protein, 27.84 ± 0.25 carbohydrates, 19.90 ± 0.32 ash, 6.86 ± 0.18 lipids, and C:N ratio of 5.54 ± 0.17. Lipids extracts revealed 46.23 ± 0.60% polyunsaturated fatty acids (PUFAs), 41.24 ± 0.72% saturated FA, with palmitic acid FA 16:0 accounting for 39.05 ± 0.66% and γ-linolenic acid FA 18:3 n-6 for 31.32 ± 0.30%. Nutritional quality indices included atherogenic index (0.75 ± 0.03%), thrombogenic index (1.32 ± 0.04%), and hypocholesterolemic/hypercholesterolemic index (0.45 ± 0.03%). Acetonic extracts contained 78.66% of chlorophyll-a, 16.18% of total carotenoids, and 5.11% of xanthophylls. The aqueous extract showed phenolics with 15.64 ± 1.76 (µg of gallic acid equivalents mg−1), sugars with 7.19 ± 0.75 (µg of glucose equivalents mg−1), chlorophyll-a with 5.84 ± 1.87 mg g−1 of dry extract (DE), and total chlorophylls with 10.02 ± 2.06 mg g−1 of DE. Phycobiliproteins content revealed concentrations of phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) at 84.88 ± 8.29, 53.11 ± 6.21, and 20.25 ± 3.12 µg mg−1 of DE, respectively.

These findings highlight the strain's strong nutritional and biotechnological potential, combining its high protein content and valuable lipid profile, as well as its rich concentration of bioactive pigments. This composition makes L. platensis LEGE 231238 a promising candidate for applications in the food, nutraceutical, cosmetic, and bioenergy industries.

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ID372 | Development of multiplex qPCR assays for rapid detection of toxic Solanaceae species in food

Author: Ricardo Oliveira

1 INIAV – National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Vairão, Vila do Conde, 4485-655, Portugal.

E-mail: ricardo.oliveira@iniav.pt

Other authors: Matilde Machado 1; Ana Campos 1,2; Joana Castro 1,2; Carina Almeida 1,2,3

2 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;

3 LEPABE, ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Background: Tropane alkaloids (TAs), notably (-)-hyoscyamine and (-)-scopolamine, are toxic plant metabolites present in Solanaceae species such as Datura stramonium, Atropa belladonna, Atropa mandragora, Hyoscyamus niger, and Solanum nigrum. These compounds pose significant food safety and public health risks. Invasive TA-producing plants are spreading globally and have caused recent poisoning outbreaks in Europe, including Portugal (2023). The aim of this work was to develop a multiplex real-time PCR methodology for rapid detection of these species in food, enabling timely assessment of potential TA contamination.

Methods: Primers and probes were designed using plastidial reference sequences (rbcL, matK, psbA-trnH, trnL-F, ycf1, accD). Candidates were evaluated in silico for specificity, sensitivity, and absence of secondary structures, then tested in vitro by qPCR. Singleplex assays were optimized for annealing temperature, primer/probe concentrations, and efficiency, and combined into two multiplex protocols. Both multiplex qPCR assays were validated on real food samples confirmed positive by HPLC-MS and on negative matrices typically associated with such contamination.

Results: The plastidial gene ycf1 provided the most promising discrimination among the studied species. D. stramonium showed best performance at an annealing temperature of 58°C with 400–500 nM primers, 200–300 nM probe, and 108.1% efficiency. A. belladonna was optimal at 58°C with 400–450 nM primers, 200–300 nM probe, and 104.5% efficiency. H. niger was optimal at 58°C with 350–600 nM primers, 200–300 nM probe, and 110.6% efficiency. S. nigrum was optimal at 52°C with 300–500 nM primers, 200–300 nM probe, and 103.1% efficiency, and M. officinarum at 52°C with 400–600 nM primers, 250 nM probe, and 99.3% efficiency. Two multiplex qPCR assays were established under compromise conditions: M1 for D. stramonium, A. belladonna, and H. niger with annealing at 58°C, and M2 for S. nigrum and M. officinarum with annealing at 52°C, both using primer/probe concentrations of 400 nM and 250 nM, respectively. Validation in real food samples confirmed detection of positives, while negatives showed no amplification.

Conclusions: The plastidial gene ycf1 effectively discriminates key Solanaceae species and enables rapid, reliable qPCR detection in food, providing a valuable tool for monitoring contamination and supporting food safety.

ID376 | Development of a colorimetric LAMP assay for Colletotrichum acutatum detection in olives

Author: Paula Martins-Lopes

1 DNA & RNA Sensing Lab, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

2 Faculdade de Ciências, Universidade de Lisboa, BioISI – Instituto de Biossistemas e Ciências Integrativas, Lisboa, Portugal

E-mail: plopes@utad.pt

Other authors: Inês Silva1, Sara Barrias1,2

1 DNA & RNA Sensing Lab, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

2 Faculdade de Ciências, Universidade de Lisboa, BioISI – Instituto de Biossistemas e Ciências Integrativas, Lisboa, Portugal

Background: Olive anthracnose is a fungal disease that affects Olea europaea L. and reduces yield and oil quality, leading to significant economic losses for producers. The different olive cultivars exhibit varying levels of susceptibility to anthracnose, with cv. Galeva Vulgar, highly relevant in Portugal, being particularly susceptible. The disease is mainly caused by fungi belonging to the Colletotrichum genus, with those from the Colletotrichum acutatum (C. acutatum) species complex being the most pathogenic. Currently, there are different methodologies based on Polymerase Chain Reaction (PCR) available for pathogen detection, but their application in-field detection and recurrent monitoring is limited by cost, time, and specialized equipment and personnel requirements. This work aimed to develop a rapid, specific, and sensitive colorimetric Loop-mediated Isothermal Amplification (LAMP) assay for C. acutatum detection in olives.

Methods: LAMP primers were designed to target a short fragment of the KLAP1 gene, previously associated with C. acutatum pathogenicity (Azevedo-Nogueira et al., 2021). The assay was evaluated with DNA extracted from fungal isolates, uninoculated and inoculated olive drupes. Results from the colorimetric LAMP reactions were validated through comparison with those from conventional PCR. The sensitivity of the developed LAMP assay was also determined.

Results: The specificity of the developed colorimetric LAMP assay was demonstrated by amplifying C. acutatum DNA opposed to DNA extracted from other endophytic fungi. The pathogen was successfully detected in inoculated olive drupes at different time points, indicating its potential for early disease diagnosis. The assay showed to be highly sensitive, as it was able to detect the presence of the targeted fragment in concentrations as low as 0.001 ng/µL.

Conclusions: A specific and highly sensitive colorimetric LAMP assay was developed for C. acutatum detection in olives. While additional optimization is required to improve robustness for application in olive oil, this work highlights the potential of the proposed assay as a valuable diagnostic tool for early detection and improved management of olive anthracnose.

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ID398 | Sustainable development of Clean Label Extruded Chocolate Breakfast Cereals containing Carob- and Yacon-based Prebiotics

Author: Clarisse Nobre

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal; 2 Associate Laboratory (LABBELS), Braga, Guimarães, Portugal

E-mail: clarissenobre@ceb.uminho.pt

Other authors: Tiago Costa 3, Sofia Amoedo 1, Fátima Martins 4, Luís Rocha 4, Ana Paula Pereira 4, Alexandre Goncalves 4,5, Cristina Caleja 5, Filipa S. Reis 5, Lillian Barros 5, Bruna Barbosa 6,7, Célia Rocha 6,7, Ana Fernandes 8, Daniela Correia 9, Iva Fernandes 3, Victor de Freitas 3, José A. Teixeira 1,2

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

2 Associate Laboratory (LABBELS), Braga, Guimarães, Portugal

3 Faculty of Sciences, University of Porto, Porto, Portugal

4 MORE – Laboratório Colaborativo Montanhas de Investigação – Associação, Bragança, Portugal

5 CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal

6 SENSE TEST Vila Nova de Gaia, Porto, Portugal

7 GreenUPorto/Inov4Agro, DGAOT, Faculty Sciences, University of Porto, Porto, Portugal

8 REQUIMTE-LAQV, Faculty of Sciences, University of Porto, Porto, Portugal

9 MC Shared Services SA, Lugar do Espido, Via Norte, Maia, Portugal

Background: To address both industrial and consumer demands, this study aimed to develop healthier breakfast cereals by replacing added sugars with prebiotics derived from sustainable sources. Three types of chocolate breakfast cereals were produced by extrusion, in which sugars were substituted with the following ingredients: (i) carob pulp flour enriched in prebiotic polyphenols and fructo-oligosaccharides (FOS), (ii) yacon flours from two varieties (Morado and Hualqui), also rich in polyphenols and FOS, and (iii) FOS enzymatically synthesized from sucrose.

Methods: The ingredients were incorporated into the base formulations to achieve a final FOS content between 1.8 and 3 g per 100 g of cereals. Extrusion was carried out under optimized conditions to ensure consistent product expansion, texture, and crispness. Carbohydrates were analyzed by HPLC and LC-MS. Sensory evaluation was performed using a panel with 100 consumers to assess both the sensory profile (using a projective mapping-approach) and overall liking using a 9-point hedonic scale. The gastrointestinal stability of the prebiotic cereals is currently being assessed using the INFOGEST 2.0 protocol.

Results: All three types of prebiotic ingredients were successfully incorporated into extruded chocolate breakfast cereals, providing prebiotic functionality while reducing sugar content. The extruded cereals exhibited desirable structural characteristics, including a crunchy, aerated texture and good expansion, which improved mouthfeel. Cereals formulated with both yacon flours obtained the highest overall liking of 5.9-6.0. Yacon Morado formulations were described as having a balanced flavor and a “hard” and “crunchy” texture, whereas carob-based cereals were characterized as “soft” with moderate crunchiness.

Conclusions: Extruded chocolate breakfast cereals incorporating prebiotic ingredients in place of traditional caloric sugars demonstrated both technological feasibility and high sensory acceptance, highlighting their strong potential for industrial application. The use of carob and yacon flours further improved the sustainability of the formulation, while contributing additional bioactive compounds,

such as polyphenols, enhancing the bioactivity of the cereals. Together, these features support the development of clean-label, low-sugar functional foods that meet consumer and industry demands.

ID401 | Authenticity Decoded: Microbes Tell the Story of PDO Cheeses

Author: Susana Serrano

1 CIISA – Interdisciplinary Centre for Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;

2 Associate Laboratory for Animal and Veterinary Science (AL4AnimalS)

E-mail: susanaserrano634@gmail.com

Other authors: Eugenio Parente 3, Maria Teresa Barreto-Crespo 4,5 and Teresa Semedo-Lemsaddek 1, 2, 6

3 Department of Science, University of Basilicata Studies, Potenza, Italy;

4 iBET, Institute of Experimental Biology and Technology, Apartado 12, 2781-901 Oeiras, Portugal;

5 António Xavier Institute of Chemical and Biological Technology, Nova University of Lisbon, Avenida da República, 2780-157 Oeiras, Portugal;

6 BioISI – Institute of Biosystems & Integrative Sciences, Faculty of Sciences, University of Lisbon, Portugal

Background: Among Portugal's eleven PDO-certified cheeses, Azeitão and Nisa are distinguished by their unique characteristics, built not only in regional specificity but also in the autochthonous microbiota of their production environments, since no external starter cultures can be introduced. While PDO certification strengthens the cultural and economic value of these cheeses, it also increases their vulnerability to fraud, highlighting the need for reliable methods of authenticity verification. This study applies a metagenomic approach to characterize the microbiota of PDO cheeses, with the aim of identifying eco-specific biomarkers that can serve as unique signatures of cheeses produced in the Azeitão and Nisa regions.

Methods: Cheese samples (2016–2022) from all PDO-certified producers in Azeitão and Nisa were analyzed via 16S rRNA (V3–V4) and ITS2 sequencing using Illumina MiSeq. Sequencing data were processed in RStudio 4.2: DADA2 was used for denoising and taxonomic assignment (SILVA v138.2 for bacteria, UNITE for fungi); phyloseq enabled microbial community analysis and visualization; DECIPHER and phangorn were applied for sequence alignment and phylogenetic inference; and microbiomeMarker was used to identify region-specific microbial biomarkers. This approach allowed for a robust characterization of the microbiota and differentiation between Azeitão and Nisa PDO cheeses.

Results: Discriminant analysis showed that microbial diversity was primarily influenced by cheese factories ($p < 0.001$) and production region ($p < 0.02$). In bacterial communities, Azeitão cheeses consistently harbor higher abundances of Leuconostoc and Lactococcus, while Nisa cheeses showed similar levels of these genera but a markedly higher prevalence of Lacticaseibacillus. Regarding fungi, both regions were dominated by Yarrowia, Geotrichum, and Kurtzmaniella. Region-specific microbial biomarkers were also identified: five unique to Azeitão and four to Nisa.

Conclusions: Preserving the authenticity of traditional foods is essential, particularly for PDO cheeses. Metagenomics offers a powerful tool to uncover microbial signatures that reinforce PDO validation, supporting producers in safeguarding regional identity while assuring consumers of product quality and authenticity.

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ID403 | Early Strategies for Alicyclobacillus acidoterrestris Spoilage Control Using Peracetic Acid and UV-C LEDs

Author: Inês Carvalho Leonardo

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal 2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

E-mail: ines.leonardo@ibet.pt

Other authors: Maria Teresa Barreto Crespo1,2, Vanessa Jorge Pereira1,2, Frédéric Bustos Gaspar1,2

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

Alicyclobacillus (ACB) spp. are Gram-positive thermoacidophilic bacteria responsible for the spoilage of fruit-based products. Their spores withstand pasteurisation and can germinate during storage, leading to off-flavour production in the final products. Vegetative cells are often overlooked in disinfection studies, under the assumption that they are eliminated during thermal fruit processing. This highlights the need for more robust preservation approaches.

In this study, exponentially growing A. acidoterrestris DSM 3922^T cells were shown to recover under optimal conditions despite their initial heat sensitivity, highlighting the importance of effective early-stage disinfection strategies. Here, the combination of Ultraviolet-C (UV-C) light-emitting diodes (LEDs) with peracetic acid (PAA) was evaluated as an early-stage disinfection strategy to inactivate A. acidoterrestris. UV-C LEDs emitting at 280 nm were first assessed as a standalone non-thermal intervention under two contamination scenarios: cells in suspension and on surfaces. They were also tested in combination with PAA to simulate a two-step industrial washing process. Exponentially growing cells and spores of A. acidoterrestris DSM 3922^T were enumerated after treatment, and their capacity to regrow under optimal conditions was subsequently assessed.

For all the conditions tested, 280 nm UV-C LEDs treatment (40, 160, and 640 mJ/cm²) achieved > 3-log reductions for both ACB forms, though higher fluences were required to fully prevent ACB regrowth. The recovery of growth was found to be fluence-dependent. For vegetative cells, no regrowth on surfaces was observed at fluences ≥160 mJ/cm². In contrast, spore treatments always resulted in some regrowth even at the highest fluence tested (640 mJ/cm²), demonstrating that, using the UV fluences tested, UV-C alone cannot achieve complete spore inactivation. When combined with PAA, complete inactivation of both ACB forms was achieved, with no recovery at fluences ≥160 mJ/cm².

This integrated approach - PAA in wash waters followed by post-wash UV-C LEDs exposure - offers a scalable, cost-effective solution for the food industry. By eliminating ACB at the processing entry point, it prevents propagation along the production line, improves product quality, extends shelf life, and reduces spoilage-related losses.

ID404 | Alicyclobacillin 24: the first bacteriocin described in the genus Alicyclobacillus

Author: Inês Carvalho Leonardo

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal 2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

E-mail: ines.leonardo@ibet.pt

Other authors: Ana Patrícia Quendera1,2, Micael Correia Freitas1, Sandra Pereira Santos1, Ana Rita Lemos1, Carolina Ferro Rodrigues2, Helena Ferreira1,2, Margarida Pimenta1,2, Carlos São-José3, Tiago Miranda Bandeiras1, Maria Teresa Barreto Crespo1,2, Frédéric Bustos Gaspar1,2

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

3 Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-019, Lisboa, Portugal.

Thermoacidophilic Alicyclobacillus (ACB) spp. are spore-forming bacteria responsible for the spoilage of fruit-based products, producing off-flavours and odours that lead to significant food waste. Their unique and sustained resilience, surviving both acidic environments and pasteurisation, renders conventional preservation strategies insufficient. This gap highlights the urgent need for novel, effective, and clean-label control strategies. Bacteriocins, ribosomally synthesised antimicrobial peptides, have emerged as promising natural candidates for replacing synthetic preservatives.

In this study, co-culture assays revealed that Alicyclobacillus acidoterrestris DSM 3922T inhibited the growth of other ACB isolates, suggesting the production of an antibacterial compound. Genome mining identified a gene encoding a geobacillin 26 family protein, and subsequent proteomic analysis confirmed its presence in the secretome. Based on its origin and molecular weight, this novel bacteriocin, representing the first described in the genus Alicyclobacillus, is designated alicyclobacillin 24 (Ali24).

Ali24 was heterologously expressed, purified, and functionally characterised. The recombinant protein exhibited broad-spectrum antibacterial activity against multiple ACB species, including spoilage-associated strains, as well as closely related Bacillus spp. Biophysical assays (dynamic light scattering and nano differential scanning fluorimetry) showed that Ali24 has optimal stability under acidic conditions, retaining functionality up to 50 °C. AlphaFold 3 structural modelling suggested a mechanism involving transient membrane association, potentially leading to membrane destabilisation rather than pore formation. This was supported by LIVE/DEAD staining, confirming a non-lytic mode of action. Together, these results align with previous reports of geobacillin 26 and support the classification of Ali24 as a Class IIIb bacteriocin.

This study reports the first functional Class III bacteriocin from A. acidoterrestris, active against spoilage Alicyclobacillus spp. Characterisation of Ali24 expands the repertoire and understanding of thermophile-derived bacteriocins and highlights new avenues for the sustainable control of ACB spoilage in the food industry.

ID428 | Valorization of Food By-Products Via Solid Submerged Fermentation for Sustainable Mycoprotein Production

Author: Vitor Sousa

Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal and LABBELS- associated laboratory, Braga, Guimarães, Portugal

E-mail: vitorsousa@ceb.uminho.pt

Other authors: Eduardo Coelho1,2, António A. Vicente1,2, Luís Abrunhosa1,2

1 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

2 LABBELS- associated laboratory, Braga, Guimarães, Portugal

Background: The growing demand for sustainable protein sources has intensified interest in alternative production systems with reduced environmental impact. Mycoprotein is a promising option in this context. This study explored solid submerged fermentation (SSmF) as a bioprocess for producing mycoprotein from Pleurotus spp. using food by-products. Brewers' spent grain (BSG), banana peel (BP), peanut peel (PL), and cocoa bean shells (CBS) were selected due to their high availability and low cost.

Methods: The C/N ratios of the by-products were determined using an elemental analyzer (vario EL cube). P. ostreatus (PO), P. ostreatus black pearl (POB), and P. eryngii (PE) were cultivated under SSmF with malt extract broth (MEB), either alone or supplemented with 10% (w/v) of each by-product. Incubations were carried out in the dark at 25 °C with agitation at 200 rpm for 15 days. Fermentations were monitored by measuring sugars consumption, dried biomass yield, and protein content. In a final trial, PO was cultivated in a 3L-bioreactor using 10% BSG in MEB.

Results: The C/N ratios obtained were: BSG (10.8 ± 1.7), BP (37.9 ± 5.9), CBS (13.6 ± 1.2), PL (32.5 ± 5.6). On MEB, PO achieved the highest biomass yield (16.0 g/L) and protein content (16.5%), compared with POB (12.4 g/L; 9.9%) and PE (18.4 g/L; 8.3%). When cultivated with by-products, PO showed lower biomass yields: PL (0.66 g/L), CBS (3.46 g/L), BP (0.83 g/L), and BSG (9.80 g/L). These differences highlight the strong influence of substrate composition on fungal growth, with BSG supporting yields closest to the corresponding control (14.3 g/L).

Conclusions: The results emphasize that the intrinsic metabolic characteristics of Pleurotus spp. are determinant for the accumulation of biomass and protein. In addition, nutrient balance, particularly the C/N ratio, strongly influences substrate utilization by PO and the resulting biomass yield. Overall, SSmF demonstrates strong potential as a bioprocess for valorizing food by-products into mycoprotein.

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ID440 | Uncovering Novel Komagataeibacter Strains from Mediterranean Vinegars: Genomic and Chemical Insights into Bacterial Cellulose Production

Author: Diogo Neves Proença

1 MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; 2 Centre for Mechanical Engineering, Materials and Processes (CEMMPRE), Advanced Production and Intelligent Systems (ARISE), Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

E-mail: daproenca@ualg.pt

Other authors: Djenabu Candé1, Célia Quintas3, Bruno Medronho1,4, Anabela Romano1

3 MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto Superior de Engenharia, Universidade do Algarve, Campus da Penha, 8005-139 Faro, Portugal; 4 Surface and Colloid Engineering, FSCN Research Centre, Mid Sweden University, SE-851 70 Sundsvall, Sweden

Background: Mediterranean ecosystems harbor remarkable microbial diversity, yet their biotechnological potential remains underexplored. Among microbial metabolites, bacterial cellulose (BC) has attracted particular attention due to its unique properties - superior mechanical properties, high water retention and excellent biocompatibility - surpassing those of plant-derived cellulose. These features make BC highly valuable for a broad range of applications. Several genera are known BC producers, especially Komagataeibacter. This study aimed to isolate, characterize and assess the biotechnological potential of BC-producing strains from the “mothers of vinegar” during artisanal vinegar acetification.

Methods: Twenty-five acetic acid bacteria were isolated and identified through 16S rRNA sequencing and phylogenetic analysis. Genomes of five representative strains were sequenced and analyzed for BC biosynthetic gene clusters and secondary metabolite potential using antiSMASH. Phylogenomic analyses were conducted for selected strains. BC production was evaluated under defined cultivation conditions using glucose, fructose and glycerol as carbon sources. The resulting BC films were chemically characterized through FTIR and XRD.

Results: The isolates belonged to Komagataeibacter (n=18)and Acetobacter(n=7). Several Komagataeibacter strains likely represent novel species (ANI<95%). Selected strains produced BC when cultured with glucose, fructose and glycerol, with yield and quality depending on both strain and carbon source. FTIR and XRD analysis revealed significant structural and chemical variability among the BC films. Genome mining confirmed the presence and organization of cellulose biosynthesis operons in producer strains, whereas non-producers lacked essential genetic elements or showed divergent operon structures. Conclusions: Mediterranean fruit-based residues constitute a valuable source of novel BC-producing bacteria, particularly within the genus Komagataeibacter. This work underscores the importance of preserving microbial biodiversity as a reservoir of biotechnological resources and highlights the potential of these strains to support the circular bioeconomy in sustainable Mediterranean agro-food systems.

ID442 | Turning Apple-Pear Ketchup Stream into Value: Fermentation for Stability and Antimicrobial Potential

Author: Ana Catarina Costa

LEAF (Linking Landscape Environment Agriculture and Food) Research Centre, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal

E-mail: acatarinacosta@isa.ulisboa.pt

Other authors: Miguel Nascimento 2; Anabela Raymundo1; Catarina Prista1

1 LEAF (Linking Landscape Environment Agriculture and Food) Research Centre, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal; 2 Mendes Gonçalves, Zona Industrial, lote 6, Golegã, 2154-909 Golegã, Portugal

Food industry waste streams, rich in nutrients, offer opportunities for valorisation through fermentation to produce valuable products, thereby reducing waste and maximising resource utilisation. Our study aims to explore the potential of using fermentation to convert Apple-Pear Ketchup (KPM) waste streams into a valuable ingredient for reuse, ensuring microbiological safety. A consortium of 2 lactic acid bacteria and a yeast was selected for lab-scale fermentations. The KPM stream was diluted 50% with demineralised water and fermented for 15 days (KPM 50%). Product stability was evaluated over 180 days by monitoring pH and total soluble solids (TSS), with additional samples taken to measure titrable acidity. Microbiological analyses were also performed to assess the viability of the inoculum. The antimicrobial potential against four spoilage yeasts (Zygosaccharomyces rouxii, Zygosaccharomyces parabailii, Pichia mashurica, Candida stellata) and two lactic acid bacteria (Levilactobacillus brevis, Lentilactobacillus parabuchneri) and a pathogenic bacterium (Bacillus cereus) was assayed, using drop tests in solid media with the incorporation of the fermented product.

KPM 50% inoculum remained viable for 180 days (lactic acid bacteria 6.40x103, yeasts 6.95x103). During 180 days of preservation at room temperature, the pH dropped from 3.81 to 3.36 at day 180. Meanwhile, TSS values showed sugar consumption, decreasing from 8.13 to 7.27ºBrix. The titratable acidity at the end of fermentation of KPM reached 2.51, decreasing to 2.99g lactic acid/100 g of fresh biomass, which is consistent with metabolically viable yeast and lactic acid bacteria. Fermented KPM 50% demonstrated antimicrobial capacity, showing complete inhibition of almost all microorganisms tested, except for Z. rouxii and Z. parabailii, known to be resistant to weak acids.

These findings highlight fermentation as a promising strategy to valorise KPM streams into a stable product with antimicrobial potential, which can be incorporated into KPM sauce as a substitute for commonly used preservatives, such as potassium sorbate.

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ID446 | Monitoring Enterococcus spp. in University Canteens: Antimicrobial Resistance in Food and Contact Surfaces

Author: Teresa Semedo-Lemsaddek

Centre for Interdisciplinary Research in Animal Health (CISSA), FMV-UL, Lisbon, Portugal/ Associate Laboratory for Animal and Veterinary Science (AI4Animals),Lisbon, Portugal /Biosystems and Integrative Sciences (BioISI), FCUL, Lisbon, Portugal

E-mail: tlemsaddek@fmv.ulisboa.pt

Other authors: Conceição S. Milindro1,2 , Joana M. Marques1,2,3, Maria T. Barreto-Crespo4,5

1 Centre for Interdisciplinary Research in Animal Health (CISSA), FMV-UL, Lisbon, Portugal 2 Associate Laboratory for Animal and Veterinary Science (AI4Animals),Lisbon, Portugal 3 National Institute of Agricultural and Veterinary Researc, IP (INIAV), Oeiras, Portugal 4 Institute of Experimental Biology and Technology (iBET), Oeiras, Portugal 5 Institute of Chemical and Biology Technology António Xavier (ITQB), Lisbon, Oeiras, Portugal

Introduction: Enterococcus spp. are Gram-positive bacteria, commensals of the gastrointestinal tract of humans and animals, but also present in environments such as food, surfaces, and water. Enterococcal detection in food raises concerns due to their role in the spread of antimicrobial resistance (AMR) along the food chain. This study aimed to characterize Enterococcus spp. from food samples and surfaces in university canteens, focusing on species identification, antimicrobial resistance profiles, and virulence potential.

Methodology: A total of 98 samples (67 foods and 31 surfaces) were collected in Portuguese university canteens between November 2023 and February 2024. The isolates were obtained using selective media, compared by RAPD-PCR and submitted to molecular identification at the species level. In addition, hemolytic activity assays and susceptibility testing against 12 antibiotics representing the main classes of antimicrobial agents were performed. The associations between resistance patterns, sample origin and species were evaluated by the chi-square test, using the R software. $P < 0.05$ was considered statistically significant.

Results: A total of 170 Enterococcus spp. were recovered, of which 98 were considered distinct based on RAPD analysis and selected for further study. Most isolates corresponded to E. faecalis (58%), followed by E. faecium (14%). Regarding safety analysis, only 3 of the 98 isolates (3.2%) from food exhibited hemolytic activity. Relevant antimicrobial resistance was observed, mainly to quinupristin-dalfopristin (54% of food isolates; 50% of surface isolates), rifampicin (38%; 63%), and erythromycin (25%; 8%). By species, E. faecalis showed resistance to quinupristin-dalfopristin (88%) and rifampicin (38%), whereas E. faecium displayed high resistance to rifampicin (90%) and low resistance to quinupristin-dalfopristin (10%). Statistical analysis revealed significant associations ($p < 0.05$) between resistance to specific antibiotics and the origin of the isolates (food vs. surfaces), as well as between species and resistance profiles. Conclusion: The results showed the occurrence of Enterococcus spp., predominantly E. faecalis followed by

E. faecium, and revealed relevant antimicrobial resistance profiles, particularly to quinupristin–dalfopristin, rifampicin, and erythromycin. Hemolytic activity was detected at low frequency but confirmed the virulence potential of some enterococci.

ID463 | Exploring Persistence Markers through Fatty Acid Profiles of Listeria monocytogenes under Distinct Temperature Conditions

Author: Rui Magalhães

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina– Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169- 005 Porto, Portugal

E-mail: rsmagalhaes@ucp.pt

Other authors: Manuela Machado (1) , Paula Teixeira(1)

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina– Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169- 005 Porto, Portugal

Background: Listeria monocytogenes is a major foodborne pathogen, partly due to its ability to persist in food processing environments. Remodeling of membrane fatty acids, essential for maintaining fluidity and functionality under stress, may contribute to this persistence. This study aimed to identify fatty acids that could serve as persistence markers in 40 isolates from the GenoPhenoTraits4Persistence collection, classified as persistent (P) or non-persistent (NP).

Methods: Lipids were extracted from pellets of cells grown at 37 ºC or 10 ºC using hexane as the solvent, followed by transesterification for analysis by GC-FID (Gas Chromatography-Flame Ionization Detection).

Results: Gas chromatography analysis showed that L. monocytogenes cultured at 37 °C and 10 °C exhibited a membrane fatty acid profile predominantly composed of branched-chain species, particularly iso-C15:0 and iso-C17:0. These fatty acids are associated with increased resilience to environmental stresses, including temperature fluctuations. Notably, a monounsaturated C19 fatty acid (C19 Δ), detected exclusively in cells grown at 10 °C, suggests an adaptive mechanism to cold stress through adjustments in membrane fluidity.

Conclusions: The GC-FID data indicate that L. monocytogenes modifies its fatty acid composition in response to growth temperature. The higher levels of iso-C15:0 and iso-C17:0, together with the appearance of C19 Δ at lower temperatures, highlight lipid traits that may serve as markers of environmental adaptation. Although fatty acid remodeling alone is not sufficient to define a strain as persistent, these features align with mechanisms that contribute to long-term survival in food-processing environments, where persistent isolates often display enhanced stress tolerance and resistance to cleaning and disinfection. These findings provide a biochemical perspective that complements genetic and ecological insights into L. monocytogenes persistence.

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ID468 | Effect of increasing gentamicin concentrations on Listeria monocytogenes cells viability

Author: Rui Magalhães

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina– Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169- 005 Porto, Portugal

E-mail: rsmagalhaes@ucp.pt

Other authors: Pedro Sousa (1), Rafaela Marques (1), Rita Calvinho (1), Rui Magalhães (1)(*), Paula Teixeira (1).

(1) Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina– Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169- 005 Porto, Portugal

Background: Listeria monocytogenes is a notorious foodborne pathogen responsible for severe illness, primarily in individuals with weakened immune systems, including pregnant women, elderly and immunocompromised. This microorganism poses a significant food safety risk due to its remarkable ability to withstand harsh environmental conditions, including high salt concentrations or low temperatures, often encountered in food processing environments. Since gentamicin is generally used to treat L. monocytogenes infections, this study aimed to explore strain-specific differences in response to this antibiotic.

Methods: In this study, various L. monocytogenes strains were exposed to increasing concentrations of gentamicin for 4 hours, with the objective of assessing the impact on cell viability.

Results: Interestingly, the results revealed strain-dependent responses. In some cases, a noticeable reduction was observed (ca. 4 log CFU/mL) as the antibiotic concentration increased, while other strains showed only a slight decrease in population under the same conditions.

Conclusions: This study demonstrates that L. monocytogenes strains differ in their susceptibility to gentamicin. Such variability may reflect underlying strain-specific traits that also influence survival under stress, helping to explain the challenges in controlling this pathogen within the food industry and its potential, in some cases, to establish as persistent strains.

ID501 | Non-Saccharomyces Yeast Genomes as a Resource for Climate-Resilient Wine Fermentation

Author: Carolina Osório

1 Centro de Biologia Molecular e Ambiental (CBMA), Braga, Portugal

E-mail: carolina.osorio.99@gmail.com

Other authors: Bruna Oliveira1, Teresa Rito2, Pedro Soares2, Ricardo Franco-Duarte 1, Maria João Sousa 1

1 Centro de Biologia Molecular e Ambiental (CBMA), Braga, Portugal 2 Institute of Science and Innovation for Bio-Sustainability (IB-S), Braga, Portugal

Background: Wine production is crucial to Portugal's secondary sector however, climate change has challenged the viticulture sector, prompting the exploration of alternative strategies, such as the use non-Saccharomyces yeasts in grape must fermentation.

Methods: This study expanded our previous work [1] on non-Saccharomyces yeast species isolated from grapes/grape must. Genome data for 229 species (1,197 genomes) were retrieved from NCBI and added 3 Saccharomyces cerevisiae genomes as reference. A bioinformatic pipeline [2] was applied for functional annotation and phylogenomic analysis. BLAST analysis was used to compare the presence/absence of 6,018 genes of S. cerevisiae S288c with the species capacity to ferment 7 carbon sources. Statistical analysis was performed to identify genes present in >95% of fermentative and 90% and <10%, respectively. Results: Functional annotation using KEGG modules, COG categories and CAZymes revealed distinct clustering patterns, reflecting both conserved and divergent genomic repertoires. While most species followed expected phylogenetic relationships, several exceptions highlighted unique genomic signatures. The BLAST-based comparative analysis provided a systematic catalogue of gene presence/absence across species, enabling the identification of candidate genes potentially linked to the utilisation of 7 carbon sources, by the identification of 18 putative genes majorly present in the fermentative species.

Conclusions: This study presents a comprehensive genomic survey of non-Saccharomyces yeasts involved in wine fermentation. By combining large-scale annotation with comparative analysis, it establishes a framework for linking genomic diversity with functional potential. The catalogue of putative genes related to carbon source utilisation provides a resource for future studies.

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ID522 | Application of Nonthermal Technologies for the Microbiological Safety and Quality of a Vegan Smoothie

Author: Nicole Moreira

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: nicolefmoreira@ua.pt

Other authors: Mariana Álvaro (2), Maria Alegria (3), Jorge Saraiva (4)

2 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 3 SUMOL+COMPAL Marcas, S.A. Estrada Nacional 118, 2080-023 Almeirim, Portugal; 4 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

This study describes the development and microbiological evaluation of a vegan smoothie selected as one of the eight finalists in ECOTROPHELIA Portugal 2024, formulated with a soy-based yogurt alternative, water kefir, vegetable byproducts such as pumpkin and carrot, with apple pieces, pear pulp, kale, lemon juice and zest, ginger, and a probiotic (Weizmannia coagulans, in sporulated form). To ensure microbiological safety while preserving sensory/nutritional/functional integrity, two nonthermal technologies were assessed: High Pressure Processing (HPP) was applied to the smoothie as a pasteurization method at 550 MPa for 3 minutes at room temperature (RT), followed by 28 days of storage at 4 °C for weekly analyses; in parallel, Hyperbaric Storage (HS) was investigated as a RT conservation alternative to refrigeration (RF), with the raw smoothie without W. coagulans (since previous tests showed it did not resist along storage) stored at 75 and 100 MPa (~21 °C) and compared to RF at 0.1 MPa/4 °C for 21 days. Microbiological analyses included Total Aerobic Mesophiles (TAM), Lactic Acid Bacteria (LAB), Enterobacteriaceae (ENT), and Yeasts and Molds (YM), and physicochemical evaluations included pH, titratable acidity (TA), °Brix, browning, and color. The HPP-smoothie showed microbiological stability, with ENT and YM undetectable and probiotic levels >6.00 log CFU/g. In contrast, the unpasteurized smoothie surpassed 7.00 log CFU/g for TAM, LAB, and YM by day 28. HPP stabilized pH and TA, whereas the unpasteurized smoothie showed a pH drop (4.55 to 4.25) and TA increase (0.55 to 1.12 g lactic acid eq/100 g). °Brix values remained stable under HPP (8.37 to 8.40), with a sharper decrease in the unpasteurized smoothie (7.95 to 6.67). Browning decreased in both samples, but HPP slightly induced greater color variation (ΔE* = 8.13 vs. 6.34). In the HS study, RF showed an overall microbiological increase, while HS at 75 MPa reduced microbial loads by 1–2 log and eliminated ENT and YM by day 7. At 100 MPa, all microbial groups dropped below the detection limit by day 7. HS preserved better pH, TA, °Brix, and browning compared to RF, but a higher ΔE* was observed under HS (13.71–15.76) than RF (7.06).

These results proved that HPP followed by RF storage maintained microbiological safety with probiotic viability of the smoothie while HS at RT extended shelf-life with better microbiological safety and globally fewer physicochemical changes than RF.

ID524 | Extraction and Application of Microbial Pigments and Biosurfactants from Rhodotorula mucilaginosa in Bio-Based Emulsions for Use as Food Colorants

Author: Mariana Brandão Nogueira Alves

1 University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal. 2 International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal

E-mail: mbna@uc.pt

Other authors: Miguel Angelo Cerqueira 2; Jorge Fernando Brandão Pereira 1

1 University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal. 2 International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal

Microbial pigments and biosurfactants have gained increasing attention as sustainable alternatives to synthetic colorants and surfactants in various industrial applications, particularly in the food sector. Rhodotorula mucilaginosa is a promising yeast capable of producing high-value carotenoid pigments and potentially biosurfactants, which can be leveraged for the formulation of stable and functional bio-based emulsions. However, challenges such as low pigment solubility, stability, and bioavailability need to be addressed to enhance their application potential.

This study developed an integrative and sustainable approach for the extraction and stabilization of microbial pigments from R. mucilaginosa. Biosurfactants derived from the yeast were characterized, and explored and compared to commercially available biosurfactants, namely rhamnolipids, to evaluate their efficiency in stabilizing pigment-based emulsions. The pigments were extracted using green, food-grade techniques, namely natural deep eutectic solvents, and compared with traditional solvent extraction methods.

The resulting extracts were formulated into bio-based colored nanoemulsions with particle sizes surrounding 200nm, with PDI around 0.3, ensuring the effective stabilization of microbial pigments while maintaining their functionality and safety for food applications.

By harnessing microbial biomolecules, this study contributed to the advancement of microbial biorefineries and promoted a circular and sustainable bioeconomy for the developments of sustainable novel food products.

ID538 | Antimicrobial potential of LAB-fermented fruit preparations as a strategy to replace common preservatives in yogurts

Author: Cristiana Santos

LEAF- Linking Landscape, Environment, Agriculture and Food Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisbon, Portugal.

E-mail: cristiana.filipe.santos@gmail.com

Other authors: Inês Magalhães 2, Daniela Correia 2, Luís Paulico 3, Cristina Rodrigues 3, Anabela Raymundo 1, Catarina Prista 1, Juliana Botelho Moreira 1

2 MC Shared Services S.A., Rua João Mendonça, 529, 4464-501 Senhora da Hora, Matosinhos, Portugal; 3 FRULACT • Headquarters & Frutech RDI Centre, Rua do Outeiro, 589 • 4475-150 Gemunde Maia, Portugal.

Growing health awareness among consumers has led to an increased demand for healthier, natural, and sustainable food products. As an alternative to replace chemical preservatives, lactic acid bacteria (LAB) have been studied due to their ability to produce organic acids, bacteriocins, and other antimicrobial metabolites during fermentation.

This study aimed to evaluate the antimicrobial activity of fermented fruit preparations and yogurts containing these preparations against Escherichia coli, Penicillium glabrum, and Aspergillus niger over 21 days. The fruit preparations (strawberry – S and red fruit – RF) were fermented for four days using two consortia: Bifidobacterium (BIF) and Milk Kefir inoculum (KF). Then, they were autoclaved and incorporated into Greek (GY) and drinkable (DY) base-yogurts, resulting in four clean-label products (BIF-GY, KL-GY, BIF-DY, and KL-DY). The controls (control-GY and control DY) were commercial yogurts containing fruit preparations preserved with potassium sorbate. The antimicrobial activity was evaluated by drop tests, using serial 10-fold dilutions, on COMPASS Ecc Agar for E. coli and Sabouraud with chloramphenicol for molds.

On day 21, E. coli was completely inhibited in all the yogurts containing the fermented fruit preparations, similarly to yogurt controls. Regarding P. glabrum, BIF-GY and KL-GY showed reductions of 1 and 2 logs, respectively, whereas in the control-GY no reduction was observed. In addition, BIF-DY and KL-DY exhibited reductions of 3 and 2 logs, respectively. For A. niger, KL GY and BIF-DY demonstrated the highest antimicrobial activity, resulting in a 1-log reduction in the number of viable cells. Neither control inhibited this pathogenic microorganism.

Overall, fermenting fruit preparations with LAB for incorporation into yogurts represents a promising clean-label strategy to replace synthetic preservatives commonly used to preserve food products.

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ID545 | Recombinant production of rat serum albumin in Pichia pastoris for capture of zearalenone

Author: Heidar Mar Adalsteinsson

Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: heidar.adalst@ceb.uminho.pt

Other authors: Tatiana Quinta Aguiar, José António Teixeira, Luís Abrunhosa

Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal; LABBELS- associated laboratory, Braga, Guimarães, Portugal

Background: Serum albumins are the most abundant proteins in plasma of mammals. One of their roles is the binding and transport of small molecules and metal ions in the body. They have also been shown to bind toxic molecules, such as mycotoxins that commonly contaminate foods. As a result, human serum albumin (HSA) has been a long standing target in studies of drug discovery, whereas other mammalian serum albumins have been studied less. Recent screening studies have shown that rat serum albumin (RSA) binds the mycotoxin zearalenone (ZEN) with significantly higher affinity than other tested serum albumins. We hypothesized a cost-effective production of recombinant RSA (rRSA) in P. pastoris for rapid immobilization in solid phase extraction (SPE) columns, which may be used for the capture and analysis of ZEN in food matrices.

Methods: P. pastoris KM71H was transformed with the pPICZαA vector for the production of rRSA bearing a His6 tag at the C-terminal. Using a previously optimized protocol, the production of stable extracellular rRSA was performed in complex medium with methanol induction (0.5 g/L) for 96 hours. Purification of rRSA was achieved using standard protocols for immobilized metal ion affinity chromatography. Circular dichroism (CD) and fluorescence quenching were used for characterization of binding properties. HPLC was used to quantify captured ZEN by immobilized rRSA in SPE columns.

Results: SDS-PAGE was used to confirm the presence of the target protein in the production medium and absence of contaminants after purification. The binding properties of purified rRSA were compared to native RSA. The recombinant protein presented identical CD spectrum to the native one, and similar changes in the presence of ZEN. Fluorescence quenching studies indicated that rRSA has a slightly higher affinity for ZEN than native RSA, or KA = 2.9x10^5 M-1 and 1.9x10^5 M-1, respectively. The recombinant protein was also immobilized directly from the production medium on a HisPur(TM) nickel charged agarose resin, packed into a SPE column, which successfully captured ZEN from a buffer solution, as confirmed by HPLC.

Conclusions: rRSA was successfully produced recombinantly in P. pastoris. Strong affinity for ZEN was shown by fluorescence quenching, and immobilized rRSA successfully captured ZEN from buffer solutions, demonstrating a proof of concept. Next steps include optimization of protocols for real food matrices.

ID546 | Microbiological characterization of Hermetia illucens larvae subjected to different types of feeding and slaughter methods

Author: Susana Maria da Silva Valente

Escola Superior Agrária, Polytechnic Institute of Beja, Rua Pedro Soares, Ap. 6158, 7801-908 Beja, Portugal

E-mail: susana.silva.valente@gmail.com

Other authors: Maria Teresa Pereira Gonçalves dos Santos (1,2); Antónia Teresa Zorro Nobre Macedo (1,3,4)

1 Escola Superior Agrária, Polytechnic Institute of Beja, Rua Pedro Soares, Ap. 6158, 7801-908 Beja, Portugal; 2 MED. Instituto Mediterrâneo para a agricultura, Ambiente e Desenvolvimento da Universidade de Évora, Universidade de Évora, Pólo da Mitra Apartado 94, 7006-554 Évora; 3 LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal; 4 CIEQV - Life Quality Research Centre (CIEQV), 2040-413 Rio Maior, Portugal;

Due to the increase in global food demand, it is essential to identify viable, sustainable and safe alternatives that ensure food security for populations. One of the alternatives with the potential is the use of edible insects, due to their high nutritional value and production efficiency. Four species have already been approved for human consumption in the European Union, although others, such as H. illucens, are of great. In regulatory terms, insects are considered “novel foods”, and microbiological safety is one of the main requirements in the approval process for new products.

This study aimed to evaluate the impact of supplementing the substrate with goat cheese whey and slaughter methods – deep-freezing and blanching – on the quality of H. illucens larvae powder. The larvae were reared on standard substrate with the addition of distilled water or whey. After most reached the pre-pupa instar, they were slaughtered by deep-freezing (24 h at -80 °C) or blanching (40 seconds in boiling water), dehydrated (60 °C) and crushed. Microbiological analyses were performed based on the regulations for already approved insect-based products.

The powders obtained were analysed for water activity (aW) and microbiological quality. The aW values ranged from 0.07 to 0.63. The total mesophilic aerobic count ranged from 3.43 to 6.08 log CFU/g, with two samples exceeding the legal limit (≤ 105 CFU/g). For yeasts, the results ranged from < 1 to 3.93 log CFU/g, with three samples not complying (limit of ≤100 CFU/g). Regarding molds, all samples (< 1 to 1.48 log CFU/g) were compliant (limit of ≤100 CFU/g). The analyses for E. coli (< 1 log CFU/g), Salmonella spp. (absent), sulfite-reducing anaerobes (absent), and coagulase-positive staphylococci (< 2 log CFU/g) had results within the corresponding legal limits. However, for Enterobacteriaceae (<1 to 2.52 log CFU/g), two samples had values above the legal limit (< 10 CFU/g).

The powders analysed showed low water activity, a factor favourable to the microbiological stability of the samples. However, some samples showed non-compliant results in relevant parameters. To date, it is not possible to state whether the slaughter under study has a significant influence on the microbiological stability of the product. These results reinforce the need for further testing and the strict application of good practices in animal production, handling, processing, and storage to ensure legal compliance and the quality of the powders obtaine

ID560 | Ethanol production by fermentation of spent coffee grounds and milk whey

Author: Lénia Lima Galante

University of Azores

E-mail: lenia1903@gmail.com

Other authors: Susana Isabel Chaves Ribeiro; Daniela Sofia Almeida Ribeiro; Célia Costa Gomes da Silva

University of the Azores, Institute of Agricultural and Environmental Research and Technology - IITAA, Angra do Heroísmo, Portugal

Background: Two prominent food industry by-products, spent coffee grounds and milk whey, offer complementary pathways for bioethanol production. Spent coffee grounds are rich in lignocellulosic polymers (cellulose 8–15%, hemicellulose 30–40%) and must first undergo pretreatment and enzymatic hydrolysis with cellulases to break down these complex structures into fermentable sugars. Milk whey, by contrast, requires lactase to hydrolyse its non-fermentable lactose (4.6–5.2%) into glucose and galactose, which are then readily fermented by Saccharomyces cerevisiae. The aim of this study is to evaluate the optimal conditions for producing ethanol from these residues and subsequent use in a distilled alcoholic drink - gin.

Methods: Pre-treatment of spent coffee (120 g/L) was performed by adding cellulase (pH 5, 50°C) and agitating at 150 rpm. To achieve higher ethanol concentrations, several parameters affecting pre-treatment (two enzyme levels) and the fermentation process with milk whey, --galactosidase and Saccharomyces cerevisiae were evaluated: the use of yeast either free or immobilised in alginate beads, two S. cerevisiae levels, fermentation at 20°C and 37°C, and fermentation times from 1 to 8 days. The fermented product was evaluated for ethanol concentration by microdistillation method [1]. Results were analysed using stepwise regression.

Results: Three parameters were predictors in the model: cellulase level, yeast immobilisation, and fermentation time. The optimal conditions for achieving higher ethanol concentration (1.8 %, v/v) were pre-treatment with 0.016 g/L cellulase, yeast immobilisation in alginate, and 8 days of fermentation. However, the increase in ethanol concentration was only 20% from 4 to 8 days. Under these optimal conditions, 120 g of spent coffee grounds subjected to acidic hydrolysis at 50 °C for 48 h and 5 days of fermentation (1 L of whey) produced 36% ethanol after distillation.

Conclusions: This study demonstrates that the combination of cellulase pretreatment, use of lactase, immobilization of yeasts in alginate and controlled fermentation allows the efficient production of ethanol from spent coffee and milk whey. The importance of critical process parameters to optimize yield is highlighted, contributing to waste recovery strategies and reducing environmental impact.

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ID563 | Irrigation with contaminated surface water alters lettuce microbiota and induces physiological stress

Author: Marta Tacão

CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: martat@ua.pt

Other authors: Carlos Barros 2, Marta Alves 1.2, Rafael Tavares 1.2, Glória Pinto 1.2

1 CESAM, University of Aveiro, Portugal; 2 Department of Biology, University of Aveiro, Portugal

Background: Water pollution threatens food safety, particularly when contaminated surface water is used for crop irrigation. Leafy greens, such as lettuce, are often eaten raw and have been frequently linked to foodborne outbreaks. The Antuã river (Portugal) is still used for agricultural irrigation despite documented contamination, raising safety concerns regarding pathogen and antimicrobial resistance dissemination. This study evaluated the impact of Antuã river water on lettuce-microbiota, including the occurrence of antibiotic-resistant bacteria (ARB) and associated resistance genes (ARGs), as well as on plant health and its suitability for irrigation.

Methods: Physicochemical and microbiological parameters were measured in river water. Butterhead lettuce seedlings were cultivated in substrate under controlled conditions for 14 days, irrigated either with distilled water (control, n=15) or with river water (n=15). Leaves (washed with tap water or unwashed) and root samples were collected at days 0 and 14 for 16S rRNA amplicon sequencing and compared to river water samples. ARGs were quantified by qPCR. Plant health was assessed through measurements of gas exchange, pigment content, and quantification of metabolic markers (e.g. flavonoids, sugars).

Results: Antuã river water showed poor quality, with high bacterial loads and fourteen pathogen-related genera detected. Irrigation with river water reduced bacterial diversity in the lettuce phyllosphere and shifted its composition when compared to the control group. Washing with tap water reduced the bacterial load in the epiphytic leaf community but river-associated taxa were still identified. Pathogen-related groups (e.g. Weeksellaceae) were found in river water and river water-irrigated plants. The integrase gene intI1 was prevalent across samples, while sul1 was detected in lettuce irrigated with river water. River water-irrigated plants exhibited increased levels of defence pigments and reduced photosynthetic activity, indicating stress responses.

Conclusions: Irrigation with contaminated surface water can alter lettuce microbiota, introduce pathogen-associated taxa and ARGs, and decline plant physiological performance. These findings highlight the need for improved management of irrigation water quality and the importance of One Health approaches to mitigate the dissemination of pathogens and antimicrobial resistance via fresh produce.

ID572 | Microbiological Safety of Raw Fish: Evaluation of Pathogens and Freezing Effect

Author: Gabriela Jorge Da Silva

Faculty of Pharmacy, University Coimbra, 3000-458 Coimbra, Portugal, CNC-UC-Center for Neuroscience and Cell Biology, University Coimbra, 3004-517 Coimbra, Portugal 3, CIBB-Centre for Innovative Biomedicine and Biotechnology, University Coimbra, 3004-548 Coimbra, Portugal

E-mail: gjsilva@ci.uc.pt

Other authors: Inês Esteves1, Tiago Lima1,2,3,4, Gonçalo Almeida5, Gabriela J. Da Silva1,2,3(*), Sara Domingues1,2,3,

1, Faculty of Pharmacy, University Coimbra, 3000-458 Coimbra, Portugal; 2, CNC-UC-Center for Neuroscience and Cell Biology, University Coimbra, 3004-517 Coimbra, Portugal 3, CIBB-Centre for Innovative Biomedicine and Biotechnology, University Coimbra, 3004-548 Coimbra, Portugal 4, Vasco da Gama Research Centre (CIVG), Department of Veterinary Sciences, Vasco da Gama University School, 3020-210 Coimbra, Portugal 5, National Institute for Agrarian and Veterinary Research, Vairão, Portugal

Background: The increasing consumption of raw fish raises safety concerns due to its perishability and potential carriage of pathogenic bacteria. This study evaluated the microbiological quality of 26 tuna and salmon samples intended for domestic consumption.

Methods: Listeria monocytogenes detection was performed by MALDI-TOF mass spectrometry, while Escherichia coli and Salmonella spp. were investigated by sequencing after partial amplification of the 16S rRNA gene. The effect of 24 h freezing on total microbial load was also assessed.

Results: All samples were negative for Salmonella spp. and E. coli. Three samples (11.5%) contained Listeria spp., specifically L. innocua (n=2) and L. welshimeri (n=1), both non-pathogenic. Other bacterial genera frequently isolated from aquatic environments were identified, including Aeromonas sp. (23%), Pseudomonas sp. (19%), and Proteus sp. (15%). Some of these comprise opportunistic pathogens, particularly relevant for immunocompromised individuals. Freezing for 24 h reduced microbial load by an average of 18%, although this reduction was not statistically significant ($P > 0.05$).

Conclusions: Overall, the absence of enteric pathogens and the predominance of environmental species indicate that the fish analyzed complied with good hygiene and safety practices from capture/production to retail. Nevertheless, domestic handling may still pose risks, especially in contexts of low food literacy, underscoring the importance of consumer awareness when preparing raw fish.

ID587 | Search on potential novel bacteriocins and bacteriocin-producing bacteria isolated from the bovine rumen

Author: Nuno Miguel Lopes Paiva

University of the Azores, Faculty of Agricultural and Environmental Sciences, Angra do Heroísmo, Portugal.

E-mail: nunop1991@gmail.com

Other authors: Susana Isabel Chaves Ribeiro, Alfredo Emílio Silveira de Borba, Célia Costa Gomes da Silva.
University of the Azores, Institute of Agricultural and Environmental Research and Technology - IITAA, Angra do Heroísmo, Portugal.

Background: The restriction of antibiotic use in livestock has intensified the search for effective alternatives, such as antimicrobial peptides. The rumen microbiome, with its complex microbial interactions, represents a promising reservoir for novel bacteriocins and bacteriocin-producing bacteria. This study aimed to isolate and characterize bacteriocin-producing bacteria from bovine ruminal fluid.

Methods: Bacterial isolates from cow ruminal fluid were screened for antimicrobial activity against a panel of foodborne and clinical pathogens: Staphylococcus aureus, Listeria monocytogenes, Listeria innocua, Bacillus cereus, Salmonella enterica subsp. enterica serovar Enteritidis, S. enterica serovar Typhimurium, Bacillus spizizenii, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa. Active isolates were further analysed by assessing the proteinaceous nature of the antimicrobial compounds via sensitivity to proteolytic enzymes (trypsin, proteinase-K, and α-chymotrypsin). Putative bacteriocin producers were identified by 16S rRNA gene sequencing.

Results: Of 97 isolated pure cultures, four strains exhibited potent antimicrobial activity. Three strains showed broad-spectrum inhibition against all tested pathogens, while one was active against four pathogens. The antimicrobial activity of all four isolates was abolished by protease treatment, confirming the proteinaceous nature of the inhibitory compounds. Sequencing identified the four isolates as belonging to the Bacillus velezensis/amyloliquefaciens/subtilis group.

Conclusions: This study successfully obtained four Bacillus spp. isolates from the bovine rumen that produce broad-spectrum, proteinaceous antimicrobial compounds, consistent with bacteriocins. These findings highlight the rumen microbiota as a valuable source of novel antimicrobial agents with significant potential as alternatives to antibiotics for improving animal health and food safety. Future work will focus on purifying and characterizing the specific bacteriocin compounds produced by these isolates.

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ID618 | Effect of wet ageing on the microbiological quality of goat meat

Author: Menalda Valeriano André

Menalda Valeriano André1, 21 LEAF – Linking Landscape, Environment, Agriculture and Food Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017 Lisboa, Portugal; 2 Faculdade de Ciências de Saúde, Universidade Lúrio, Campus Universitário de Marrere, Nampula 4250, Mozambique

E-mail: menaldaandre@isa.ulisboa.pt

Other authors: Vítor D. Alves; Teresa J. S. Matos; Catarina Prista

1 LEAF – Linking Landscape, Environment, Agriculture and Food Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017 Lisboa, Portugal; 2 2Associate Laboratory Terra, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017 Lisboa, Portugal

Background: Post-mortem aging of meat is an effective strategy for improving meat quality attributes. Among aging methods, wet aging is the most commonly used due to its flexibility, accessibility and ease of storage (1). However, previous studies have identified concerns regarding the microbiological safety of aged meat, mainly due to the lack of specific regulations. This regulatory gap hinders accurate risk assessment and compromises the assurance of food safety (2). Therefore, the main objective of this study was to evaluate the microbiological safety of goat meat wet aged for 14 days, as well as the effects of cooking.

Methods: Four female Serpentina goats (8-12 years old) were slaughtered according to European guidelines. On day 3 post-mortem, the left LTL muscle was vacuum-packed and aged for 14 days at 2 ± 2ºC. Microbiological analyses were carried out on days 0, 7 and 14 without cooking; and at day 14 after cooking the aged meat until reaching 68ºC internal temperature. Analyses followed ISO standards and included: total count bacteria (TCB), psychotropic bacteria, Escherichia coli, Salmonella spp., Listeria spp., Pseudomonas spp., moulds and yeasts, lactic acid bacteria (LAB) and Staphylococcus aureus. Data were analyzed using one-way ANOVA in RStudio ($P \leq 0.05$).

Results: A significant increase in the counts of aerobic bacteria, psychotropic microorganisms, Escherichia coli, Pseudomonas spp and LAB was observed over the 14-day ageing period. However, this microbial growth was significantly reduced at day 14 after cooking. The highest microbial load that was detected in cooked samples was from TCB, while the remaining parameters remained close to the detection limit. Salmonella spp. and Listeria spp. were not detected. Ageing did not significantly affect yeast growth, but it promoted an upward trend in Escherichia coli and Pseudomonas spp., which was completely mitigated by cooking. A reduction in Staphylococcus aureus counts was observed over the aging period.

Conclusions: Wet aging for 14 days increases microbial load in goat meat, but cooking effectively reduces it, ensuring the microbiological safety of the final product.

ID625 | Development of an oleogel as a strategy to reduce saturated fats in biscuit filling cream

Author: Vasco Lima

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal; 2 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

E-mail: vjlima@ucp.pt

Other authors: Catarina Vila-Real 2, Andrea García-Guzmán 3, Rita Peixoto 4, Ana Maria Gomes 2

2 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal; 3 KU Leuven Ghent Technology Campus, Faculty of Engineering Technology, Gent, Belgium; 4 Vieira de Castro Produtos Alimentares S.A., V. N. Famalicão, Portugal

Background: Food reformulation to improve the nutritional profile is a key strategy to promote balanced diets and offer additional benefits but the addition, substitution, or elimination of ingredients can affect functional and sensory properties. This work aimed to improve the nutritional profile of a biscuit filling cream, by creating an oleogel with reduced saturated fats.

Methods: High-oleic sunflower oil was used as the liquid oil phase and two natural waxes (rice bran wax (RBW) and candelilla wax (CDW)) were tried as oleogelators at varied concentrations (2-14%). Oleogels were added sugar and cocoa powder to make the creams. Textural profile analysis, oil binding capacity (filter paper method) and microstructure (polarized light microscopy) of oleogels and creams were assessed. Total saturated fat content (gas chromatography with a flame ionization detector) was evaluated in experimental and commercial biscuits.

Results: The hardness of filling creams, simple oleogel and oleogel with sugar was similar: as oleogelator's concentration increases, oleogel's hardness increases (not proportionally). 14% RBW (0.04%/h) and 10% CDW (0.03%/h) creams had the lowest oil migration rate, with a final oil loss <2% after 5 days for both. CDW oleogels had a feather and needle-like structure, forming a continuous network and RBW oleogels had a spherulitic structure without a continuous network. Micrographs of the oleogels with sugar showed white, shiny structures, while those with cocoa particles were brown, opaque and homogeneously distributed throughout the network. The most promisor cream was prepared with 14% RBW. Experimental biscuit's saturated fat content was ~50% lower than commercial biscuit's value (9.7 ± 0.7 g/ 100g vs. 19.9 ± 0.5 g/ 100g).

Conclusions: The high unsaturated fat oleogel-based filling cream developed shows potential to replace traditional high saturated fat creams. This strategy helps enhance confectionery foods, offering health-conscious consumers indulgent, yet nutritious snacks.

Acknowledgements: Thanks are due to Cindy Serafim for the microscopy analysis support. We also thank the scientific collaboration under the FCT project UIDB/50016/2020.

Funding: This work was financially supported by the project Agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o Agroalimentar, financed by Plano de Recuperação e Resiliência de Portugal ("PRR"), and by European Commission through the Erasmus Mundus programme.

ID627 | Alicyclobacillus acidoterrestris and Clostridium perfringens germination and development control at room temperature by hyperbaric storage

Author: Carlos Alberto Cruz Pinto

LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: carlospinto@ua.pt

Other authors: Alireza Mousakhani Ganjeh (2), Jorge A. Saraiva (1)

(1) LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; (2

Background: Bacteria from the genera Bacillus and Clostridium spp. are responsible for the production of highly resistant spores that can survive conventional and emergent pasteurization processes, being, this way, a threat to food quality and safety. This way, pasteurized foods are generally kept under RF conditions to temporarily delay spore germination and development. Hyperbaric storage (HS) is a new food preservation technique that uses storage pressure control, instead of temperature control as in refrigeration (RF), to inhibit microbial development. HS offers several advantages over RF, particularly because is more efficient in delaying microbial development, causing also microbial inactivation, and additionally is quasi energetically costless, since it is applied at RT.

Methods: This study aimed evaluating the potential of HS (75-250 MPa, up to 30 days) at RT (18-23 ºC) in inhibiting the development of the spores of Alicyclobacillus acidoterrestris (ACB) and Clostridium perfringens spores and comparing it with RF (4 ºC). C. perfringens spores were inoculated in coconut water (pH 5.60), and ACB spores were inoculated in commercial apple juice (pH 3.70).

Results: The results showed that HS was able to gradually inactivate ACB spores (up to 5 log units) in commercial apple juice after 24 hours at 150 MPa and at RT. Interestingly, a pressure increase to 200 or 250 MPa did not accelerate the inactivation of ACB spores, possibly due to changes in the inactivation mechanisms that occur around 150 MPa. In coconut water, C. perfringens spores were gradually inactivated during the 30 days of storage experiments, with an overall reduction of about 3 log units after 30 days at 200 MPa at and at RT. These results contrast with RF that only inhibited spore development. Additionally, for most cases, it was possible to fit the Weibull model to describe the inactivation process. Moreover, while under HS conditions, the spores did not reach the vegetative state despite being at RT, which considerably reduces the risk, for example, of guaiacol or toxins' production in the case of ACB and C. perfringens, respectively.

Conclusions: This new food preservation methodology, HS, can simultaneously be seen as a food preservation methodology at RT better than RF in what concerns microbial growth inhibition, but even being able to inactivate ACB and C. perfringens at RT and so with no thermal input, and additionally is quasi energetically costless, since it is applied at RT.

ID630 | Influence of ultrasound and pulsed electric field on the hyperbaric inactivation of Alicyclobacillus acidoterrestris spores s in apple juice

Author: Jorge Manuel Alexandre Saraiva

LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

E-mail: jorgesaraiva@ua.pt

Other authors: Carlos Alberto Cruz Pinto (1) Maria João Alegria (2)

(1) LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal (2) SUMOLCOMPAL Marcas, S.A. Estrada Nacional 118, 2080-023 Almeirim, Portugal

Background: Alicyclobacillus acidoterrestris (ACB) spores are responsible for acidic fruit juices spoilage, surviving conventional pasteurization and posing quality challenges to the industry. Truly, in the vegetative form, ACB can produce guaiacol, a halophenol that will confer off-flavours and odours to the contaminated products, leading to severe economic losses. Hyperbaric inactivation (HI) is a new nonthermal processing method that uses moderate hydrostatic pressures (125-250 MPa) for a few hours to destroy spores without thermal input. To further accelerate spore inactivation, other emergent technologies (such as ultrasound and pulsed electric field (US and PEF) can be used to induce the germination of spores, leading them to lose resistance.

Methods: This study evaluated the impact of US and PEF pre-treatments on the hyperbaric inactivation (HI, 150–250 MPa, up to 24 h, 18–23 °C) of ACB spores inoculated in apple juice. Commercial apple juice (pH 3.7) inoculated with ~5-6 log CFU/mL of ACB spores was subjected to PEF (30 kV, 80 µs, 1400 Hz) or US (67 W, 24 kHz, 5 min) prior to HI at 150, 200, or 250 MPa. Spore loads were quantified as total endospore load (TEL) and heat-resistant endospore fraction (HREF) and inactivation kinetics were modelled with Biphasic, Log-logistic, and Weibull equations.

Results: US alone did not significantly reduce spore counts but induced structural changes evidenced by dipicolinic acid release. During HI, US pre-treatment showed no major effect on inactivation kinetics compared to untreated spores, yielding ~4–5 log reductions after 24 h. In contrast, PEF pre-treatment slowed down HI inactivation, with lower reductions (≈3.0–3.4 log units) compared to ~4.5 log units without pre-treatment. Microscopy suggested that PEF may alter germinant receptor responsiveness, delaying nutrient-like pressure-induced germination.

Conclusions: Ultrasound did not enhance HI efficacy under the tested conditions, whereas PEF unexpectedly increased spore resistance to HI, likely through structural modifications interfering with pressure-triggered germination. These findings highlight the complexity of spore responses to emerging technologies and suggest that US and PEF are not straightforward sensitizers for HI. Further work is needed to optimize treatment parameters and explore potential synergistic combinations.

ID641 | Preservation of watermelon juice by hyperbaric storage: impact on microbial behavior and volatile profile

Author: Vasco Lima

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal; 2 CBQF—Centro de Biotecnologia e Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

E-mail: vjlima@ucp.pt

Other authors: : Rebeca Cruz 3, Susana Casal 3 & Jorge Saraiva 1

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal; 3 LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto – Portugal

Background: Hyperbaric storage (HS), an emerging method for food preservation under mild pressures at room temperature (RT), offers a potential alternative to conventional refrigeration (RF). It has almost no energy costs and substantially reduced greenhouse gas emissions. Watermelon juice (WJ), known for its health benefits and rich nutritional profile, is very perishable due to its high pH (5.20–6.70) and water activity (0.97–0.99), causing rapid microbial growth and enzymatic activity. Its aroma, a key quality attribute, consists mainly of volatile C6 and C9 aldehydes, ketones, and alcohols.

Methods: WJ (pH 6.50) was inoculated with Escherichia coli, Listeria monocytogenes and Saccharomyces cerevisiae and stored by HS at 25–100 MPa/RT (18–23 °C) for up to 24 days. Controls included RT and RF at atmospheric pressure. Changes in the volatile profile were assessed by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC-MS) after 4 weeks of storage under HS/RT (75 MPa, 18–23 °C) or RF (4 °C).

Results: HS/RT promoted microbial inactivation at all tested pressures for E. coli and S. cerevisiae and was effective against L. monocytogenes at ≥63 MPa, with higher pressures (75–100 MPa) yielding greater reductions compared with controls.

Volatile-profile analysis showed distinct effects of HS and RF. RF led to higher concentrations of compounds related with microbial activity (like ethanol), a ~41% increase in total volatile content due to the rise in alcohols (like 3-nonen-1-ol and 3,6-nonadien-1-ol) and terpenoids and loss of aldehydes like hexanal (and other less concentrated aldehydes), with ketones remaining relatively stable. Conversely, HS produced a ~37% decrease in total volatiles mostly due to alcohol loss, while ketones and terpenoids like 6-methyl-5-hepten-2-one and geranial increased, and aldehyde levels remained mostly stable. The increase in volatiles linked to microbial activity was avoided during HS.

Conclusions: HS at mild pressures and RT achieved strong microbial control in WJ while maintaining or enhancing key aroma attributes and suppressing spoilage-related volatiles. These results highlight HS as a low-energy, climate-friendly preservation technology with clear potential for broader adoption in high-pH fruit juices and similar beverages.

ID657 | From Waste to Bioactives: Green Extraction of Natural Antioxidants and Antimicrobials from Tomato By-products

Author: Ana Fernandes

Laboratório Associado, Escola Superior de Biotecnologia CBQF—Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto, 4169-005, Portugal.

E-mail: aclpfernandes@ucp.pt

Other authors: Daniela Magalhães, Hiléia K. S. Souza, Débora A. Campos, Ana A. Vilas Boas and Manuela Pintado
Laboratório Associado, Escola Superior de Biotecnologia CBQF—Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto, 4169-005, Portugal.

Background: Global tomato production reached 192 million tons in 2023. Industrial processing generates around 25% by-products (peels, seeds, pulp), often discarded, yet rich in phenolics (e.g. quercetin, lutein), carotenoids (e.g. lycopene, β-carotene), fiber and proteins. With growing demand for sustainable, biodegradable, and clean-label packaging materials, there is increasing interest in using natural bioactive compounds to replace synthetic additives [1]. The NOVAPACK project explores the potential of tomato by-products as sources of antioxidants and antimicrobials and pigments for use in active edible packaging, thereby supporting circular economy goals and reducing environmental impact.

Methods: Tomato by-products were characterized in terms of proximal composition, and bioactive compounds were extracted using environmentally friendly, food-safe solvents: ethanol/water, citric acid, lactic acid, and ascorbic acid (HCl as reference). Extracts were analyzed using HPLC to determine the levels of carotenoid and phenolic compounds. FTIR spectroscopy was used to identify functional groups. Antioxidant capacity was tested with DPPH and ABTS assays. Antimicrobial effectiveness was evaluated against pathogens such as E. coli, B. cereus, S. aureus and Salmonella spp.

Results: Tomato by-products showed proteins (20.50 ± 0.20%), total dietary fiber (65.89 ± 2.25%) and ash (2.96 ± 0.09%). Ethanol/water extracts yielded higher quercetin concentrations, while acidic solvents improved phenolic compounds diversity extracting rutin and naringenin. Carotenoids were efficiently recovered, yielding a total carotenoid content reaching 2.24 ± 0.03%, which played an important role in the observed antioxidant activity. FTIR analysis revealed hydroxyl and aromatic C=C groups, confirming the presence of phenolics and carotenoids. Antioxidant assays showed strong radical-scavenging activity, and antimicrobial tests indicated effective inhibition of the referred foodborne pathogens, with growth suppression at concentrations ranging from 0.01 % to 1 % w/v, with further optimization being still performed.

Conclusions: This study highlights tomato by-products as sources of natural antioxidants and antimicrobials for active packaging. Green extraction methods exhibit strong potential, especially when applied within Mediterranean agrosystems. Findings support bio-based packaging that improves food safety, shelf-life, and waste valorization, aligning with NOVAPACK goals.

ID665 | Staphylococcus aureus in the Food Chain: Antimicrobial Resistance in Ready-to-Eat Foods and Food Handlers

Author: Joana Paiva

1 Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal; 2 Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

E-mail: jpaiva@utad.pt

Other authors: Kamila Soares 1,2,†, Sónia Saraiva 1,2,3, Cristina Saraiva 1,2,3

1 Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal; 2 Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal 3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 5000-801 Vila Real, Portugal † These authors contributed equally to this work

Background: The emergence of antimicrobial-resistant Staphylococcus aureus in ready-to-eat foods and food handlers' hands highlights a growing public health concern, as this bacterium acts both as a pathogen and a reservoir of resistance.

Materials and Methods: A total of 251 samples, including ready-to-eat foods (RTE) and food handlers' hands, were collected and subjected to microbiological analysis and antimicrobial resistance testing. Strains were identified using morphological and molecular methods, and antibiotic resistance was determined by the Kirby-Bauer disk diffusion method against a panel of 15 antimicrobials.

Results: The overall prevalence of S. aureus among screened samples was 11.55%: 20 from handlers' hands and 9 from RTE foods. The nuc gene, used as a species-specific marker, was detected in all isolates. In food isolates, antimicrobial resistance was most frequently observed for gentamicin (17.2%) and penicillins (oxacillin and benzylpenicillin, 17.2% each), as shown in Table I. Among handlers' isolates, resistance was highest to penicillins (20%) and mupirocin (15%). Methicillin-resistant S. aureus (MRSA) was identified in both groups, with an overall prevalence of 13.79% (1 food isolate, 11.11%; 3 handler isolates, 15.0%). All MRSA isolates displayed a multidrug-resistant profile. Table I. Frequency of antimicrobial resistance in S. aureus isolates from RTE foods and food handlers in university food service establishments.

Conclusions: These findings demonstrate the presence of antimicrobial resistance, including MRSA with multidrug-resistant profiles, in S. aureus from RTE foods and food handlers. The occurrence of S. aureus in RTE foods suggests post-processing contamination, likely associated with inadequate hygiene or cross-contamination. The detection of resistance to antimicrobials essential for human medicine underscores a major public health concern and emphasizes the need for continuous monitoring and strict hygiene practices to mitigate foodborne risks.

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ID671 | Genomic Insights into Bacteriocin-Producing Lactococcus garvieae Isolated from Raw Milk Cheese

Author: Susana C. Ribeiro

University of the Azores, Institute of Agricultural and Environmental Research and Technology, Angra do Heroísmo, Portugal

E-mail: susana.ic.ribeiro@uac.pt

Other authors: Henrique J.D. Rosa, Célia C.G. Silva

University of the Azores, Institute of Agricultural and Environmental Research and Technology - IITAA, Angra do Heroísmo, Portugal.

Background: Bacteriocins from lactic acid bacteria are promising antimicrobial peptides with applications as biopreservatives in food or as therapeutics to prevent intestinal diseases. Lactococcus garvieae has been recognised as a major component of the autochthonous microbial populations of artisanal cheeses made from raw milk. The aim of this study was to conduct a comprehensive phenotypic and genetic characterisation of a bacteriocin-producing L. garvieae strain SJ17, previously isolated from a traditional Azorean cheese made from raw milk.

Methods: Genomic DNA extraction of strain was performed using the UltraClean® Microbial DNA Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer procedure. The sequencing was performed at Stabvida, utilizing a Illumina Novaseq technology, using 150bp paired-end sequencing reads. The analysis of the generated raw sequence data was carried out using CLC Genomics Workbench 12.0.3. The genome assemblies were evaluated with QUAST 5.0.2. The assembly statistics and cumulative plots were created. For species prediction, the SpeciesFinder v.2.0 tool was used. Acquired antibiotic resistance genes were identified using ResFinder v.4.2. Genes encoding bacterial virulence and pathogenicity factors were analysed using VirulenceFinder v.2.0.3 and PathogenFinder v.1.1 tools. The bacteriocin mining tool BAGEL4 was used to identify putative bacteriocins.

Results: WGS of L. garvieae. SJC17 generated 9661720 reads. Assemblies resulted in 31 contigs with a mean coverage of 55- and 41-fold and a GC content of 38.34%. Virulence genes were not detected. Additionally, the PathogenFinder v.1.1 tool identified L. garviae SJ17 as a non-human pathogen. This strain showed resistance to tetracyclines (doxycycline, minocycline, and tetracycline), but not to other classes of antibiotics. Bacteriocin detection tool identified Garvieacin Q as one putative bacteriocin cluster present in the genome. This bacteriocin is a class II, with 70 amino acids, and contains a 20-amino-acid N-terminal leader peptide.

Conclusions: The application of this strain can contribute to the development of foods with improved functional and safety qualities. Further studies are needed to evaluate its impact on gastrointestinal functions.

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ID672 | Whole-genome sequencing of Enterococcus faecalis L3A21K6 reveals a new lasso-peptide with activity against Listeria monocytogenes

Author: Célia C. G. Silva

Institute of Agricultural and Environmental Research and Technology (IITAA), Universidade dos Açores, Angra do Heroísmo, Portugal

E-mail: celia.cg.silva@uac.pt

Other authors: Susana C. Ribeiro

Institute of Agricultural and Environmental Research and Technology (IITAA), Universidade dos Açores, Angra do Heroísmo, Portugal

Background: Consumer demand for natural preservatives is increasing, positioning bacteriocins as a promising solution for the food industry. Previously, we isolated an Enterococcus faecalis strain from an artisanal cheese that produced an unidentified bacteriocin active against Listeria monocytogenes. This study aims to provide a genetic characterisation of this strain via whole-genome sequencing, identify the operons responsible for bacteriocin production, and determine the bacteriocin sequence.

Methods: Genomic DNA was extracted using the UltraClean® isolation kit (MoBio, Carlsbad, CA), and whole-genome sequencing (WGS) was performed on the Illumina Novaseq platform (StabVida). Genome assemblies were evaluated with QUAST 5.0.2. Bacteriocin biosynthetic clusters were identified using BAGEL4. Acquired antibiotic resistance genes were identified using ResFinder v.4.2, and genes encoding bacterial virulence factors were analysed using VirulenceFinder v.2.0.3. Genome annotation was performed using the RAST tool (RASTtk).

Results: A single bacteriocin operon was identified in the strain, showing 44% similarity to Propeptin 2, a member of the lasso peptide family. The leader peptide comprised 22 amino acid residues, and the core peptide consisted of 23 amino acid residues, with an isopeptide bond linking the N-terminus to the side β-chain carboxyl group of Asp at position 9. This lasso peptide is classified as class II, lacking a disulphide bridge. Instead, the structure is stabilised by the bulky side chains of tyrosine residues in the C-terminal tail. Genome mining of the strain revealed an acquired resistance gene for lincosamides, 16 virulence genes, and 7 mobile genetic elements (MGEs).

Conclusions: A novel class II lasso peptide bacteriocin was identified in E. faecalis L3A21K6, featuring a unique 23-amino acid core peptide. While this bacteriocin represents a promising anti-listerial agent, the concomitant presence of acquired antibiotic resistance and virulence genes in the producer strain requires caution. For safe application, future work should focus on the heterologous expression of the purified bacteriocin, separating its beneficial antimicrobial properties from the potential risks associated with the native host.

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ID691 | Development of Bagels Fortified with Chlorella vulgaris: Technological, Nutritional and Functional Impact of Different Strains

Author: Sérgio Sousa

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

E-mail: sdsousa@ucp.pt

Other authors: Grishma Mehta1, Ema Dias2, Ana Gomes1

1 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal; 2 Germen – Moagem de Cereais S.A., Matosinhos, Portugal

The development of bakery products fortified with Chlorella vulgaris addresses current challenges in nutritional sustainability through microalgal biomass valorisation and functional food diversification. Bagels constitute a suitable formulation for the delivery of bioactive compounds due to their versatility and global consumers acceptance.

Bagels were developed using organic wheat and chickpea flours and 4% (w/w) freeze-dried C. vulgaris (White [WC] or Honey [HC] strains). Analyses included proximal composition, texture profile (TPA), oscillatory rheology, antioxidant capacity (ABTS, DPPH, ORAC, FRAP), phenolic composition (Folin-Ciocalteu, HPLC-DAD), lipid profile (GC-FID), minerals (ICP-OES) and protein (Kjeldahl).

Microalgal fortification promoted significant mineral enrichment: calcium (27-48%), phosphorus (12-22%), zinc (14-49%) and manganese (3-11%), with a slight decrease in sodium (5-6 %). Protein content increased to 10.96-11.06% vs 10.20% in the control. The textural profile differed substantially: WC decreased adhesiveness by 80% while maintaining hardness, cohesiveness and chewiness equivalent to the control; HC decreased cohesiveness (25%) and resilience (23%), increasing adhesiveness (4.1×) and cutting work (105%). Rheological analysis revealed that HC provided improved pre-fermentation structuring but marked degradation during fermentation (G'/G":80-86%), while WC demonstrated structural stability (38-39%). Total phenolics were found to be higher with HC (8.9%); 4-hydroxybenzoic acid and (+)-catechin were identified by HPLC-DAD. Antioxidant capacity remained equivalent to the control (ABTS, ORAC), with selective reductions in DPPH (22.5%) and FRAP (16.5%) in the WC formulation. The lipid profile revealed a 13.9% increase in total fatty acids with HC, predominantly in oleic (25.9%) and palmitic (21.4%) acids.

The developed bagels comply with EU regulatory requirements for “source of protein”, “source of phosphorus and copper” and “high manganese content” claims, demonstrating the feasibility of C. vulgaris enrichment. The strains demonstrated distinct technological impacts, allowing textural modulation for different market segments.

The work was developed under the framework of project Agenda VIIAFOOD, financed through the Program for Recovery and Resilience. The authors would like to acknowledge GERMEN-Moagem de Cereais S.A for their support in carrying out the project, and the scientific collaboration under the FCT project UIDB/50016/2020

ABSTRACTS

TOPIC #3: Health Biotechnology

ORAL COMMUNICATIONS

ID219 | Viruses by design – the future of phage therapy

Author: Martin J. Loessner

Department of Health Science and Technology, ETH Zurich, Switzerland

E-mail: martin.loessner@ethz.ch

Background: The looming antibiotic resistance crisis requires novel strategies to control pathogenic bacteria causing infections. While bacteriophages offer targeted host killing, their therapeutic potential is limited by narrow host-ranges, insufficient antimicrobial activity, lysogeny, and rapid emergence of resistance. However, synthetic biology and engineering of phage genomes can overcome these limitations and enable design of smart and effective phage-based antimicrobials.

Methods: We use an engineering approach based on synthetic in-vitro DNA assembly and subsequent reactivation (rebooting) of phage genomes within suitable cells. For rebooting phage genomes of Gram-positive cells, we developed an L-form based approach, providing less stringent surrogate hosts for first-round amplification. To enhance recombination-based engineering of large phage genomes unsuitable for synthetic fragment assembly, CRISPR/Cas-based counterselection systems were established in various phage hosts.

Results & Conclusions: Using these platforms, we (i) converted temperate phages to virulent ones, (ii) produced phages carrying a broad variety of additional payload genes for expression in the infected host,

(iii) created phages showing extended killing of heterologous bacteria by phage-encoded cross-acting antimicrobials, (iv) designed transducing but non-replicating killer phage, (v) broadened phage host ranges by structure-guided design of receptor-binding proteins, and (vi) provided a corresponding arsenal of nanoluciferase reporter phages for companion diagnostics prior to clinical application.

Besides bacteriophages, another successful approach is to harness their bacteriolytic endolysins. Here, we have made significant progress by optimizing enzyme activity and in vivo half-life through domain shuffling and fusion to non-phage sequence, but also targeted modification of endolysins for fine-tuned application in serum and blood, tissue, and intracellular environments.

ID263 | Fungal DNA barcoding on a chip – sequence-based fungal infection diagnosis

Author: Maria Zolotareva

1 iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon Av. Rovisco Pais 1049-001 Lisboa, Portugal; 2 INESC Microsystems and Nanotechnologies, Rua Alves Redol, No. 9, 1000-029 Lisbon, Portugal; 3 Flash - Rapid Diagnostics Lda, Avenida Dom João II 1990-083, Lisbon, Portugal;

E-mail: maria.o.zolotareva@gmail.com

Other authors: Francisco Cascalheira (1,2), Cátia Caneiras (3,4,5,6), Cristina Bárbara (4,5,6), Diogo Caetano (2,3,7), Miguel Cacho Teixeira (1,8)

1 iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon Av. Rovisco Pais 1049-001 Lisboa, Portugal; 2 INESC Microsystems and Nanotechnologies, Rua Alves Redol, No. 9, 1000-029 Lisbon, Portugal; 3 Flash - Rapid Diagnostics Lda, Avenida Dom João II 1990-083, Lisbon, Portugal; 4 Environmental Health Institute, University of Lisbon Faculty of Medicine, Avenida Professor Egas Moniz, 1649-028 Lisbon, Portugal; 5 Associate Laboratory TERRA, University of Lisbon Faculty of Medicine, 1649-026 Lisboa, Portugal; 6 North Lisbon University Hospital Centre, Av. Prof. Egas Moniz MB, 1649-028 Lisboa, Portugal; 7Department of Electrical and Computer Engineering, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal; 8Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal.

Background: The global burden of fungal infections continues to rise due to expanding endemic regions, broader host ranges of certain mycoses, and the emergence of novel opportunistic, drug-resistant, and multi drug resistant fungal pathogens. Many deaths are to blame on delayed, inaccurate, or absent fungal infection diagnosis, leading to the late administration of effective antifungal treatments. Panfungal PCR followed by sequence identification has the advantage of accurately detecting slow growing, unculturable and even rare, unexpected or novel pathogens. However, integrating sequence-based fungal diagnostics into routine clinical practice remains challenging[1].

Methods: We designed ITS rDNA hybridization probes targeting four critical priority pathogens: Candida albicans, Candida auris, Nakaseomyces glabratus and Cryptococcus neoformans. We evaluated their diagnostic value, first in silico against a dataset of 58 pathogenic species (1239 DNA sequences) retrieved from the ISHAM barcoding database, and then in vitro, in cross hybridization assays using the lab strain and 2 clinical isolates of each species. In vitro assays were performed on a magnetoresistive detection platform[2], harnessing the giant magnetoresistive effect throughout spin-valve biosensors and paramagnetic DNA labelling. Reactions were conducted inside a PDMS microfluidic channel integrated into the portable device, designed to decentralize molecular testing and support point-of-care applications. We further validated the hybridization sensitivity against 30 unsequenced clinical isolates of N. glabratus, and against femtomolar DNA concentrations.

Results: Our in silico results demonstrate the feasibility of designing hybridization probes that are both sensitive and specific across a broad range of common and rare fungal species and strains. On-chip testing yielded 95% hybridization accuracy, 95% sensitivity, and 96% specificity, with a detection limit of approximately 600 single-stranded amplified ITS DNA molecules.

Conclusions: We discuss the relevance, feasibility, and challenges of implementing sequence-based diagnostics for fungal infections. Our work highlights the potential of lab-on-chip platforms to meet ASSURED criteria, enabling taxonomic identification at the point of care. We will present our latest developments and a multidisciplinary perspective on the future of fungal diagnostics.

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2 doi.org/10.1016/j.jmmm.2009.02.141

ID269 | Investigating Human Host Adaptations to Infection by the Gut Bacterium Bilophila wadsworthia in a Multicellular Intestinal Co culture

Author: Sara Matos

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras 2780-157, Portugal

E-mail: sara.matos@itqb.unl.pt

Other authors: Inês Cardoso Pereira, Andreia Pimenta

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras 2780-157, Portugal

Background: The interplay between gut microbiota and the human host is recognized as a key determinant of gastrointestinal health. The Western diet, rich in animal protein, fat and sugar, has been implicated in the pathogenesis of inflammatory bowel diseases and colorectal cancer, by promoting the proliferation of sulfide-producing bacteria like Bilophila wadsworthia. The elevated levels of sulfide produced are cytotoxic, contributing to gut barrier dysfunction and inflammation. Moreover, Bilophila has demonstrated pathogenicity by infecting host cells, adhering to the intestinal epithelium and disrupting the protective mucus layer.

Methods: The mechanisms by which Bilophila disrupts the intestinal barrier remain unclear, largely due to limitations in traditional in vitro models that fail to recapitulate the complexity of the human gut. To address this gap, we developed a transwell triple co-culture, comprising EA.hy926 endothelial cells, Caco-2 enterocytes and HT29-MTX goblet-like cells, mimicking key features of the intestinal epithelium like polarization, microvilli formation and mucus production. Barrier integrity was validated by transepithelial electrical resistance (TEER) measurements.

Results: Our findings reveal that monolayer tightness increases over time as cells differentiate. The highest TEER values are seen for Caco-2 cells, alone and in co-culture, in agreement with current literature highlighting their predominant role in maintaining tight junctions. Additionally, preliminary infection assays indicate that Bilophila exposure may compromise barrier integrity, as evidenced by initial TEER fluctuations. These will be followed by total RNA extraction for qRT-PCR of target markers of cellular structure, barrier function and inflammation, complemented by ELISA quantification of pro-inflammatory cytokines.

Conclusions: Our fully differentiated co-culture model lays the groundwork for disclosing host-microbe interactions at the gut interface. In the future, we aim to translate this system to a microfluidic gut-on-chip, enhancing physiology by incorporating fluid flow, enabling a dynamic analysis of host-microbe interactions and paving the way for targeted therapies of gut-related diseases.

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ID276 | Harnessing Keratin's Versatility: From Waste to Innovative Biomedical Applications

Author: Iuri Machado

CICECO – Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal.

E-mail: iurimachado@ua.pt

Other authors: Polesca, Cariny 2; Coutinho, João A.P. 1; Freire, Mara G. 1

1 CICECO – Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal. 2 Department of Chemical Engineering, Imperial College London, South Kensington, London, U.K.

Keratin is a fibrous structural protein and the third most abundant biopolymer present in the environment. It is available from various sources (e.g. chicken feathers, wool and human hair) and has emerged as a highly versatile biopolymer with significant potential for diverse technological and biomedical applications. Keratin's unique physicochemical properties, including biocompatibility, biodegradability, and renewal capability highlight its potential applications across biotechnology, materials science, and medicine. Despite its abundance, the efficient recovery and application of keratin has been considered a challenge due to its low solubility in water and most organic solvents, primarily caused by intra- and intermolecular disulfide cross-links and other structural features. However, recent advancements in green chemistry, particularly using biocompatible ionic liquids (ILs) such as cholinium acetate, have enabled the efficient recovery and processing of keratin from poultry waste. This approach not only enhances resource sustainability through waste valorisation but also contributes to circular economy concepts by converting waste into high-value materials.

In this study, cholinium-based ILs were used to dissolve keratin-rich waste, and by adding a coagulant solvent, keratin was recovered. This protein has been used to develop sustainable and innovative biomaterials, ranging from innovative hydrogels with drug delivery capabilities for treating ocular infections, to novel bioinks for 3D bioprinting for tissue engineering applications.

The recovery of keratin from waste sources, such as chicken feathers and human hair, was successfully achieved using different cholinium-based ILs, with recovery yields up to 93 wt%. The developed biomaterials exhibited exceptional cytocompatibility as well as cell adhesion and proliferation, biodegradability, remarkable UV protection capabilities, antioxidant and anti-inflammatory effects and tunable physicochemical properties. All these properties underscore the promise of the keratin-based materials for tissue engineering, drug delivery, photoprotection, and other advanced healthcare solutions.

The sustainable transformation of keratin-rich waste into high-performance biomaterials demonstrates not only a successful approach to waste valorisation but also highlights the versatility and potential of keratin as a building block for the next-generation of sustainable materials.

ID282 | Mechanical and biological assessment of metal nanoparticles functionalised bacterial nanocellulose (BNC) mesh

Author: Liliana Melro

1 Centre for Textile Science and Technology (2C2T), Department of Textile Engineering, University of Minho, Campus of Azurém, 4800-058 Guimarães, Portugal

E-mail: liliana.melro@2c2t.uminho.pt

Other authors: Marta Fernandes 1, Ana Isabel Ribeiro 1, Behnaz Mehravani 1, Teresa Campos 2,3, João Pedro Silva 4,5, Nuno Dourado 2,3, Fernando Ferreira 6, Jorge Padrão 1, Andrea Zille

1 Centre for Textile Science and Technology (2C2T), Department of Textile Engineering, University of Minho, Campus of Azurém, 4800-058 Guimarães, Portugal; 2 Center for MicroElectroMechanical Systems (CMEMS-UMINHO), University of Minho, 4800-058 Guimarães, Portugal; 3 LABBELS–Associate Laboratory, 4800-058 Guimarães, Portugal; 4 Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal; 5 UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal; 6 University of Porto, Faculty of Medicine, Senhora da Hora, 4464-513 Matosinhos, Portugal

Hernia mesh devices are commonly used in surgery to support weakened or damaged muscle tissue and are typically made from synthetic polymers. However, these materials often induce foreign body reactions or become susceptible to microbial colonization. An alternative is to use bacterial nanocellulose (BNC). Produced by different bacteria species (e.g. Komagataeibacter hansenii), it consists of highly pure, randomly entangled cellulose nanofibers. BNC meshes were obtained using a template during the fermentation process. To impart antibacterial activity, metal nanoparticles (NPs): silver (Ag), copper (Cu), and copper oxide (Cu2O) were incorporated within BNC via in situ synthesis. Because when BNC is dried the number of hydrogen bonds dramatically increases, which impacts the mechanical properties, BNC meshes in the hydrated and rehydrated states were tested. The elastic modulus (E) was determined through true stress-strain curves of tensile strength testing. Poisson ratio (-) was taken from the literature, being appropriately validated upon numerical modelling with experimental data by means of a developed inverse procedure. All BNC meshes revealed - of 0.01. Higher E values were found for rehydrated meshes, particularly for BNC/Cu2O, 20.5 MPa. However, these values are low compared to commercial polypropylene (PP) mesh (48 g/m2, Optilene B. Braun-Portugal), 164 MPa. The release profile of the NPs was determined by atomic absorbance spectroscopy. No significant difference was observed between hydrated and rehydrated meshes. There was, though, a variation in NPs release: Ag was ~5x higher, and Cu2O ~3x higher than Cu after 6 months. This minimal release proved insufficient to exert antibacterial effect via diffusion, as confirmed by the shake flask method. Both Cu and Cu2O NPs exhibited moderate disinfectant activity against Staphylococcus aureus and Pseudomonas aeruginosa in the contact killing assays. This effectiveness upon direct contact is particularly relevant for the proposed application, preventing postoperative infections while minimizing the risk of NPs systemic dispersion. Cellular adhesion and metabolic activity of L929 fibroblasts were assessed on the meshes over 7 days by cell counting and MTT reduction assay, respectively. Results showed similar adhesion between functionalised BNC and PP meshes, with less than 50% adhesions. However, highly statistically significant differences were found between rehydrated BNC and hydrated BNC/Ag ($p < 0.001$).

ID283 | Bacterial Nanocellulose–Kraft Lignin Composites as Antioxidant and Antimicrobial Biomaterials for Neural Applications

Author: Cátia Alves

Centre for Textile Science and Technology (2C2T), University of Minho, Campus de Azurém, 4800-058, Guimarães, Portugal

E-mail: catia.alves@2c2t.uminho.pt

Other authors: Rui D. V. Fernandes 1, Liliana Melro 1, Marta Fernandes 1, Jorge Padrão 1, Diego Moldes 2, António Salgado 3,4 and Andrea Zille 1

1. Centre for Textile Science and Technology (2C2T), University of Minho, Campus de Azurém, 4800-058, Guimarães, Portugal; 2. Department of Chemical Engineering, University of Vigo, Lagoas Marcosende s/n, E-36310 Vigo, Spain; 3. Life and Health Sciences Research Institute (ICVS), University of Minho, Campus de Gualtar, 4701-057 Braga, Portugal; 4. ICVS/3B's—PT Government Associate Laboratory, 4710-057 Braga/Guimarães, Portugal

Neuralgias are associated with demyelination and neurovascular compression, leading to intense pain and reduced quality of life. Current treatments, including pharmacological therapies and synthetic-material implants, often exhibit limited long-term success and carry a risk of complications, emphasising the need for biofunctional materials that facilitate nerve repair and prevent infection. Bacterial nanocellulose (BNC) is a biocompatible, porous, and mechanically robust material with strong potential for biomedical use. However, its limited tissue adhesion, lack of antimicrobial and antioxidant properties hinder its neural applications. This study envisaged to functionalise BNC membranes, synthesised by Komagataeibacter hansenii and Komagataeibacter sucrofermentans, with Kraft lignin (KL), a naturally abundant macromolecule known for its antioxidant (reactive oxygen species neutralisation) and antimicrobial properties.

KL particles (6 wt%), dispersed in an ethanol/methanol solution, were synthesised via solvent/antisolvent method with aqueous solutions containing different concentrations (0.01-10 wt%) of non-ionic surfactants (Tween 20/Tween 80/Triton X-100) as antisolvent. Production parameters, such as KL solution addition rate (1-7.5 mL/min), stirring speed (250-1500 rpm), and initial KL concentration (0.1-6 wt%), were optimised. The resulting colloidal particles were characterised by scanning electron microscopy (SEM) and dynamic light scattering, confirming their spherical morphology and size distribution (125-700 nm). Among the tested conditions, Tween 80 at 0.5 wt% (3 mL/min, 1500 rpm) produced the smallest and monodisperse particles (polydispersity index<0.1). Optimised dispersions were used to functionalise BNC membranes by exhaustion. The membranes were evaluated via SEM, contact-killing antimicrobial assays (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans), and antioxidant activity assays via inhibition of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical cation. The functionalised composites demonstrated a maximum log reduction of 5 against S. aureus, and a considerable increase in antioxidant activity. Therefore, the proposed bioimplant has a high potential for mitigating the risk of postoperative infections. Furthermore, antioxidant activity of KL is expected to promote Schwann cell proliferation and axonal remyelination, thereby enhancing the safety and efficacy of BNC-based neural implants.

ID313 | Disarming and Destroying Listeria Biofilms: Cationic Dendrimers as Dual-Action Anti-Biofilm Agents

Author: Sandra N. Pinto

iBB and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal.
E-mail: sandrapinto@ist.utl.pt

Other authors: Laura Pereira1, Maria Carvalho1,2, Adriana Cruz1, Ana Godinho-Santos3, José M. Andrade4, Manuel Melo4, Marta M. Alves5, Vasco D.B. Bonifácio1

1 iBB and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal. 2 Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal. 3 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal. 4 Instituto de Tecnologia Química Biológica António Xavier, Universidade NOVA de Lisboa, Portugal. 5 Centro de Química Estrutural (CQE), Instituto Superior Técnico, Lisbon, Portugal.

Background: Listeria monocytogenes is a foodborne pathogen whose biofilm-forming ability enhances its persistence in food processing environments and compromises treatment. The ineffectiveness of conventional antimicrobials against biofilms necessitates novel therapeutic strategies. Thus, this study evaluates the efficacy of synthetic cationic polyurea dendrimers in disrupting and eradicating L. monocytogenes biofilms.

Methods: Two cationic polyurea dendrimers, PUREG4-OEI48 and PUREG4-OCEI24, were synthesized and first evaluated for their membrane selectivity using giant unilamellar vesicles (GUVs) mimicking bacterial membranes. The more promising dendrimer was then selected for comprehensive anti-biofilm assessment. We determined minimum inhibitory and bactericidal concentrations (MIC/MBC), performed live-dead assays via spectral flow cytometry, quantified biofilm inhibition and eradication, and analyzed the dendrimer's impact on the expression of key virulence and biofilm-related genes.

Results: PUREG4-OEI48 exhibited a higher zeta potential (+43.52 mV) and greater selectivity for bacterial membrane mimics than PUREG4-OCEI24. It demonstrated potent bactericidal activity against L. monocytogenes (MIC = MBC = 0.78 μM). The dendrimer significantly inhibited biofilm adhesion and disrupted pre-formed biofilms, even under a high bacterial load (10^8 CFU/mL). Mechanistic studies revealed that PUREG4-OEI48 treatment suppressed critical virulence pathways, including quorum sensing and biofilm formation genes. Furthermore, it restored normal cytoskeletal labeling in host cells, indicating impaired bacterial recruitment of host components for spread.

Conclusions: The cationic polyurea dendrimer PUREG4-OEI48 is a highly promising anti-biofilm agent against L. monocytogenes. Its dual mechanism of action, direct bacterial membrane disruption and downregulation of quorum sensing and biofilm formation genes, potentiates its efficacy. These findings position cationic dendrimers as an innovative strategy to prevent and treat recalcitrant Listeria biofilms.

ID339 | PipeUCare: A Novel Biofilm-Targeted Disinfectant for Hospital Greywater Systems

Author: Isabel Carvalho

CEB, Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
E-mail: isabel.carvalho@ceb.uminho.pt

Other authors: Débora Castro1,2, Isabel Ferreri2, Mariana Henriques1,3

1 CEB, Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; 2 Success Gadget - Nanotecnologia e Novos Materiais, Lda, Rua Filipa Borges, 1245, 4750-823 Barcelos, Braga, Portugal ; 3 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Hospitals are hotspots for pathogenic microorganisms, many resistant to antibiotics. In Portugal, healthcare-associated infections affect ~11% of inpatients, above the European average. Greywater systems are overlooked sources of such infections, acting as reservoirs and dissemination routes for resistant bacteria. This study validates PipeUCare, the first antimicrobial disinfectant developed for non-porous greywater system surfaces, addressing a critical regulatory and technological gap.

Methods: The antibacterial and antibiofilm activity of PipeUCare was tested on common hospital piping surfaces: rigid/flexible PVC and stainless steel. Performance was compared with two conventional disinfectants (Products A and B) using mono- and multi-species biofilm models. Target microorganisms — Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus hirae — are key hospital pathogens and biofilm formers. Biofilms were grown under simulated greywater flow, on the selected surfaces, with and without fluid accumulation, and after up to five water passages.

Results: PipeUCare achieved >5-log reduction in viable cells for all strains after five water cycles. It fully eradicated preformed biofilms in dry zones and greatly reduced biomass in wet zones. Inhibition halos persisted 48h post-treatment on all surfaces, confirming sustained antibacterial activity. Additionally, PipeUCare prevented bacterial adhesion, showing a preventive effect against biofilm formation absent in Products A and B.

Conclusions: PipeUCare demonstrates strong efficacy in controlling clinically relevant biofilms on critical hospital infrastructure. These findings support integrating biofilm-targeted disinfection into sanitation protocols and underline the need for agents adapted to the complex microbiology of greywater systems.

ID421 | LNA-based ASO-siderophore conjugate: a new potential Trojan-horse drug to outsmart antibiotic-resistant bacteria

Author: Igor Resende

LEPABE, AliCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; UMR7242 Biotechnology and Cell Signaling, CNRS/University of Strasbourg, 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France; University of Strasbourg, Research Institute of the Strasbourg's School of Biotechnology (IREBS), 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France

E-mail: up201706005@edu.fe.up.pt

Other authors: Johan Revol-Tissot 2,3; Rita S. Santos 1; Gaëtan L.A. Mislin 2,3; Nuno F. Azevedo 1

1 LEPABE, AliCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 2 UMR7242 Biotechnology and Cell Signaling, CNRS/University of Strasbourg, 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France; 3 University of Strasbourg, Research Institute of the Strasbourg's School of Biotechnology (IREBS), 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France

Background: Bacterial resistance to antibiotics undermines infection treatment, highlighting the need for new therapeutics. Antisense oligonucleotides (ASOs) composed of nucleic acid mimics (NAMs) are a promising species-specific approach by silencing essential bacterial genes. NAMs enhance RNA-binding affinity and nuclease resistance. Locked nucleic acid (LNA), for example, shows strong hybridization and is often combined with 2′-O-methyl RNA (2′-OMe) to balance affinity, specificity, and safety. However, the bacterial envelope is impermeable to naked ASOs. Iron-uptake systems act as selective entry points in this barrier. Bacteria secrete siderophores that scavenge iron from the environment and are then imported via specific transporters. This pathway has been exploited for antibiotic delivery through siderophore-antibiotic conjugates using a Trojan-horse strategy and could similarly enable ASO delivery.

Methods: A novel enterobactin analog, termed tris-catechol vector (TCV), was synthesized and functionalized with a dibenzocyclooctyne (DBCO) moiety via a multi-step route and purified by flash chromatography. TCV was conjugated to an azide-bearing LNA/2′-OMe ASO targeting the essential acpP gene in Escherichia coli (E. coli) using strain-promoted alkyne-azide cycloaddition (SPAAC). Reaction progress was assessed by denaturing PAGE, and the conjugate was characterized by MALDI-TOF, with yields quantified by HPLC. Minimum inhibitory concentration assays were performed under iron-depleted conditions.

Results: The yield of the TCV-DBCO synthesis was 57%. SPAAC conjugation with the anti-acpP ASO proceeded efficiently, with complete conversion confirmed by gel electrophoresis. MALDI-TOF verified the expected mass, and the conjugate was isolated in yields up to 97%. Preliminary antibacterial testing in E. coli showed no detectable inhibitory activity. Growth resembled that seen with siderophore alone, suggesting internalization may occur, but the antisense effect may be insufficient to counterbalance the growth benefit from iron uptake.

Conclusions: This work expands the chemical toolbox for conjugating complex biomolecules to siderophores. Antibacterial testing of the conjugate in E. coli is ongoing, with further optimization needed. Future efforts will also focus on investigating iron-uptake pathway mutants, employing fluorescence-based assays to monitor cellular uptake, and testing a validated anti-acpP peptide nucleic acid sequence as a positive control.

ID541 | Discovery of a Novel Synthetic Antimalarial Class Active Against Multidrug-Resistant Plasmodium falciparum

Author: Pedro Eduardo Ferreira

1Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho. Gualtar, 4710-057, Braga, Portugal 2Life and Health Sciences Research Institute (ICVS)/ Biomaterials, Biodegradables and Biomimetics Research Group (3B's)-PT Government Associate Laboratory, 4710-057 Braga, Portugal

E-mail: pedroferreira@med.uminho.pt

Other authors: Adriana F. Gonçalves 1,2 (*), Ana Lima-Pinheiro 1,2, Gustavo Capatti Cassiano 3, Pedro Cravo 3, and Maria Alice Carvalho4

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho. Gualtar, 4710-057, Braga, Portugal 2 Life and Health Sciences Research Institute (ICVS)/ Biomaterials, Biodegradables and Biomimetics Research Group (3B's)-PT Government Associate Laboratory, 4710-057 Braga, Portugal 3 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 4 Chemistry Centre of University of Minho (CQ-UM), Campus de Gualtar, 4710-057 Braga, Portugal and Departamento de Química, Escola de Ciências da Universidade do Minho, Braga, Portugal;

Background: Antimalarial drug resistance, particularly to artemisinin-based combination therapies (ACTs), threatens malaria control and eradication efforts. Resistance has been documented in Southeast Asia and is emerging in sub-Saharan Africa. Current treatments rely heavily on plant-derived compounds with long production cycles and limited scalability. There is an urgent need for novel, fully synthetic antimalarial compounds with new mechanisms of action.

Methods: A chemical screening platform was used to identify a new class of synthetic compounds with antimalarial activity applying chemigenomics. In vitro susceptibility assays were conducted using drug-sensitive and multidrug-resistant P. falciparum strains. Parasite stage-specific profiling and resistance selection assays were performed. A panel of recombinant progeny lines from a genetic cross was used to identify loci associated with drug response. In vivo tolerability and efficacy were assessed in a murine malaria model.

Results: The lead compounds showed low-nanomolar potency against both sensitive and resistant P. falciparum lines. Activity was enhanced in the trophozoite stage and showed delayed resistance emergence in mature stages. Resistance selection revealed a locus on chromosome 13 not linked to known resistance genes (pfmdr1, pfcrt). Seven derivatives demonstrated low cytotoxicity with selectivity indices >10. Oral administration in mice showed good tolerability and moderate parasite clearance.

Conclusions: This novel synthetic antimalarial class demonstrates potent activity against resistant P. falciparum, a unique resistance profile, and favorable safety in preclinical models. Its synthetic accessibility and distinct mode of action position it as a promising candidate for further development toward clinical use.

ID615 | Exploiting Bacteriophages for Targeted Control and Rapid Detection of Klebsiella pneumoniae

Author: Filipa Campos

CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: filipapscampos@gmail.com

Other authors: Diana Pires, Sílvio B Santos

1 CEB - Centre of Biological Engineering, Braga, Portugal and LABBELS – Associate Laboratory, Braga, Guimarães, Portugal; 2 CEB - Centre of Biological Engineering, Braga, Portugal and LABBELS – Associate Laboratory, Braga, Guimarães, Portugal.

Antimicrobial resistance (AMR) is a critical global health challenge, with Klebsiella pneumoniae recognised by the World Health Organization as a high-priority pathogen due to its major role in healthcare-associated infections and multidrug resistance. Rapid detection tools and alternative therapeutic strategies are urgently needed to mitigate the burden caused by this bacterium. Bacteriophages represent a dual opportunity, both as therapeutic agents and as a source of receptor-binding proteins (RBPs), which can be harnessed for the development of innovative diagnostic platforms.

A lytic K. pneumoniae bacteriophage was isolated from wastewater using clinical strains as hosts. The phage was morphologically characterised by transmission electron microscopy (TEM) and evaluated for host range, latent period, and burst size. Whole-genome sequencing and annotation enabled the identification of putative RBPs. Candidate RBPs were further cloned in Escherichia coli fused to GFP, expressed, purified, and tested for their ability to bind to the target pathogen. Binding activity and specificity were assessed by fluorescence microscopy using a panel of K. pneumoniae isolates and other relevant bacteria.

TEM analysis revealed a myovirus morphology. The phage displayed infectivity against 31% of tested clinical isolates, with a latent period of 10 minutes and a burst size of 41 PFU per cell. Genome annotation enabled the identification of two RBPs with affinity to K. pneumoniae. These proteins were successfully cloned, expressed, and purified, maintaining correct folding as GFP fusions. Fluorescence microscopy confirmed their ability to specifically bind to the bacterial cell wall, validating their potential as recognition elements for diagnostic applications.

This work reports the isolation and characterisation of a novel K. pneumoniae phage with both therapeutic relevance and biotechnological potential. The identified RBPs demonstrated strong and specific binding capacity, highlighting their value as innovative tools for the development of rapid phage-based detection methods. These findings underscore and highlight the potential of phages as new, efficient alternative therapeutics and their versatility for the development of next-generation diagnostics.

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ID621 | Discovery and Functional Characterization of a Novel Kv1.3 Channel Modulator Derived from Physalia physalis Venom

Author: Zuzanna Tomkielska

1 Center of Biotechnology of Azores (CBA), University of Azores, 9500-321 Ponta Delgada, Portugal 2 Mesosystem Investigação & Investimentos by Spinpark, Barco, 4805-017 Guimarães, Portugal;

E-mail: zuziatomkielska@gmail.com

Other authors: David Mona 1, Miriam Rando Cabello 1, Jorge Frias 1, Ana Casas 2, Nelson Simões 1, and Duarte Toubarro 1 *

1 Center of Biotechnology of Azores (CBA), University of Azores, 9500-321 Ponta Delgada, Portugal 2 MesosystemInvestigação & Investimentos by Spinpark, Barco, 4805-017 Guimarães, Portugal;

Marine venoms, although present in nature for millions of years, remain insufficiently characterized. Among their diverse bioactive components, ion channel modulators have emerged as molecules of significant interest. Peptide neurotoxins that selectively target potassium channels have been shown to be promising therapeutic leads, since channel dysfunction is directly implicated in nervous, cardiac, immune, and endocrine systems disorders. Here, we report the identification and characterization of a novel peptide from the venom of Physalia physalis, predicted to target vertebrate Kv channels.

Crude venom was partially fractionated by size-exclusion chromatography (SEC), and each fraction was administered to a Drosophila W1118 to assess their neurotoxic activity. The most neuroactive fraction was analyzed by LC-MS/MS, and proteins were identified using an in-house database. A neurotoxic peptide candidate identified in the active fraction was subjected to structural prediction by AlphaFold and docked against Kv channels by ZDOCK. The peptide was heterologously expressed in E. coli, and its activity was validated by whole-cell patch-clamp and by radioligand competition assay using α-dendrotoxin to assess selectivity within Kv channels.

Oral administration of the low molecular weight venom fraction induced pronounced neurotoxic phenotypes in flies, suggesting the presence of neuromodulators. Further LC-MS/MS analysis of this fraction identified the presence of a cysteine-rich peptide. In silico modeling revealed 3D structure consistent with the ShK family, Kv channel binding peptides with predicted affinity for Kv1.3. Recombinant peptide named ShK20_D1 was successfully expressed and purified, and electrophysiological assays confirmed its inhibitory activity against Kv1.3. Competition assay demonstrated that ShK20_D1 doesn't compete with α-dendrotoxin for Kv1.1, Kv1.2, and Kv1.6 even at concentrations 200-fold higher than the reference compound.

ShK20_D1 represents a newly identified selective Kv1.3 channel modulator from P. physalis venom. These findings highlight ShK20_D1 as a selective Kv1.3 channel modulator with potential therapeutic relevance. However, further studies in mice should evaluate its therapeutic efficacy and safety.

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ID638 | A ferulic acid derivative transformed by Lactococcus lactis is a potential inhibitor of endometrial cancer

Author: Gabriel Amaro Monteiro

1iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; gabAssociate Laboratory i4HB—Institute for Health and Bioeconomy, Portugal

E-mail: gabmonteiro@tecnico.ulisboa.pt

Other authors: Sara Leonor Ferreira Azevedo 1,3; Maria Luísa Guedes Sousa 2,3; Bruno Fonseca 2,3; Natércia Teixeira 2,3; Georgina Correia da Silva 2,3; Gabriel Amaro Monteiro 1,3; Sofia Oliveira Dias Duarte1,3

1 iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; 2 UCIBIO-REQUIMTE, Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal; 3 Associate Laboratory i4HB —Institute for Health and Bioeconomy, Portugal

Lactic acid bacteria (LAB) are Gram-positive bacteria well known for their role in food production, probiotic properties, and contribution to human health. Their versatility is closely linked to their ability to metabolise and secrete a wide variety of bioactive compounds. For example, they can convert ferulic acid (FA), a hydroxycinnamic acid found widely in nature and food products. FA has attracted attention because of its antioxidant properties and its potential to be transformed into valuable metabolites in food (eg vanillin) and therapeutics (eg hydroferulic acid).

This study investigates the ability of Lactococcus lactis subsp. lactis strain LMG19460 to metabolise ferulic acid and convert it into bioactive metabolites. L. lactis LMG19460 was cultured in three different media, each supplemented with 2 mM FA. The FA concentration was performed by HPLC over a period of 72 hours. Across all culture media, HPLC analysis revealed a gradual decrease in FA concentration over time. After 24 hours of growth, a new chromatographic peak appeared suggesting that L. lactis LMG19460 can metabolise FA and convert it into an unknown compound. This compound was purified by HPLC, followed by mass spectrometry and nuclear magnetic resonance analysis, to identify it. Preliminary data suggests that this compound may be a geranyl derivative, which is known for its antioxidant, anti-inflammatory and anti-cancer properties.

In addition, to evaluate the therapeutic properties of the unknown compound, preliminary assays were performed using endometrial cancer cell lines (Ishikawa and Hec50co) and primary healthy endometrial stromal cells (ESCs) exposed to different concentrations of the unknown compound (0.01- 50 µg/ml) for different periods (24-72h) to assess its effects on cell morphology and viability. Cell assays reveal a reduction in viability in endometrial cancer cells, which is most evident at higher concentrations (25 and 50 µg/ml). In contrast, healthy endometrial stromal cells show a decrease in viability appearing only at 50 µg/ml, indicating a differential sensitivity between malignant and non-malignant cells.

This study may contribute to the discovery of new bioactive molecules derived from probiotic LAB, highlighting the therapeutic potential of these bacteria.

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ID651 | Antioxidant and Neuroprotective Potential of Mushroom Biomass: Effects on ROS Production in Human Microglial Cell Line (HMC3) and Alzheimer's Disease Caenorhabditis elegans Models

Author: Helena Araújo-Rodrigues

1 Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina –Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho, 1327, 4169-005 Porto, Portugal.

E-mail: hrodrigues@ucp.pt

Other authors: Lidia Garzón-García3, Ana Sofia Salsinha1, João B. Relvas2,4, Freni K. Tavaria1, Celestino Santos- Buelga3, Ana M. González-Paramás3, and Manuela E. Pintado1

1 Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina –Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho, 1327, 4169-005 Porto, Portugal. 2 Glial Cell Biology, i3S Instituto de Investigação e Inovação em Saúde, Universidade do Porto (Porto, Portugal). 3 Grupo de Investigación en Polifenoles (GIP-USAL), Campus Miguel de Unamuno, Universidad de Salamanca, 37007 Salamanca, Spain. 4 IBMC Instituto de Biologia Molecular e Celular, Universidade do Porto (Porto, Portugal).

Background: Mushrooms exhibit diverse bioactive compounds with prebiotic, immunomodulatory, antioxidant, and neuroprotective effects. These benefits may arise from direct metabolite absorption and gut microbiota modulation, a key regulator of brain health through the gut-brain axis1. This study explored the neuroprotective potential of macromolecules from the mushroom biomass (MB) from Trametes versicolor (TV), Hericium erinaceus (HE), and Pleurotus ostreatus (PO) before and after gastrointestinal digestion (GID), together with microbiota-derived metabolites. Their effects were evaluated in the human microglial cells (HMC3) by ROS measurement and in transgenic Caenorhabditis elegans Alzheimer's disease (AD) models by chemotaxis and paralysis assays.

Methods: Bioactive compounds in MBs were quantified by enzymatic assays, HPLC, and spectrophotometric methods. GID was simulated by the INFOGEST standardized method, and the microbiota-derived metabolites were determined by human fecal fermentation. Intracellular ROS in HMC3 cells were measured using a fluorescent probe. Transgenic C. elegans AD models were used for chemotaxis and paralysis assays.

Results: Biochemical characterization of MBs confirmed the presence of bioactive compounds with antioxidant potential. TV showed higher levels of γ-aminobutyric acid (GABA; 125.99 mg/100 g), ergosterol (8.97 mg/100 g), ascorbic acid (3.31 mg/100 g), carotenoids (18.05 mg/100 g), and free phenolic compounds (113.31 mg GAE/100 g). HE possessed higher tocopherol levels (816.74 µg/100 g), while PO was richer in zinc content (2.37 mg/100 g). Total glucan content (76.15-80.45%) and selenium content (87.68–93.04 µg/100 g) were similar across species. All biomass species inhibited ROS production in microglia (15.35–21.29%) at 0.63–1.25 mg/mL. Digestive fractions of all mushroom species significantly improved chemosensory responses in the BR5270 strain, which expresses the hyperphosphorylated tau protein in neurons. TV, HE, and PO also significantly delayed paralysis progression in the CL4176 strain. Moreover, butyric acid and GABA restored chemotaxis deficits in CL2355 and delayed progressive paralysis in CL4176, both strains with amyloid beta peptide expression, indicating protective effects against amyloid-induced neuronal dysfunction.

Conclusions: Mushroom biomass-derived compounds and microbiota metabolites show neuroprotective potential, mitigating Aβ- and Tau-induced toxicity, two of the main hallmarks of AD.

ID653 | A non-coding RNA modulates plasmid copy number and antibiotic resistance in Listeria monocytogenes

Author: Gonçalo Filipe Moreno

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
E-mail: goncalo.moreno@itqb.unl.pt

Other authors: Ana Patrícia Quendera, Cecília Maria Arraiano, José Marques Andrade

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Background: Plasmids are major drivers of antimicrobial resistance and essential vectors in biotechnology. Their replication is classically regulated by plasmid-encoded proteins and antisense RNAs, yet the influence of host-encoded non-coding RNAs (ncRNAs) has remained largely unexplored. Listeria monocytogenes, a major foodborne pathogen and model organism encodes hundreds of ncRNAs, many involved in virulence and stress adaptation. Here we demonstrate that a bacterial chromosomally-encoded ncRNA modulates plasmid behaviour. This expands our understanding of host-plasmid interplay and reveals new opportunities for plasmid engineering in biotechnology.

Methods: Two Listeria ncRNAs (Rli159 and Rli161) were cloned into well-established and wide used shuttle vectors pMK4P and pMK3P. Antibiotic susceptibility was assessed by minimum inhibitory concentration (MIC) assays and growth-curve analysis under selective pressure. RT-qPCR quantified plasmid-encoded genes (cat, repC, bla), a variant of qPCR determined plasmid copy number, and IntaRNA software predicted potential interactions between Rli159 and plasmid replication initiator mRNAs.

Results: Overexpression of Rli159 (but not Rli161) markedly increased resistance to plasmid-borne selective markers: chloramphenicol in pMK4P and kanamycin in pMK3P. Rli159-overexpressing strains showed faster adaptation to high antibiotic concentrations. RT-qPCR revealed higher transcript levels of cat (+57%), repC (+320%), and bla (+1345%), while PCN analysis showed a dramatic increase (~10-fold) from ~20 to ~200 copies per cell, in Rli159-overexpressing strains. Computational predictions identified RNA-RNA interactions between Rli159 and rep mRNAs, consistent with enhanced replication initiation. Conclusions: This study identifies, for the first time and to the best of our knowledge, a chromosomally encoded ncRNA that increases plasmid copy number and resistance in bacteria. This discovery expands our view of how host–plasmid interactions shape bacterial antibiotic resistance. At the same time, it uncovers a regulatory principle that can be repurposed for biotechnology, where precise control of plasmid dosage is central to applications ranging from synthetic biology, industrial applications to therapeutic vector design. By linking RNA biology, antimicrobial resistance, and plasmid engineering, this work opens a new conceptual space at the intersection of microbiology and biotechnology.

ID654 | Amino acid-based ionic liquids as the next generation of excipients for RNA with improved stability during storage

Author: Ana F. Pereira

CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago 3810-193, Aveiro, Portugal

E-mail: anafilipa98@ua.pt

Other authors: Augusto Q. Pedro1, Ana P. M. Tavares1, Luís C. Branco2, João A. P. Coutinho1, Fani Sousa3, Mara G. Freire1

1 CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago 3810-193, Aveiro, Portugal; 2 LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Lisboa, Portugal; 3 CICS-UBI – Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Convento de Sto. António 6201-001, Covilhã, Portugal

One of the major challenges of the widespread application of RNA-based therapeutics continues to be related with RNA stability, mainly during its long-term storage. In fact, current RNA bioprocessing technologies still lack efficient strategies that prevent its degradation and thus allow RNA to perform its therapeutic function. Amino-acid-based ILs (AA-ILs) may significantly contribute to this field, by taking advantage of their tunable and biocompatible character and their ability to establish preferential interactions with RNA nucleobases.

This work aims to investigate AA-ILs as a new class of excipients to maintain and/or enhance the structural stability of RNA, with the purpose of creating alternative and competitive RNA stabilization platforms.

A novel set of AA-ILs combining amino acids in the cation and/or anion was synthesized and characterized. Then, several aqueous solutions of AA-ILs in concentrations ranging from 0.1 to 10 mM were investigated to act as excipients in stabilizing RNA during long-term storage at room temperature. Circular dichroism (CD) spectroscopic measurements were performed and [Cholinium][Aspartate], [Histidinium][Aspartate], [Argininium][Aspartate] and [Lysinium][Aspartate] arise as the most promising AA-ILs in maintaining the structural stability of RNA after a 10 month-storage period, at room temperature, in contrast with the control solution, where RNA stabilization was not verified. Complementary cell viability studies performed onto two different human cell lines confirmed the non-cytotoxic nature of the synthesized AA-ILs.

Overall, the AA-ILs herein exploited are capable of meet the critical demand of high-quality RNA, with potential use as novel excipients in RNA-based biotherapeutics.

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ID656 | Rabbit-Derived Single-Domain Antibody Fused to Zag Protein for Potent SARS-CoV-2 Neutralization and Extended Half-Life

Author: Isa Moutinho

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).

E-mail: isasoraiia_@hotmail.com

Other authors: Rafaela Marimon1,2, João Gonçalves3, Lurdes Gano4, João Galamba4, Ruben Silva4, LuísTavares1,2, Frederico Aires-da-Silva1,2

3 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal. 4 Centro de Ciências e Tecnologias Nucleares and Departamento de Engenharia e Ciências Nucleares, IST, Universidade de Lisboa, Lisbon, Portugal

Background: SARS-CoV-2 remains a health concern due to its rapid evolution and the emergence of new variants. Vaccination has reduced morbidity and mortality, but requires frequent updates, and vulnerable groups often respond poorly. Monoclonal antibodies (mAbs) provide an important complement to vaccines, but conventional IgGs (~150 kDa) have limitations: costly production, limited hidden epitope access, risk of antibody-dependent enhancement (ADE) and reduced efficacy against mutated variants. Single-domain antibodies (sdAbs) are a promising alternative. Their small size (~15 kDa), stability, and microbial production enable rapid and low-cost manufacturing. They access conserved epitopes, lack Fc-mediated ADE and are suitable for inhalation delivery. Their main drawback is short half-life due to renal clearance, which can be overcome by fusion with albumin-binding domains (ABDs) that exploit FcRn recycling. Here, we aimed to develop a broadly neutralizing rabbit-derived sdAb against SARS-CoV-2 and improve its pharmacokinetics through ABD fusion. Methods: A New Zealand White rabbit was immunized with the SARS-CoV-2 RBD from Delta variant and the antibody titres were measured by ELISA. A rabbit-derived sdAb library was constructed and subjected to phage display selection. Candidates were screened for expression, RBD binding, and neutralizing activity. To enhance pharmacokinetic properties, the lead sdAb, B3, was fused to the ABD derived from Streptococcus zooepidemicus Zag protein, enabling binding to albumin and prolonging serum half-life.

Results: The rabbit developed a strong antibody response (titer 1:640.000). Three rounds of phage display enriched clones with increased RBD binding. The lead candidate B3 showed strong RBD binding and potent neutralizing activity, with 94% inhibition of SARS-CoV-2 wild-type and 97% and 81% against Delta and Omicron, respectively. B3-ABD(Zag) preserved neutralization potency, increased expression yields by~ 5-fold and acquired cross-species albumin binding (rat, mouse and human). In vivo, ABD fusion improved pharmacokinetics, extending blood retention 5.9-fold (13.5%I.A./g for B3-(ABD)Zag vs. 2.3%I.A./g for B3) and reducing renal uptake by >50% (31.0 vs. 69.7%I.A./g), demonstrating the feasibility of this strategy. Conclusions: Rabbit-derived sdAbs fused to ABD(Zag) represent a promising strategy for next-generation biologics against SARS-CoV-2 and a versatile platform adaptable to future emerging infectious diseases.

POSTERS

ID194 | Amphiphilic dendrimers as delivery vectors for nucleic acid mimics against E. coli

Author: Mariana Gomes

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 in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr Roberto Frias, 4200-465 Porto, Portugal

E-mail: up201303475@edu.fe.up.pt

Other authors: Igor Resende 1,2, Yana Zamoshchak 1,2,3, Daniela Araújo 4,5, Joana Castro 4,5, Dinesh Dhumal 6, Ling Peng 6, Rita S. Santos 1,2, Nuno F. Azevedo 1,2

3 Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium; 4 INIAV - National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Lugar da Madalena, Vairão, 4485-655 Vila do Conde, Portugal; 5 CEB - Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; 6 CINaM - Interdisciplinary Center of Nanoscience of Marseille, Aix-Marseille Université, CNRS UMR 7325, 13288 Marseille, France

Background: The growing threat of antimicrobial resistance highlights the urgent need for alternatives to traditional antibiotics. Antisense nucleic acid mimics (NAMs) can selectively silence bacterial genes but face major delivery challenges due to the impermeability of bacterial envelopes. This study evaluates amphiphilic dendrimers (ADs) as delivery vehicles for NAMs targeting the essential acpP gene in Escherichia coli.

Methods: Two poly(amidoamine)-based ADs - 1a (primary amines) and 1b (tertiary amines) - were combined with PNA- and LNA/2′O-methyl-based NAMs. Their physicochemical properties were characterised using TEM, zeta potential, and size exclusion chromatography. NAM uptake and membrane permeabilisation were assessed via flow cytometry and fluorescence microscopy. Antibacterial efficacy was measured in vitro against reference and clinical E. coli strains, and in vivo toxicity was evaluated in a Galleria mellonella model.

Results: Both ADs enhanced membrane permeabilisation and NAM uptake, with 1b showing greater permeabilisation and 1a achieving higher internalisation. The 1b-PNA combination reduced E. coli viability by up to 8 log10 CFU/mL and maintained activity for 24 hours. This formulation also showed activity against the drug-resistant clinical isolate. No toxicity was observed in G. mellonella larvae.

Conclusions: This is the first report of effective bacterial delivery of antisense NAMs using amphiphilic dendrimers. The 1b-PNA complex demonstrated potent, specific, and non-toxic antibacterial activity, supporting its potential as a precision therapy agent against resistant bacteria. Further studies will focus on targeting within mixed microbial communities and advancing toward clinical use.

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ID210 | Pre-clinical evaluation of a divalent liposomal vaccine to control invasive candidiasis

Author: Paula Sampaio

1Centre of Molecular and Environmental Biology (CBMA) /Aquatic Research Network (ARNET) Associate Laboratory, Universidade doMinho, Campus de Gualtar, 4710-057 Braga , Portugal

E-mail: psampaio@bio.uminho.pt

Other authors: AugustoCosta-Barbosa1,2, Maria Inês Pacheco1,2 ,AndreiaC.Gomes1,2, TonyCollins1,2 ,Manuel Vilanova3,4, Célia Pais1, Alexandra Correia4

1Centre of Molecular and Environmental Biology (CBMA) /Aquatic Research Network (ARNET) Associate Laboratory, Universidade doMinho, Campus de Gualtar, Braga, Portugal. 2Institute of Science and Innovation for Sustainability (IB-S), Universidade do Minho, Campus de Gualtar, Braga, Portugal. 3i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal. 4IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Background: Candida albicans is a common fungal pathogen that causes severe infections, particularly in immunocompromised individuals. Currently, no human vaccine is available for these infections. Patients undergoing organ transplantation, cancer chemotherapy, or hemodialysis are at high risk for C. albicans infection and would benefit from a prophylactic vaccine that could boost their immunity before major medical intervention.

Methods: In this study, we prepared a novel divalent vaccine utilizing DODAB:MO liposomes incorporating recombinant C. albicans Cht3 and Sap2 as target antigens. The immunomodulatory and protective effects were evaluated iin the mouse model of disseminated candidiasis using BALB/c mice.

Results: Vaccination induced a long-lasting response with a protective Th1/Th17 profile of CD4+ T cells, a favorable Th1/Th2 ratio, and antigen-specific antibodies. It also triggered enhance bone marrow-derived macrophages inflammatory activity, suggestive of trained immunity.

Conclusions: The liposomal vaccine significantly improved survival rate in a mouse model, demonstrating its potential as a divalent vaccine candidate against systemic candidiasis.

ID231 | Mapping Genomic Safe Harbors in Lactobacillus acidophilus for Synthetic Circuit Integration

Author: Daniela Pinto

Phage Biology Research and Infection Control (PhaBRIC) lab, Research Institute for Medicines (iMed.ULisboa),Faculdade de Farmácia, Universidade de Lisboa, Portugal

E-mail: dspinto@ff.ulisboa.pt

Other authors: Carlos São-José

1 Phage Biology Research and Infection Control (PhaBRIC) lab, Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal

Background: Genetic engineering of probiotic bacteria is a promising strategy to develop live biotherapeutics with enhanced or novel functionalities. Lactobacillus acidophilus, a dominant species in the vaginal microbiota, is a prime chassis candidate. However, precise integration of synthetic circuits requires genomic “safe harbors” - loci that ensure stability and orthogonality while preserving host viability and probiotic traits.

Methods: We combined pangenome analysis using Roary, genome annotation inspection, and in silico promoter localization using BPROM to systematically identify candidate integration sites in the genome of L. acidophilus ATCC 4356. Divergently oriented ORFs were manually curated to identify intergenic regions flanked by outward-facing promoters.

Results: We identified 224 divergently oriented ORF pairs with intergenic regions harboring oppositely oriented promoters, which can enable insertion of synthetic circuits without transcriptional interference from adjacent ORFs. Many of these pairs include pseudogenes or hypothetical proteins, suggesting non-essentiality and, therefore, the potential for safe integration without disrupting core cellular functions. Promoter confidence scores typically fell in a moderate range, and several intergenic regions offered sufficient space for well-insulated insertions. To assist synthetic biologists in selecting optimal insertion regions based on their circuit design constraints, we developed an interactive visualization for intuitive genome exploration. Experimental validation of selected sites is ongoing and will assess circuit expression and stability, and host fitness.

Conclusions: This study provides a systematic roadmap for rational genomic integration in L. acidophilus, offering a foundation for more predictable and stable synthetic circuits. Engineered strains with well-insulated synthetic circuits may support future applications in women’s health, including responsive vaginal probiotics delivering antimicrobials.

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ID290 | Polymyxin-based analogues featuring unnatural amino acids: synthesis and enhanced activity against bacterial-associated infections

Author: Susana Patrícia Lopes

CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal; LABBELS-Associate Laboratory, Braga/Guimarães, Portugal

E-mail: supat@ceb.uminho.pt

Other authors: Tânia Grainha 1,2; Mariana Linhares 3; Elsa Mariana Martins 3; Susana Paula Costa 3; Paula Jorge 1,2

1-CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal; 2-LABBELS-Associate Laboratory, Braga/Guimarães, Portugal; 3-CQ-Centre of Chemistry, University of Minho, Braga, Portugal

Background: Polymyxins (PM), notably B (PMB) and E (PME), are last-resort antibiotics for Gram-negative bacterial infections, but their clinical use is jeopardized by their limited spectrum, along with rising resistance and significant toxicity. As most infections are polymicrobial and biofilm-related, structural modification is a highly promising approach to respond to the stall in the discovery of novel compounds. But while numerous PM analogs have been reported, improvements in activity/toxicity remain inconsistent. Unnatural amino acids (UNAA), such as α,α-dialkylglycines (DAG), offer advantageous properties, including enhanced bioactivity, metabolic stability, and hydrophobicity, positioning them as valuable tools for next-generation PM analog design.

Methods: Six UNAA, including DAG, structurally related to native Phe, Leu, or Dab, were synthesized (Ugi multicomponent reaction) and site-specifically incorporated at PM positions 1, 3, 6, and/or 7 via solid-phase peptide synthesis, generating twelve novel analogs - three PME-based (PMEa–c) and nine PMB-based (PMBa–i). Antibacterial activity was determined (broth microdilution) against Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923, while antibiofilm activity was assessed on 24-h P. aeruginosa biofilms.

Results: Five analogs modified at positions 1 (PMBd), 3 (PMBe), 6 (PMBi), and 7 (PMBa/PMEa) showed the most promising activity against P. aeruginosa. PMB analogs exhibited the best MIC values (4–8 mg/L), approaching that of native PMB (~1 mg/L). PMBa also reduced P. aeruginosa biofilm cell counts by 3.7 log (CFU/mL), closely matching PMB (~3.9 log) but at an estimated lower concentration. Strikingly, an unexpected yet highly significant enhancement in antistaphylococcal activity (MIC=8-128 mg/L) was found for PMEb (Leu6→DAG) and seven PMB analogs incorporating DAG or other UNAA at positions 1, 3, or 7, outperforming native PM' activity (MIC≥128 mg/L), in some cases by 4-fold.

Conclusions: These findings support the strategic use of UNAA in PM analog design to enhance antibacterial, antibiofilm, and broad-spectrum activity. Current studies target S. aureus biofilms, optimize compound purity, and assess nephrotoxicity.

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ID302 | Enhanced Albumin Loaded Liposome Optimization for Targeted Delivery of Anti-HPV Drug in Cervical Cancer Treatment

Author: Miguel Ferreira

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, 6201-506 Covilhã, Portugal

E-mail: miguel.r.ferreira@ubi.pt

Other authors: Diana Gomes 1,2,3, Samuel Silvestre 1,4,5,6, Diana Costa 1, Adriana O. Santos 1, Ângela Sousa 1

1 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, 6201-506 Covilhã, Portugal; 2 UCIBIO—Applied Molecular Biosciences Unit, Departament of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 3 Associate Laboratory i4HB—Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA, 2819-516 Caparica, Portugal; 4 Laboratório de Fármaco-Toxicologia - UBIMedical, University of Beira Interior, 6200-284 Covilhã, Portugal; 5 CNC - Centro de Neurociências e Biologia Celular, University of Coimbra, 3004-504 Coimbra, Portugal; 6 C4 - Cloud Computing Competence Centre, UBIMedical, University of Beira Interior, Estrada Municipal 506, 6200-284 Covilhã, Portugal.

Cervical cancer is the fourth leading cause of cancer in women worldwide, resulting in more than 340,000 deaths annually. Its incidence is directly related to the human papillomavirus (HPV), which is responsible for between 70 and 100% of cases. E6 and E7 oncoproteins, expressed by HPV, are particularly relevant due to their ability to inhibit the tumour suppressor proteins p53 and pRb, respectively, leading to apoptosis inhibition and uncontrolled growth of cancer cells. Although preventive vaccines for the most aggressive types of HPV are now available, they are not widely distributed and administered in less developed countries, making this cancer the second leading cause of cancer death in women in those countries. The treatment for this type of cancer often involves chemotherapy through the administration of cisplatin or paclitaxel, resulting in numerous undesirable side effects. In addition, surgery to remove the uterus is also very common, leading to infertility.

In this regard, new approaches are being developed to create an effective and targeted delivery for cervical cancer, with the main target being the oncoproteins E6 and E7.

The aim of the study was to develop a small molecule that inhibits the E6 oncoprotein and encapsulate it in a lipid system functionalized with albumin to increase its bioavailability and enable targeted delivery to cancer cells.

The properties of size, polydispersity index (PDI), yield, and encapsulation efficiency of the lipid system consisting of phosphatidylcholine, cholesterol, and DSPE-PEG2000 were optimized by varying the lipid composition, preparation methodology, purification method, and drug:lipid ratio.

After an optimization process, it was possible to obtain liposomes with a mean size of 88.52± 1.65 nm, PDI of 0.235± 0,009, yield of 77± 0,63% and encapsulation efficiency of 92.16± 0,58%. Functionalization with albumin was performed subsequently, and it was possible to obtain a functionalization efficiency of over 60%.

Future perspectives include in vitro release assays, cytotoxicity assays on HPV-positive and HPV-negative cancer cells and also on healthy cells, and evaluation of the expression of proteins such as E6 and p53. Finally, in vivo assays will be carried out in a mice HPV cancer model to assess the therapeutic efficacy of the delivery system.

This work opens a promising avenue for the development of precision therapies (more effective and less toxic) in the treatment of cervical cancer.

ID304 | Process Optimization and Biofunctional Profiling of Enzymatic Hydrolysates from Fucus vesiculosus

Author: Paulo Nova

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

E-mail: pnova@ucp.pt

Other authors: Sara A. Cunha1, Marta Coelho1, Manuela Machado1, Ana R. Costa-Pinto2,3, Ana Maria Gomes1

1. Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal; 2. i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; 3. IPATIMUP – Instituto de Patologia Molecular e Imunologia da Universidade do Porto, Porto, Portugal

Fucus vesiculosus (FV) is a brown macroalga (phaeophyceae) spread all over the world in the cold temperature waters of rocky intertidal habitats, with a prevalence on the northeast Pacific and North Atlantic coastline. It is a rich reservoir of bioactive compounds that exhibit antitumor, antioxidant, anti-inflammatory and antidiabetic activities and as such is of high interest for the biotechnological, medical and food industries. This research work focused on the optimized production of enzyme-assisted water-soluble hydrolysates from F. vesiculosus and their chemical and biological profiling. Two Box-Behnken designs of experiments (DOE) were performed to establish the optimal conditions to produce hydrolysates with higher levels of biological activity. Three experimental factors were considered for F. vesiculosus extracted with cellulase (FVc) (cellulase percentage, hydrolysis temperature and duration) and four for F. vesiculosus extracted with cellulase and alcalase (FVca) (cellulase percentage, protease percentage, hydrolysis temperature and duration). The analysis of the responses included: a) extraction yield; b) total phenolic content; c) total antioxidant capacity; d) oxygen radical absorbance capacity (ORAC). The optimal conditions determined by the DOE enabled the production of two hydrolysates: FVc, with a 31.02% of yield,

100.9 µmol TE/g dry extract total antioxidant capacity, 252.8 GAE/g dry extract for total phenolic content and 216.1 µmol TE/g dry extract for ORAC; and FVca, with a 40.6% yield, 149.0 µmol TE/g dry extract total antioxidant capacity, 256.0 GAE/g dry extract for total phenolic content and 487.2 µmol TE/g dry extract for ORAC. The hydrolysate with the greatest potential (FVca) was further characterized, revealing a rich amino acid profile comprising several essential amino acids (histidine, isoleucine, leucine, methionine, phenylalanine, threonine and tryptophan), peptides with low molecular weight and biological potential (3-5 kDa and 5-10 kDa), as well as cytotoxicity against CaCo-2 human colorectal adenocarcinoma cell lines - especially pronounced at concentrations above 2.5 mg/ml. These results reveal the promising potential of F. vesiculosus hydrolysates for applications in the medical, food and biotechnological industries.

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ID319 | Evaluation of surface morphology and toxicity profiles of encapsulated LipoNAMs targeting E. coli

Author: Joana Castro

1INIAV National Institute for Agrarian and Veterinary Research, Vila do Conde, Portugal; 2CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

E-mail: joana.castro@iniav.pt

Other authors: Emílio Gomes 1,3,4, Daniela Araújo 1,2, Carmo Serrano 5,6, Beatriz Fernandes 5,7, João Santos 3,4,Nuno Guimarães 3,4, Carina Almeida 1,2,3,4, Nuno F. Azevedo 3,4

3LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal 4ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal 5INIAV, National Institute for Agrarian and Veterinary Research, Av. da República, Quinta do Marquês, Oeiras 6LEAF-Linking Landscape, Environment, Agriculture and Food Research Unit, Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Lisbon, 7CERENA, DEQ, Instituto Superior Técnico, University of Lisbon, Lisbon

Background: Nucleic acid mimics (NAMs) are synthetic molecules designed to replicate the structure of DNA or RNA. Due to their ability to specifically target pathogens and their resistance to enzymatic degradation, which increases their half-life, they offer significant therapeutic potential. However, effective delivery systems are essential to protect NAMs and ensure their precise release at target sites.

Methods: In this study, we developed and evaluated liposomes with two different formulations (DOPE/DOTAP at 50:50 and 25:75 ratios with 1% DSPE-methoxy-PEG) and used them to encapsulate NAMs targeting essential genes for the survival of E. coli. The resulting LipoNAMs were then encapsulated using an atomization process with Hi-CapTM 100, a modified starch designed to protect sensitive molecules from degradation.

The encapsulation process began with the hydration of Hi-CapTM in distilled water under magnetic stirring and heating, followed by overnight agitation. The next day, the LipoNAMs were added to the solution and homogenized using an Ultra-Turrax at 16,000 rpm for 6 minutes. The final emulsion was atomized using a lab-scale atomizer at 140 °C, resulting in dry particles containing the LipoNAMs.

Results: Morphological characterization was performed using scanning electron microscopy (SEM), confirming successful encapsulation. The particles were spherical with sizes ranging from 10-30 µm. Physicochemical analysis showed that the best zeta potential values were obtained when particles were suspended in water at acidic pH, indicating good stability under simulated gastric conditions.

Finally, the toxicity was evaluated using the Galleria mellonella model, with concentrations of 0.25 and 0.5 g/mL of the obtained particles. No adverse effects were observed, with high health index scores (>7) and no mortality during the 72 hours of the study.

Conclusions: These results support the potential of this encapsulation strategy to protect and deliver the LipoNAMs specifically to the intestinal environment, presenting a novel approach for combating intestinal infections in veterinary settings.

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ID320 | Diatomaceous Earth and SWIPER Monolith: A Groundbreaking Strategy for DNA Vaccine Isolation

Author: Matilde Costa

RISE-Health, Departamento de Ciências Médicas, Faculdade de Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

E-mail: matilde.costa@ubi.pt

Other authors: Ângela Sousa

RISE-Health, Departamento de Ciências Médicas, Faculdade de Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

Cervical cancer remains a leading cause of death among women, ranking as the fourth most common cancer globally. Its progression is primarily associated with persistent Human Papillomavirus (HPV) infection, particularly due to the oncogenic E6 and E7 proteins that promote the degradation of tumor suppressor proteins p53 and pRb, respectively. Recent advancements in nucleic acid-based vaccines, especially DNA vaccines, offer a promising strategy to combat this public health issue. DNA vaccines are favored for their superior thermal stability and ability to elicit both humoral and cellular immune responses. However, the production of DNA vaccines faces significant challenges, including time-consuming extraction protocols, the use of hazardous organic solvents, and high salt concentrations. In this context, Diatomaceous Earth (DE) emerges as a potential filter aid due to its porous structure, which can enhance plasmid DNA recovery, reduce processing time, and minimize environmental impact.

This study aimed to optimize the DNA vaccine isolation process, by eliminating the use of salts and organic solvents of the conventional approach, through the application of DE, and exploring the Swiper monolithic column for the purification step. Plasmid DNA production was conducted using Escherichia coli cells, followed by extraction. Conventionally, this step requires isopropanol and ammonium sulfate, however in this study, DE was tested as a filter aid in three different proportions, 25%, 50%, and 75% of the pellet's weight. Process efficiency was evaluated qualitatively, through agarose gel electrophoresis, and quantitatively, using an analytical chromatographic column. The conventional method yields

approximately 34% purity and 62% recovery, while the addition of 50% DE achieved around 33% purity and 87% recovery. This condition, having shown the best results, was further purified using the CIMmultus SWIPER column. After optimizing the purification step, the column effectively removed high molecular weight RNA, one of the major contaminants from the DE extraction, yielding a final recovery of 77% and purity of 82%, approximately. Overall, the findings highlight the potential of the optimized process developed in this study, demonstrating the ability to capture or directly purify a DNA vaccine with high-yield through a rapid and environmentally friendly method.

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ID321 | Development of a Prevotella species PNA probe and in vivo evaluation alongside a P. bivia7specific PNA probe in vaginal swabs

Author: Taynara Maria Mulinde Marques Pereira

LIBRO, CEB, University of Minho, Braga, Portugal

E-mail: taynarammmarques@gmail.com

Other authors: Lúcia G. V. Sousa 1, Joana Castro1,2, Sheridan D. George 3, Christina Muzny 3, Nuno Cerca 1,4

1 Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 INIAV - National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Lugar da Madalena, Vairão, 4485-655 Vila do Conde, Portugal; 3 Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL 35233, USA; 4 LABBELS - Associate Laboratory, 4710-057 Braga, Portugal

Background: Bacterial vaginosis (BV) is the most common vaginal infection in women of reproductive age, and it is associated with an increased risk of adverse pregnancy outcomes. BV involves a shift from a Lactobacillus-dominated microbiota to a polymicrobial biofilm enriched in anaerobes, including Prevotella bivia. A peptide nucleic acid (PNA) probe targeting P. bivia was previously developed to investigate its role as a potential early colonizer, in parallel with Gardnerella species. However, our unpublished findings raised doubts about the specificity of this association, suggesting a broader involvement of other Prevotella species. To clarify this, we developed a new PNA probe targeting Prevotella species and compared its in vivo performance to the existing P. bivianspecific probe in vaginal swabs.

Methods: A PNA probe targeting all Prevotella species by fluorescence in situ hybridization (FISH) was designed in silico based on 23S rRNA gene sequences, and compared its performance to the existing P. bivia-specific probe. To determine in vitro sensitivity and specificity, the new probe, along with a previously developed P. bivia-specific probe [1], was tested against a panel of 28 Prevotella strains representing 24 different species, and 34 non-Prevotella BV-associated bacteria. Following optimization of hybridization conditions, both probes were applied to 18 vaginal swabs, 9 from women clinically diagnosed with BV and 9 from healthy controls.

Results: In vitro validation demonstrated that the new Prevotella probe achieved a specificity of 94% and sensitivity of 92%. As expected, its broader detection design allowed identification of a wider range of Prevotella species compared to the P. bivia-specific probe, which was intentionally restricted to a single species. Application to clinical samples revealed that the new probe identified a significantly higher count of Prevotella in 6 out of 9 BV- positive samples compared to the P. bivia-specific probe. In 2 out of 9 healthy control samples, elevated Prevotella detection was also observed.

Conclusions: These results support the hypothesis that multiple Prevotella species, not only P. bivia, may be involved in the polymicrobial profile of BV. The broader detection achieved with the Prevotella spp. probe highlights its potential as a more informative tool for studying the vaginal microbiota.

ID348 | Exploring snake venom molecules for new strategies in antimalarial drug development

Author: Inês Morais

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: ines.morais@ihmt.unl.pt

Other authors: Sofia Cortes 2, Paula Dantas 2, Paula Gomes 3, Sonia Chudzinski 2, Fatima Nogueira 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 Biopharmaceuticals, Butantan Institute, Sao Paulo, Brazil; 3 LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences of the University of Porto, Porto, Portugal

Background: The spread of multidrug-resistant Plasmodium falciparum in Southeast Asia and Africa poses a major challenge to malaria control, underscoring the urgent need for novel therapeutic agents. Snake venoms (SVs) are complex mixtures of bioactive molecules, including phospholipases A2, metalloproteinases, proteases, disintegrins, and neurotoxins, some of which have demonstrated antimalarial properties. Building on this, we explored the potential of Brazilian snake venom fractions as a source of new antimalarial compounds.

Methods: A panel of purified fractions was screened for activity against erythrocytic stages of P. falciparum 3D7HT-GFP clone (MRA-1029). Fractions that in screening assays had an inhibition higher than 70%. Cytotoxicity assays in mammalian cell lines (V79) and hemolysis assays to evaluate safety. Fractions displaying significant activity were further characterized in preliminary mechanism of action studies to identify possible molecular targets and biological pathways involved. To evaluate broader antiparasitic potential, fractions were also tested against Leishmania infantum.

Results: IC50 values indicate modest activity. However, the fractions display low cytotoxicity and low hemolysis effects. Preliminary mechanistic studies are underway, including invasion assays with the most active fractions, to explore potential interactions with parasite biological pathways.

Conclusions: Our findings highlight snake venom fractions as a rich source of novel bioactive molecules with selective antimalarial activity. These results support the continued exploration of SV-derived compounds as candidates for drug development, with the potential to address the pressing challenge of antimalarial drug resistance.

ID354 | Enhancing the delivery efficiency of Nucleic Acid Mimics into bacteria: Influence of liposome composition/functionalization

Author: João Santos

LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

E-mail: up202103293@up.pt

Other authors: Nuno Guimarães 1,2, Eva Pinho 3, Rita S. Santos 1,2, Nuno Azevedo 1,2

1. LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 2. ALiCE - Laboratório Associado em Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 3. Innovayt Portugal, Lda

The development of antimicrobial resistance (AMR) by bacteria is putting an end to the “golden-era” of antibiotics and is already responsible for thousands of annual deaths worldwide [1], requiring the development of novel therapeutic solutions [2]. Nucleic Acid Mimics (NAMs) are one of the potential alternatives. However, being poorly internalized by bacteria, they must be delivered into the cytosol, being liposomes one of the vectors with greater potential for that purpose [3]. The presence of cationic lipids may favour both the encapsulation of anionic oligonucleotides and the interaction with the negatively charged bacterial cell envelope [4].

In this work, different liposome formulations were evaluated, via flow cytometry and fluorescence microscopy, regarding the delivery efficiency of NAMs into important foodborne pathogens. The oligonucleotide was designed to target and inhibit the essential acpP gene, being fluorescently labelled with Cy3.

Using 75% of cationic DOTAP and 25% of fusogenic DOPE resulted in an increase of at least 3-fold in delivery efficiency, comparing to the 50:50 ratio, and allowed the inclusion of low percentages of PEG in the formulation (necessary for its stabilization), without compromising the delivery. The conjugation of E. coli polyclonal antibodies (ImmunoLipoNAMs) improved the delivery into that species, in comparison to those which were not the specific target of the formulation, with a difference of approximately 20% in labelling efficiency.

Future work will include the optimization of ImmunoLipoNAMs and the application of different targeting strategies. Furthermore, cytotoxicity assays will be performed to check for the in vivo applicability of the formulations. The effect of LipoNAMs in biofilms, both during their growth or after formation, will also be assessed.

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ID355 | Silver Sulfobenzoate Coordination Polymers as Bioactive Dopants in Antibacterial and Antibiofilm Cellulose Films

Author: Paula Jorge

1 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 LABBELS − Associate Laboratory, Braga/Guimarães, Portugal

E-mail: paulajorge@ceb.uminho.pt

Other authors: Rafaela Cabral 3,4, Tiago Fernandes 3,5, Chris Franco 3, Ivo Bragança 6,7, Ana Catarina Sousa 3,4, Nuno Cerca 1,2, Alexander Kirillov 3

3 Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisboa, Portugal; 4 Departamento de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, R. Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal; 5 Departamento de Ciências e Tecnologia, Universidade Aberta, 1000-013 Lisboa, Portugal; 6 IDMEC, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisboa, Portugal; 7 CIMOSM, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, R. Conselheiro Emídio Navarro, 1, 1959-007 Lisboa, Portugal

Background: Bacterial biofilms are complex microbial communities that cause persistent infections, as their protective matrix shields them from antimicrobials and the immune system, while close cell proximity enhances resistance and communication. Biofilm formation begins with microbial adhesion to surfaces such as hospital equipment and medical devices. Developing antibiofilm materials offers a promising strategy to prevent adhesion, reduce resistance, and limit infection spread in clinical settings.

Methods: This study developed antibacterial and antibiofilm biopolymer materials incorporating bioactive coordination polymers (bioCPs). Two new Ag(I)-based bioCPs, [Ag(msba)]n (CP1) and [Ag(Ksba·H2O) ·3H2O]n (CP2), were synthesized from Ag(I) salts and structurally related organic linkers: 4-(methylsulfonyl) benzoic acid (Hmsba, C8H7O4S) and 4-sulfobenzoic acid monopotassium salt (Ksba, C7H4O5K). These bioCPs were embedded as dopants in ethylcellulose (EC) and cellulose acetate (CA) films. Antibacterial activity was assessed against four pathogens (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis) using agar diffusion and biofilm inhibition assays by measuring growth inhibition halos and quantifying CFU on the films after 24 h, respectively. Data was normalized for the molar content of silver(I).

Results: Both CA biopolymer films (CP1@[CA]n and CP2@[CA]n) and CP2@[EC]n, with low CP dopant loadings of 1, 2.5 and 5 wt%, demonstrated superior antimicrobial activity against all species when compared with their silver control equivalents. All biopolymer films in the three concentrations tested were also more effective than their silver control equivalents in inhibiting the proliferation of bacterial biofilms, including reductions up to almost 4 log (CFU/cm2) in the case of CA and EC films doped with CP2.

Conclusions: By describing new bioCPs and derived biopolymer films made from renewable polysaccharides, this interdisciplinary study expands the use of coordination polymers as components of antibacterial hybrid materials.

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ID358 | Aptamer-enhanced prostate-specific antigen detection using aqueous biphasic systems and microfluidic platforms

Author: Maria S. M. Mendes

CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

E-mail: msilvinamm@ua.pt

Other authors: Virginia Chu2, Mara G. Freire1, Francisca A. e Silva1, João P. Conde2,3

1 CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2 Instituto de Engenharia de Sistemas e Computadores-Microsistemas e Nanotecnologias (INESC MN), Rua Alves Redol 9, Lisbon, 1000-029, Portugal; 3 Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, Lisbon, 1049-001, Portugal

Background: Prostate-specific antigen (PSA) detection in clinical samples is often challenged by matrix effects from abundant proteins, which can compromise analytical performance. To overcome these obstacles, we developed an integrated strategy combining sample pretreatment via aqueous biphasic systems (ABS) with microfluidic immunoassays based on aptamers for sensitive and specific PSA detection.

Methods: ABS pretreatment was applied to human serum to deplete high-abundance proteins prior to PSA detection. Two microfluidic detection strategies were evaluated: one employing a conventional antibody-antibody sandwich assay and the other featuring an aptamer-based assay, in which the detection antibody was replaced by a PSA-binding aptamer.

Results: Comparative evaluations in phosphate-buffered saline (PBS) and untreated serum revealed that the aptamer-based assay consistently outperformed the antibody format, showing higher analytical sensitivity and greater resistance to matrix-induced interferences. Although both assays were influenced by serum components, the antibody platform was more severely affected. ABS pretreatment mitigated these effects, most strongly enhancing detection in the aptamer assay, which achieved a detection limit of 7.49 ng·mL-1, consistent with clinically relevant PSA screening levels.

Conclusions: These results underscore the complementary advantages of ABS pretreatment and aptamer biosensing in microfluidic devices, extending detection approaches beyond conventional antibody-based immunoassays. This combined strategy provides a foundation for improved point-of-care diagnostic tools for prostate cancer screening.

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ID389 | Galleria mellonella as an infection model for Leishmania sp.

Author: Sofia Cortes

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

E-mail: scortes@ihmt.unl.pt

Other authors: Eunice Gaspar, Mariana Andrade, Joana Marques, Sofia Santos Costa

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

Background: Galleria mellonella is an alternative invertebrate model to study various aspects of infectious diseases. A key feature is the similarity between its innate immune response, particularly hemocytes, and that of vertebrates. Leishmaniasis is a vector-borne disease caused by intracellular protozoan parasites of the genus Leishmania, with an estimated one million new cases each year. Drug development studies with Leishmania spp. rely heavily on in vitro and in vivo models, such as mouse macrophages and murine infection. Thus, there is a growing demand for new models complying with the 3R policy, such as G. mellonella hemocytes. This study establishes culture conditions for G. mellonella hemocytes and evaluates their applicability as a model for Leishmania infection.

Methods: G. mellonella hemocyte cultures were obtained in Grace medium supplemented with 10% FBS, L-cysteine and a drug cocktail (PEN–STREP, vancomycin, fluocytocin) to minimize contamination. Additionally, a 3-day pretreatment of larvae with doxycycline and/or ciprofloxacin was also done prior to hemolymph extraction. Hemocyte's cytotoxicity was evaluated using the drug cocktail and Leishmania reference drugs (amphotericin B and miltefosine). Hemocyte's infection with L. donovani promastigotes was evaluated for 24 and 48 h.

Results: Hemocytes were maintained in culture at 28 °C for 25 days with a mean viability of 82%, but they did not proliferate substantially (~ 3-fold increase). The use of the drug cocktail and antibiotic pre-treatment successfully prevented contamination by the larvae microbiome. The cocktail and reference drugs caused no cytotoxicity, maintaining 100% hemocyte's viability. Successful infection of G. mellonella hemocytes by L. donovani promastigotes was achieved, with amastigotes observed inside hemocytes, validating the designed protocol for infection assays.

Conclusions: Our study provided new culture conditions for G. mellonella hemocytes that support Leishmania infection, demonstrating the potential of G. mellonella as a sustainable alternative model for infection studies. Our findings validate this approach for L. donovani and pave the way for extending its applicability to other Leishmania species.

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ID394 | High-throughput phenotypic screening of the PT-openscreen library identifies novel antiplasmodial hits

Author: Maria Manuela da Silva

1Global 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), 1349-008 Lisbon, Portugal.

E-mail: a21001276@ihmt.unl.pt

Other authors: Sofia Benfeito2,3; Fernando Cagide2; Daniel Chavarria4; Sara Mendes1; Ana Paula Arez1; Pedro Cravo1; António Pombinho5*; Fernanda Borges2,3*; Gustavo Capatti Cassiano1*

1Global 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), 1349-008 Lisbon, Portugal. 2MedInUP, Center for Drug Discovery and Innovative Medicines, Department of Biomedicine, Unit of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal 3Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal. 4 CIQUP-IMS− Chemistry Research Center of the University of Porto, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal 5i3S - Institute for Research and Innovation in Health, University of Porto, Porto, Portugal.

Background: Malaria is a disease caused by protozoa of the genus Plasmodium and transmitted to humans by female Anopheles mosquitoes. According to WHO, about 263 million cases were reported in 2023, with 597,000 deaths, mainly among children under 5. A major challenge in combating malaria is emergence and spread of resistance to antimalarial drugs. This is concerning given reports of P. falciparum resistance to ACTs in Southeast Asia (1), and recent detection of mutations in kelch13 gene, a marker of artemisinin resistance, in Africa (2). These highlight the urgent need for new antimalarial therapies with novel mechanisms of action.

One of the strategies for the discovery of new antimalarials is based on phenotypic screenings using high-throughput assays (HTS). This approach relies on a straightforward assessment of parasite viability in the presence of test compounds and more faithfully reflects the parasite's physiological conditions.

This project aimed to perform phenotypic screening of synthetic compounds against the asexual blood stage of P. falciparum using an HTS assay validated with SYBR Green fluorescence.

Methods: The HTS was carried out at i3S with 283 compounds from PT OPENSCREEN, tested at 2 µM in 384-well plates, following Guiguemde et al. (3). Parasite growth was assessed after 72 h. Hits had their IC50 values determined against sensitive (3D7) and multidrug-resistant (Dd2) strains. Hemolysis was evaluated according to Pereira et al. (4), and cytotoxicity was assessed in HepG2 cells using an HTS assay (5). Gametocytocidal activity was determined according to Fivelman et al. (6).

Results: Thirty compounds showed over 70% inhibition at 2 µM in the HTS, without significantly affecting HepG2 cell viability. Of these, ten were selected based on availability for dose-response assays against the 3D7-sensitive strain, displaying IC50 values ranging from 0.045 to >20 µM. No cross-resistance was observed against the multidrug-resistant Dd2 strain. Additionally, no hemolysis was detected for the tested compounds at 50 µM. Importantly, five compounds also displayed gametocytocidal activity, effectively killing the transmissible parasite stages when incubated at 10 µM.

Conclusion: These findings highlight promising antiplasmodial candidates with selective activity and good safety profiles.

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ID400 | Antimicrobial and potential adjuvant activities of new azole derivatives against norA over-expressing Staphylococcus aureus

Author: Marta Leal

2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Portugal

E-mail: a21001253@ihmt.unl.pt

Other authors: Humberto Medeiros Barreto 1, Jonas Nascimento de Sousa 1 , Noelia Marcela Ceballos 3 , Carlos Emidio Sampaio Nogueira 4 , Walter Jose Peláez 3, Miguel Viveiros 2 , Sofia Santos Costa 2 , Isabel Couto 2

1 Federal University of Piauí, Teresina, Brazil; 2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Portugal; 3 Instituto de Investigaciones en Fisicoquímica de Córdoba, Universidad Nacional de Córdoba, Argentina; 4 Regional University of Cariri, Crato, Brazil

Background: Staphylococcus aureus is the agent of several infections and is also known for its high capacity to develop resistance to antimicrobials, including resistance mediated by multidrug efflux pumps, such as NorA. In this work, we aimed to evaluate the anti-bacterial activity of new azole derivatives, comprising 4-imidazolidinones (T1-T4) and 4-thiazolidinones (T5-T8), and to investigate their adjuvant effect on the activity of different antimicrobials against a S. aureus strain overexpressing the norA gene.

Methods: For the evaluation of antimicrobial activity, the minimum inhibitory concentration (MIC) values of the azole derivatives were determined using the broth microdilution method. The potential adjuvant effect of the compounds was initially assessed by their capacity to reduce the MICs of norfloxacin, ciprofloxacin, the biocide tetraphenylphosphonium bromide, and ethidium bromide (EtBr). Then, their potential as efflux inhibitors was evaluated by real-time fluorometry, using EtBr accumulation and efflux assays.

Results: The 4-imidazolidinone derivatives did not present significant antimicrobial activity against S. aureus (MICs: 512 to 1024 mg/L), unlike the 4-thiazolidinone derivatives that showed antimicrobial activity against the tested strains (MICs: 4 to 16 mg/L). The 4-imidazolidinones, T2, T3, and T4, exhibited an adjuvant effect, reducing the MICs of effluxable antimicrobials and EtBr by at least 4-fold, which for compound T3 was associated with inhibition of efflux activity, evaluated by fluorometric assays. Molecular dynamics analysis suggests that T3 stably binds at the NorA binding site with a high affinity (-22.9 Kcal/mol).

Conclusions: 4-thiazolidinones could be considered as potential anti-staphylococcal agents, while the 4-imidazolidinone T3 could function as an adjuvant of fluoroquinolones and tetraphenylphosphonium bromide against efflux-mediated resistance in S. aureus.

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ID408 | Targeting efflux and biofilms: new candidate drugs for repurposing againstantimicrobial-resistant Staphylococcus aureus and Staphylococcus epidermidis

Author: Mariana Andrade

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

E-mail: a21001252@ihmt.unl.pt

Other authors: Joana Neves1, Matilde Nunes1, Sofia G. Seabra1, Ana Armada1, Miguel Viveiros1, Liliana Rodrigues1, Isabel Couto1, Sofia Santos Costa1

1Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

Background: Staphylococcus aureus and Staphylococcus epidermidis are key pathogens linked to chronic infections that are difficult to treat. This resilience is partly due to efflux-mediated resistance and biofilm formation, for which new therapeutic options are needed. This study aims to evaluate the potential of 57 candidate drugs, identified through an in silico drug repurposing strategy, as efflux and biofilm inhibitors in these pathogens.

Methods: Efflux and biofilm inhibitory activities were evaluated in isogenic and reference strains differing in the expression of norA (encoding NorA, a major staphylococcal efflux pump), as well as in clinical strains. Efflux inhibitory activity was assessed by evaluating the drugs' modulation of the MICs of effluxable antimicrobials and fluorometry. Drugs with a significant effect [modulation factor (MF) score ≥ 4 and/or relative final fluorescence value (RFF) ≥ 1] were classified as efflux inhibitors (EIs) and further evaluated by checkerboard assays to determine their lowest effective concentrations. EIs' ability to inhibit biofilm formation or eradicate mature biofilms was assessed using the crystal violet method and minimum biofilm eradication concentration (MBEC) assays. The efficacy of the two most promising drugs, amlodipine (AML) and imipramine (IMI), was tested in the Galleria mellonella infection model at different doses. Drugs' targets are being identified by an omics approach.

Results: Of the 57 drugs tested, only two presented mild antimicrobial activity (MICs 8 – 32 mg/L). Twenty-four drugs showed potential as EIs. Eight drugs presented dual EI and antibiofilm activity in both staphylococcal species at concentrations of 16 – 1024 mg/L with AML, IMI and fluoxetine being the most promising ones. Moreover, IMI and doxepin eradicated mature S. aureus biofilms. G. mellonella infection assays indicate that AML and IMI have a mild positive effect, with AML being the most promising drug, increasing larva survival by 20%. Future studies will focus on assessing AML adjuvant effect with ciprofloxacin in G. mellonella.

Conclusions: This study highlights the potential of clinically approved drugs to be repurposed as efflux and biofilm inhibitors against antimicrobial-resistant staphylococci.

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ID450 | Recombinant expression, purification and characterization of Shiga toxin B subunits

Author: Ana Maria Campos

National Institute for Agricultural and Veterinary Research, Vairão, Porto, Portugal; CEB–Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal.

E-mail: anamaria.campos@iniav.pt

Other authors: Marta Gomes, Sónia Silva, Carina Almeida, Hugo Oliveira.

1 CEB–Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal; 2 CEB–Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal, LABBELS—Associate Laboratory in Biotechnology and Bioengineering and Microelectromechanical Systems, Braga, Portugal; 3 National Institute for Agricultural and Veterinary Research, Vairão, Porto, Portugal, ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal, LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto; 4 CEB–Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal, LABBELS—Associate Laboratory in Biotechnology and Bioengineering and Microelectromechanical Systems, Braga, Portugal.

Background: Shiga toxin-producing Escherichia coli (STEC) is a public health threat, associated with a range of clinical outcomes, from bloody diarrhea to more severe complications such as hemolytic-uremic syndrome (HUS), especially in children. STEC main virulence factor is the presence of lambdoid bacteriophages carrying genes encoding Shiga toxins (Stx). Among these, Stx1a and Stx2a are commonly found subtypes in humans, but Stx2d stands out due to its increased toxicity after proteolytic activation by host elastase. These AB5-type toxins comprise a catalytic A subunit and a pentamer of five receptor-binding B subunits, the latter playing a key role in host cell intoxication. This work aims to establish a workflow for recombinant production of B subunits of Stx1a, Stx2a and, importantly, Stx2d, which remains poorly represented in scientific literature despite its clinical relevance. Through recombinant expression in E. coli and purification by affinity chromatography, we aimed to obtain soluble and properly assembled toxin subunits, to foster as essential targets for downstream optimization of anti-toxin strategies.

Methods: Nucleotide sequences encoding the B subunits of Stx1a, Stx2a and Stx2d were synthesized and cloned into individual pET28a plasmids by an external provider. Plasmids were transformed into electrocompetent E. coli BL21 (DE3) cells, and protein expression was induced at the exponential growth phase. After lysis and centrifugation, soluble and insoluble fractions were visualised by SDS-PAGE to confirm expression. Lastly, protein purification was performed by Immobilized Metal Affinity Chromatography (IMAC), followed by concentration through Amicon ultrafiltration. The oligomeric state of the purified proteins was evaluated by Native-PAGE, and the structure and thermal stability was analysed by Circular Dichroism.

Results: Expression of Stx1a, Stx2a and Stx2d B subunits was confirmed by SDS-PAGE, and only Stx2d was detectable in the soluble fraction, before and after IMAC. Afterwards, the soluble fraction of Stx2d was ultrafiltered and then analysed by Native-PAGE, which revealed that it assembles into the anticipated pentameric complex.

Conclusions: These preliminary findings establish Stx2d as a priority target and provide the basis for expanding purification to all three proteins. This work also provides a foundation for recombinant production of Stx B subunits, an important step toward developing therapeutic tools targeting Stx.

ID454 | Applying a drug repurposing strategy to identify new efflux inhibitors and antibiofilm agents for Enterococcus faecalis and Pseudomonas aeruginosa

Author: Sofia Santos Costa

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

E-mail: scosta@ihmt.unl.pt

Other authors: Pedro Rita1, Mariana Andrade1, Miguel Viveiros1, Liliana Rodrigues1, Isabel Couto1

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

Background: A previous in silico drug repurposing study identified 24 approved drugs as potential inhibitors of efflux pumps and biofilm formation in staphylococci. The present work is an expansion of that study, in which we investigate if these drugs also affect other clinically significant pathogens associated with antimicrobial resistance and chronic infections, namely Enterococcus faecalis (EF) and Pseudomonas aeruginosa (PA).

Methods: Reference strains of EF (n=2) and PA (n=1), with basal to increased efflux activity and strong biofilm producers, were used to evaluate the efflux inhibitory activity of the 24 candidate drugs. Efflux inhibitory activity was assessed by the drugs ability to modulate MICs of effluxable antimicrobials and to promote intracellular EtBr accumulation, measured by fluorometry. Drugs showing efflux inhibitory activity were further tested for their ability to inhibit biofilm formation using the microplate adhesion method with crystal violet staining. The efficacy of the most effective drug was assessed in the Galleria mellonella infection model.

Results: None of the 24 drugs presented antimicrobial activity against EF or PA (MICs 32 – 64 mg/L). For EF, 9 drugs showed potential efflux inhibitory activity [(Modulation factor ≥ 4 and/or relative final fluorescence (RFF) ≥ 1]. Of these, 6 drugs also presented an effect against biofilm formation. For PA, 11 drugs were categorized as potential efflux inhibitors, but none of them presented antibiofilm activity. Amlodipine, the most promising candidate drug in the above assays, showed no impact on the survival of G. mellonella infected by an EF reference strain. Yet it may affect survival as an adjuvant, a condition to be further tested.

Conclusions: Overall, 6 drugs showed dual efflux and antibiofilm activities in EF whereas 11 drugs were only able to inhibit efflux in PA. This study demonstrates the potential of approved drugs as candidates for repurposing as efflux inhibitors and/or antibiofilm agents against infections caused by the clinically relevant pathogens E. faecalis and P. aeruginosa.

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ID466 | Establishment of Canine B-Cell Lymphoma Xenografts as Preclinical Models for Non-Hodgkin Lymphoma Therapy

Author: Ana Leonardo

Vector B2B – Drug Development from bench 2 bedside, Lisbon, Portugal

E-mail: aleonardo@fmv.ulisboa.pt

Other authors: Joana Nunes Ribeiro Dias, Ana André, Solange Gil, Luís Tavares, Frederico Aires da Silva

1 CIISA – Centro de Investigacão Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisbon, Portugal; 2 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

Background: One of the most common neoplasia in dogs is canine diffuse large B-cell lymphoma (DLBCL), which shares great similarities with its human counterpart, making it an excellent model for research of novel therapeutic agents. However, partially due to the lack of suitable xenograft mouse models, the application of canine lymphoma in comparative studies has been limited. To overcome these limitations, we established and characterized a localized subcutaneous canine DLBCL xenograft mouse model, with and without bioluminescence, to monitor tumor progression and treatment response in preclinical studies.

Methods: The established CLBL-1 cell line, proven to faithfully represent canine DLBCL, was used for tumor inoculation in mice. Bioluminescent imaging with an IVIS system was performed as a noninvasive monitoring of tumor growth. Further validation of the model was done with the evaluation of therapeutic responses to multiple compounds, including small molecules (e.g., panobinostat), antibody–drug conjugates, and nanoparticles (liposomes). The xenograft model was further applied to in vivo phage display for antibody selection against canine DLBCL.

Results: The tumors demonstrated high engraftment efficiency, consistently localized at the inoculation site. Histological and immunophenotypic analyses confirmed that the xenograft tumors retained features consistent with canine DLBCL. Bioluminescent imaging successfully detected and quantified tumor growth. Therapeutic studies demonstrated measurable responses to the tested compounds, validating the model's utility for preclinical research. Furthermore, the in vivo phage display led to the successful selection of a promising antibody candidate targeting canine DLBCL.

Conclusions: These findings establish the model as a valuable preclinical platform for testing novel therapies with translational relevance to both veterinary and human oncology.

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ID478 | Antimicrobial Potential of Antisense Peptide Nucleic Acids Conjugated with Cell-Penetrating Peptides Against Gram-Negative Bacteria

Author: Joana S. Oliveira

1 LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal. 2 i3S- Institute for Research and Innovation in Health, University of Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal. 3 IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, University of Porto, Rua Júlio Amaral de Carvalho 45, 4200-135 Porto, Portugal

E-mail: up201202544@edu.fe.up.pt

Other authors: Rita S. Santos1, Nuno F. Azevedo1

1 LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Background: The escalating problem of antimicrobial resistance is worsening globally, largely due to the increasing use of broad-spectrum antibiotics. Developing antibacterials with species-specific activity is a key strategy for tackling this challenge. Antisense oligonucleotides (ASOs) offer a targeted approach to eliminate specific bacteria. By hybridizing with mRNA, they inhibit the expression of essential bacterial genes. This approach is effective only if the ASOs are delivered into the bacterial cytosol, since they are too large to cross the bacterial envelope without assistance. Cell-penetrating peptides (CPPs), such as (KFF) 3K and (RXR)4XB, are widely used as carriers for oligonucleotides in bacteria. Chemically-modified peptide nucleic acid (PNA) stands out as a highly promising oligonucleotide chemistry due to its nuclease resistance and strong target affinity. However, if species-specific effects can be achieved with PNA conjugated to these CPPs remains to be investigated. This study investigates the effects of PNA conjugated with these two CPPs designed against Escherichia coli (E. coli).

Methods: Each conjugate designed to target the acpP gene (involved in fatty acid biosynthesis), was added to a suspension of E. coli K12 or P. aeruginosa PAO1, incubated, and cultivable cell counts were assessed at 0 and 4 h. (KFF)3K, (RXR)4XB and the PNA sequence were used separately as controls.

Results: The anti-acpP PNA-(KFF)3K conjugate, created to target E. coli, markedly reduced E. coli growth by 5 logs at 30 μM, with no observed effect on P. aeruginosa, thereby demonstrating its specific antibacterial properties. However, the conjugate intended to inhibit P. aeruginosa showed no antibacterial activity. Further experiments revealed that (KFF)3K permeabilized the E. coli cell envelope, but not that of P. aeruginosa, suggesting a barrier to PNA entry into the P. aeruginosa cytosol. Evaluation of the anti-acpP PNA-(RXR)4XB conjugate against both bacteria is forthcoming.

Conclusions: This study emphasizes that the PNA sequence provides species-specific effect, but also that the CPP carrier can be selective. This highlights the need to consider both the PNA sequence and the carrier to develop effective, specific conjugates as alternatives to broad-spectrum antibiotics.

ID486 | Efficient mRNA Isolation through Amino-Acid-Based Chromatography

Author: Marco Ângelo Luís

RISE-Health, Faculty of Sciences, University of Beira Interior, 6201-506 Covilhã, Portugal

E-mail: marco.luis@ubi.pt

Other authors: Bruno Baptista 1, Augusto Pedro 2, Chantal Pichon 3, Mara G. Freire 2, Fani Sousa 4

1 RISE-Health, Faculty of Sciences, University of Beira Interior, 6201-506 Covilhã, Portugal; 2 CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 3 ART-ARNm ISERM, Research Unit at French Institute of Health and Medical Research, University of Orléans, France; 4 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

Messenger RNA (mRNA) has gained significant attention due to its success as a vaccine platform and its potential in therapeutic applications, including protein replacement, immunomodulation, cell therapy, and cancer treatment [1]. The main strategy for its production is in vitro transcription (IVT), which, however, generates undesirable contaminants such as double-stranded RNA (dsRNA), known to induce strong inflammatory and immunogenic responses due to its physicochemical similarity to mRNA [2]. Moreover, current purification methods rely on expensive affinity ligands and are ineffective at removing dsRNA.

In this study, we present the application of an arginine-functionalized chromatographic support for the single-step purification of mRNA. mRNA encoding p53 was produced via IVT from plasmids previously extracted and linearized. Two increasing sodium chloride gradients were established, prioritizing either higher recovery or higher purity.

The strategy globally enabled the removal of plasmid DNA, as confirmed by agarose gel electrophoresis and quantitative PCR (qPCR). Moreover, dot-blot analysis showed a significant reduction in dsRNA content in both gradients. Finally, the gradients were compared with two commercial transcript purification kits, and the results showed that both established gradients provide more efficient purification of mRNA, resulting in higher removal of pDNA and dsRNA.

For this, the application of an arginine functionalized support for the purification of mRNA provides a rapid (~10 min), cost-effective, and efficient alternative for mRNA purification, with potential for large-scale applications, when compared to commercial purification kits.

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ID488 | Biotechnological advances for the use of microalgae and seaweed species in obesity and diabetes

Author: Ralph Urbatzka

CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Av. de General Norton de Matos s/n, 4450 -208 Matosinhos, Portugal

E-mail: rurbatzka@ciimar.up.pt

Other authors: Javier Sanz 1, Ana Catarina Fonseca 1, Marielle Frank 1, Rita Pimenta 1, Filipe Henriques 1, Maria Silva 1, Diogo Martins 1

1 CIIMAR

Background: Novel molecules to fight metabolic diseases are urgently needed, and algae are important resources for valuable compounds with human health benefits (e.g. polyunsaturated fatty acids, pigments, vitamins). The aim of this research was to analyze the bioactivities of microalgae and seaweeds for obesity and diabetes.

Methods: Organic extracts were prepared and screened using phenotypic assays with zebrafish larvae, namely Nile red fat metabolism, 2NBDG glucose uptake, appetite suppression of fluorescent liposomes, beta-cell regeneration after cell ablation. Metabolite profiling with LCMS-MS was performed on the GNPS platform, and mRNA expression with quantitative real-time PCR.

Results: The results revealed that extracts from Chlorella vulgaris and from Chlorococcum sp. reduced the appetite > 40% in zebrafish larvae. Aqueous extracts from Tetraselmis chuii stimulated glucose uptake at 40%, and lipidic extracts of both Pavlova gyrans and of Phaeodactylum triocornutum reduced neutral lipid level at 50%. Nanofrusturum shiloi and Nannochloropsis oceanica were bioactive in beta-cell regeneration. The macroalgae Porphyra dioica reduced neutral lipids at 40%, while Codium tomentosum increased glucose uptake at 50%. Promising extracts were submitted to metabolite profiling with LC-MS/MS and the GNPS platform to receive insights into responsible compounds. Some unsaturated fatty acids (oleic acid, linoleic acid, palmitoleic acid) and nucleosides (adenosine, methylthioadenosine) were selected from those clusters, and confirmed their appetite reducing activity at 5 – 20 µM. The analysis of mRNA expression of appetite regulating genes demonstrated increased pomc and cartp mRNA, involved in anorexigenic, neuroendocrine signaling of appetite. The identification of the responsible compounds from the other bioactive extracts is ongoing.

Conclusions: In summary, promising activity was observed for various microalgae and seaweed species, which could lay the ground for the development of nutraceuticals for the treatment of obesity and diabetes.

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ID507 | AI-Based Prediction of HIV-1 Drug Resistance from Partial pol Sequences: Genome-wide DRM Inference and Prediction

Author: Matheus Tavares

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal. 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: id11290@uminho.pt

Other authors: Ana Santos Pereira 1,2, João Correia 3, Nuno S. Osório 1,2

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal. 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

Background: Antiretroviral therapy (ART) improved survival and quality of life, but drug resistance mutations (DRMs) continue to undermine treatment. In many settings, routine genotyping is limited to the pol region (protease, reverse transcriptase), while integrase and other regions are not routinely sequenced. Partial or lower-quality data further constrain clinical use. We propose AI models that, using pol sequences and essential clinical parameters, infer DRMs beyond pol and forecast resistance emergence under different ART regimens, providing decision support for clinicians.

Methods: We curated 8,036 HIV-1 pol sequences (PR+RT, nucleotides 2253–3870), applied quality control (removal of low-quality/ambiguous/duplicated sequences; length ≥90% of pol), and subtype/metadata annotation. Baseline machine-learning models (Random Forest, SVM, XGBoost) were trained to predict integrase DRMs from pol-derived features. The research plan includes: (i) model baselines integrating clinical covariates (e.g., ART regimen, viral load, CD4); (ii) deep learning (CNNs, Transformers); and (iii) fine-tuning of a Genome Language Model (e.g., Evo) on curated HIV-1 data. We will implement bias detection/correction across subtypes, regions, and treatment status, validate on independent datasets, and perform sensitivity analysis to identify influential features.

Results: From pol alone, models accurately recover multiple integrase DRMs. Across most positions—P50, P74, P101, P201—precision, recall and F1 ≥0.98. Additional loci show strong performance (P140 F1 ~0.86–0.91; P149 F1 ~0.84–0.89; P157 F1 ~0.79–0.82), while P138 presents lower but informative signal (F1 ~0.52–0.62). These results support the feasibility of inferring clinically relevant DRMs from routinely available pol sequences.

Conclusions: AI models trained on partial pol data can provide cost-effective resistance insights beyond the sequenced region and support personalized ART. Combining fine-tuned sequence models with essential clinical parameters can deliver predictions to improve treatment selection, reduce failure, and strengthen resistance surveillance – aligned with clinical needs in settings where full-genome sequencing is not routine.

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ID518 | Uncovering SF1: A new marine bioactive with potent antifungal activity

Author: Carolina Vitorino Mariano

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: cv.mariano@itqb.unl.pt

Other authors: Susana Ribeiro 1, Nuno Miguel Saraiva 1, Cláudia Malta-Luís 1, Teresa Pissarro 1, Américo Gonzalez Duarte 1, Oscar Lenis Rojas 1, Maria Rita Ventura 1, Catarina Pimentel 1

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Background: Invasive fungal infections (IFIs) have been increasing in recent years, causing substantial morbidity and mortality. Current therapeutic strategies rely predominantly on three systemic antifungal classes: azoles, polyenes, and echinocandins. However, rising resistance to these agents has significantly reduced their clinical efficacy, underscoring the urgent need for novel antifungal therapeutics with distinct mechanisms of action. This study explores SF1, a bioactive compound isolated from a marine sponge for its antifungal potential and mechanism of action.

Methods: Antifungal susceptibility of SF1 was assessed against several clinically relevant fungal species. Time-kill assays were performed to determine fungicidal activity. Checkerboard assays evaluated interactions with marketed antifungal drugs. SF1 stability, solubility and lipophilicity were analyzed using UHPLC-MS/MS. To explore its mechanism of action, similarity searches in PubChem and literature reviews identified potential targets, including Hsp90 and the plasma membrane Hn-ATPase (Pma1). HSP90 gene expression and C. albicans filamentation were assessed by RT-PCR and microscopy, respectively. Pma1 activity was tested using everted vesicles from Saccharomyces cerevisiae.

Results: SF1 exhibited broad-spectrum antifungal activity, with remarkable fungicidal effects at concentrations twice its minimal inhibitory concentration against C. albicans. Furthermore, SF1 addresses gaps in the current antifungal pipeline, showing activity against species such as C. krusei and C. guilliermondii. Checkerboard assays revealed an antagonistic interaction with fluconazole. No significant changes in HSP90 expression or filamentation patterns were observed after SF1 treatment, ruling out Hsp90 as a target. Initial assays to test Pma1 inhibition were limited by compound availability, prompting purification of FLAG-tagged Pma1 for future assays. To ensure a scalable and sustainable supply, efforts are currently underway to establish a synthetic route for SF1.

Conclusions: SF1 demonstrates strong antifungal potential and appears to act through a novel mechanism of action distinct from standard antifungal pathways. These findings support further investigation of SF1 as a promising candidate for treating IFIs.

ID519 | Biofilm Dynamics in Cystic Fibrosis: Application of NAM-FISH in Air3Gel, a 3D airway mucus model

Author: Isadora Maia

1 LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

E-mail: unbisadora@gmail.com

Other authors: Natalia Suárez4, Daniela Pacheco4, Nuno F. Azevedo1,2,3, Andreia S. Azevedo1,2,3

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 in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 3 FEUP - Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 4 Bac3Gel, TagusPark - Avenida Jacques Delors, Edifício Inovação II, nº 421, Laboratório 2, 2740-122 Porto Salvo, Portugal

Biofilms are structured microbial communities embedded in a self-produced extracellular matrix that protect microorganisms from antimicrobial agents and host immune responses. In cystic fibrosis (CF), these communities are central to the persistence and treatment resistance of respiratory infections. In vitro models are essential to replicate clinically relevant conditions, enabling the study of biofilm physiology and interactions. In this study, we investigated biofilm formation by three CF-associated pathogens, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae. A three-dimensional airway model, Air3Gel, was employed to mimic the CF lung environment [1]. Bacterial populations were mapped using PNA-FISH (Peptide Nucleic Acid – Fluorescence in situ Hybridization) with confocal laser scanning microscopy (CLSM) [2]. Quantitative analysis was performed through colony-forming unit (CFU) counting and DAPI staining [3].

The integrated approach enabled the visualization of heterogeneous communities formed by P. aeruginosa, S. aureus and K. pneumoniae within the 3D airway model. Rather than forming clearly separated, species-specific structures, the microorganisms established polymicrobial biofilms where the predominance of one species over the others could be distinguished and varied over time. PNA-FISH with CLSM allowed spatial mapping of these dynamic shifts, revealing how bacterial aggregates and microcolonies evolved during incubation. In single-species biofilms, PNA-FISH detected after 24h log 8.6 and log 8.9 cells/mL of P. aeruginosa and K. pneumoniae, respectively, and after 48h log 9.3 and log 8.9 cells/mL. Quantitative data from CFU and DAPI confirmed changes in biomass and validated bacterial load. Together, these results highlight the robustness of the model in capturing both the heterogeneity and temporal evolution of polymicrobial biofilms in a CF model, mimicking features of chronic respiratory infections.

This study demonstrates the feasibility of integrating PNA-FISH, CLSM and Air3Gel as a reliable framework for investigating CF-associated polymicrobial biofilms. By enabling quantification and visualization of heterogeneous communities, the model provides insights into spatial organization and temporal dynamics in a CF-relevant context. These findings establish a versatile platform for future studies on interspecies interactions and pave the way for a robust disease model of chronic respiratory infections.

ID521 | Hyperbaric storage of stem cells: A potential alternative in cell preservation

Author: Ana Patrícia Martins

LAQV-REQUIMTE & CICECO, Department of Chemistry, University of Aveiro, Portugal

E-mail: ana.patricia.martins@ua.pt

Other authors: Tiago Correia, João Mano, Jorge Saraiva

1 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Portugal; 2 CICECO, Department of Chemistry, University of Aveiro, Portugal; 3 CICECO, Department of Chemistry, University of Aveiro, Portugal

Background: Stem cells are pivotal for cell-based therapies and regenerative medicine due to the self-renewal and differentiation capacity. Effective preservation strategies are critical to maintain their therapeutic potential. While cryopreservation remains the gold standard for extending cell lifetime and storage, it presents limitations such as the need for cryoprotective agents (e.g. DMSO), risk of cryoinjuries, and high energy consumption. Hyperbaric storage (HS) is an emerging preservation technique with potential for preservation of foods and biological products under pressure. When applied at room temperature (RT), HS can be considered a quasi-energetically costless procedure, as no additional energy is needed during storage after the initial pressurization, leading to significant energy savings. This study evaluates the feasibility of HS at RT as a novel, cryoprotectant-free, and low-energy alternative for preserving stem cells using human adipose stem cells as a relevant study model.

Methods: Cells were stored under pressure (20, 30, and 40 MPa) at RT, up to 21 days, and compared to cells preserved at −80 °C. To confirm the potential of HS as a cell preservation method, in vitro characterization assays were done, namely, cell viability, metabolic activity, DNA quantification and phenotype determination.

Results: After 3 days of storage, cells kept at 30 MPa/RT presented the best results with similar viability, and metabolic activity compared to cryopreserved controls while phenotypic markers were maintained. Moreover, cells stored under HS (despite the pressure used during storage) exhibited a shorter adaptation period post-storage, indicating potential advantages for rapid downstream applications. Results after 7 days of storage showed a reduction in metabolic activity and viability, however phenotypic markers were maintained and cells kept normal morphology for adherent hASCs. Storage for longer periods (14 and 21 days) resulted in reduced viability and adherence, although stemness markers remained similar throughout storage timeframe.

Conclusions: These findings suggest that short-to-mid-term storage under HS/RT (30 MPa/RT) can preserve stem cells function effectively with low energy costs and without the need for additives such as cryoprotective agents. This initial evaluation supports the feasibility of HS/RT as a possible alternative to conventional cryopreservation, particularly for applications involving transport or short-term storage.

ID528 | Smart extracellular vesicles for targeted therapy in breast cancer

Author: Débora Ferreira

1 CEB Centre of Biological Engineering, University of Minho, 4710 057 Braga, Portugal 2 LABBELS Associate Laboratory, Braga/ Guimarães Portugal

E-mail: deboraferreira@ceb.uminho.pt

Other authors: Leonor Gandarela 1, Catarina Gonçalves 1, Inês Novo 1, Mariana Sousa 1, Ana Catarina Pereira 1, Diana Andrade Sousa 1 and Lígia Raquel Rodrigues 1,2

1 CEB Centre of Biological Engineering, University of Minho, 4710 057 Braga, Portugal 2 LABBELS Associate Laboratory, Braga/ Guimarães Portugal

A central challenge in cancer nanomedicine is achieving selective drug delivery that directs therapeutic agents specifically to tumor cells while minimizing adverse effects on healthy tissues. Over the past decades, drug delivery systems have advanced considerably, with nanocarriers explored as promising vehicles to improve treatment outcomes. Among these, extracellular vesicles (EVs), which are lipid bilayer enclosed particles naturally secreted by cells during both physiological and pathological processes, have attracted great interest. Their inherent biocompatibility, low immunogenicity, and natural ability to transport biological material position them as strong candidates for clinical translation. Building on these properties, we sought to improve the tumor specificity of EVs for the treatment of triple negative breast cancer (TNBC), an aggressive subtype that currently lacks effective targeted therapies. To this end, we developed an in-house TNBC-specific peptide, termed 231-Pep, through phage display screening, and conjugated it to PEG-phospholipid micelles, which were subsequently integrated into cell-derived EVs. Comprehensive analyses demonstrated that the engineered EVs exhibited markedly improved recognition and uptake by TNBC cells both in vitro and in vivo compared with unmodified controls, confirming their selective targeting capability. Furthermore, we showed that these EVs could be loaded with a broad range of therapeutic molecules, including small interfering RNAs, microRNAs, and functional proteins, underlining their versatility as adaptable drug delivery vehicles. Collectively, these findings suggest that peptide guided engineering of EVs offers a promising strategy to achieve precise drug delivery in TNBC and may pave the way for personalized therapeutic approaches that could be extended to other difficult to treat cancers.

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ID531 | Development of Highly Specific and Potent Degrader-Antibody Conjugates for Triple-Negative Breast Cancer

Author: Rafaela Marimon

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: rmarimon@fmv.ulisboa.pt

Other authors: Isa Moutinho 1,2, Ana Leonardo 1,2, Rita Pedrosa 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).

Background: Triple-Negative Breast Cancer (TNBC) represents 15-20% of all breast cancers and is characterized by high aggressiveness, poor prognosis, and lack of targeted therapies. Similarities between TNBC and Feline Mammary Carcinoma (FMC), which is predominantly triple-negative, offer a unique opportunity for comparative oncology. PROTACs (PROteolysis-Targeting-Chimeras) represent a novel therapeutic class inducing targeted protein degradation but face challenges such as off-target toxicity and poor pharmacokinetics. Degrader-Antibody Conjugates (DACs), which combine PROTACs with monoclonal antibodies, emerge as an innovative strategy to enhance specificity and therapeutic efficacy.

Methods: We generated rabbit-derived single-domain antibodies (sdAbs) targeting TROP-2, a validated TNBC receptor, using phage display in vitro and in vivo screening platforms. In parallel, a high-throughput screening of PROTAC libraries have identified compounds targeting TNBC-associated proteins. Selected PROTACs will be conjugated to anti-TROP-2 sdAbs via a cleavable linker, yielding our DAC molecule. These will be characterized for binding, stability, and anti-tumor activity in feline and human TNBC cell lines, followed by pharmacokinetics, biodistribution, and efficacy/safety studies in TNBC xenograft mouse models. Results: We identified highly specific anti-TROP-2 sdAbs with strong internalization properties. In addition to that, a PROTAC library is being tested, and preliminary studies have already identified compounds with activity against both FMC and TNBC. Conjugation will generate stable DACs with superior tumor penetration, improved pharmacokinetics, and reduced off-target effects compared to PROTACs alone. In vivo validation is anticipated to demonstrate significant tumor growth inhibition and favorable safety profiles. Conclusions: This study pioneers the development of DACs for TNBC, integrating antibody engineering, targeted protein degradation, and comparative oncology. By leveraging FMC as a translational model, our approach aims to provide a next-generation therapeutic platform with high precision and reduced toxicity, paving the way for clinical translation in both human and veterinary oncology.

ID532 | AptaVerse – Unveiling the full potential of aptamers in oncology

Author: Débora Ferreira

1 CEB Centre of Biological Engineering, University of Minho, 4710 057 Braga, Portugal 2 LABBELS Associate Laboratory, Braga/ Guimarães Portugal

E-mail: deboraferreira@ceb.uminho.pt

Other authors: Lígia Raquel Rodrigues

1 CEB Centre of Biological Engineering, University of Minho, 4710 057 Braga, Portugal 2 LABBELS Associate Laboratory, Braga/ Guimarães Portugal

Cancer continues to pose a major global health burden, largely due to its complexity and heterogeneity, which limit the effectiveness of conventional therapies. Developing new strategies that selectively target malignant cells while sparing healthy tissues remains an urgent priority. An emerging approach in tumor-targeted therapies involves the use of aptamers, short single-stranded oligonucleotides capable of binding molecular targets with high affinity and specificity. Aptamers offer several advantages, including straightforward synthesis, facile chemical modification, low immunogenicity, rapid tissue penetration, and minimal toxicity. They are typically identified through Systematic Evolution of Ligands by EXponential Enrichment (SELEX), an iterative process of selection and amplification that can be applied to a broad spectrum of targets, from purified proteins and small molecules to complex cellular structures. Over time, SELEX has evolved into specialized variants, including cell-SELEX, which uses intact living cells as targets and enables the discovery of aptamers that recognize cell-surface molecules in their native context. Based on this, SELEX was integrated with next-generation sequencing and bioinformatics analysis to identify aptamers specifically binding to metastatic cancer. The candidate aptamers were subsequently validated for binding affinity and selectivity using flow cytometry and fluorescence microscopy, and their translational potential was examined on tumor tissue sections. After aptamer selection, bioinformatic tools were employed to further explore the interaction with the candidates. Our findings highlight the promise of aptamers as precise molecular tools for cancer targeting, emphasizing their potential to advance tumor diagnostics and therapeutics.

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ID533 | Advancing Precision Medicine in Canine Lymphoma: Identification of Potent Payloads for Antibody-Drug Conjugates

Author: Rafaela Marimon

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: rmarimon@fmv.ulisboa.pt

Other authors: Ana André 1,2, Rita Pedrosa 1,2,Gonçalo Vicente 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).

Background: Canine lymphoma (cL) is the most common hematopoietic malignancy in dogs, commonly treated with multi-agent chemotherapy that lacks specificity and often leads to relapse. Antibody-Drug Conjugates (ADCs) combine antibody selectivity with potent cytotoxic payloads, offering a more targeted and safer approach. Building on an ADC previously developed by our group, this study aimed to identify cytotoxic compounds with distinct mechanisms of action as candidate payloads to improve therapeutic efficacy. Beyond veterinary oncology, ADC development in dogs contributes to comparative oncology and supports precision medicine strategies with translational relevance to human cancer therapy.

Methods: We evaluated a panel of cytotoxic compounds, including microtubule inhibitors and PROTAC molecules, for their activity in two canine B-cell lymphoma cell lines (CLBL-1 and 17-71). Cytotoxicity was assessed by WST-1 assays following 24-48 h of incubation. ICnn values were calculated, and drug interactions were tested for potential synergism.

Results: All tested compounds induced dose-dependent cytotoxicity. MMAE and DM1 displayed the highest potency, followed by ARV-825. Notably, SN38 and ARV-825 showed synergistic effects when combined. The data highlight compounds with distinct mechanisms of action that may be exploited as payloads for ADC development.

Conclusions: Our study identified extremely potent molecules suitable for incorporation into canine lymphoma-targeting ADCs. This approach not only holds promise to improve therapeutic efficacy and safety in veterinary oncology, but also strengthens the role of dogs as a comparative oncology model, contributing to the development of precision medicine strategies with direct translational relevance to human cancer therapy.

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ID539 | Anti-inflammatory potential in algae refinery residues

Author: Carolina Cruz

Laboratory of Microbiology, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal

E-mail: carolina.cruz@nms.unl.pt

Other authors: Lúcia Rodrigues1, Diego O. Hartmann3, Patrícia Sequeira3, Hugo Pereira4, Maria Jesus Chasqueira1,2, Paulo Paixão1,2

1Laboratory of Microbiology, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal 2CHCR, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal 3Sea4Us - Biotecnologia e Recursos Marinhos, S.A, Sagres, Portugal 4GreenColab - Associação Oceano Verde, Universidade Do Algarve, Faro, Portugal

Background: Marine algae have long been recognized as a source of potentially bioactive compounds with a wide range of biological activities. Its diversity of applications and recent consumer focus on more sustainable alternatives have made algae production and refinery a growing industry. A circular economy approach can be applied to waste products of algae refinery practices, as these may possess high-value bioactive compounds, easily accessible in an already established production pipeline. Our team, incorporated in the Blue Bioeconomy Pact, sought to identify anti-inflammatory potential in algae fractions. Methods: Fractions of 22 algae species, provided by GreenCoLab, Universidade do Minho and Aveiro were evaluated in RAW 264.7 macrophages. Cytotoxicity activity was assessed with the CellTiterGlo bioluminescent assay for a range of four concentrations [100, 10, 1, 0.1 ug/mL]. Anti-inflammatory potential was assessed through quantification of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) release after stimulation with LPS or 2'3'cGAMP using commercial ELISA kits according to the manufacturer's instructions.

Results: Of the 87 samples analysed, 4 revealed cytotoxicity at the highest concentration tested. A wide range of cytokine reduction rates (RRs) was obtained in 19 of the 22 species tested, in at least one of the studied fractions for one of the stimuli. 39 fractions of 11 species presented a RR above 50% of which 15 fractions of 6 species belonging to Nannochloropsis, Tisochrysis, Cylindrotheca, Chaetoceros and Pavlova spp. achieved a RR of 100%. Two species demonstrated potent anti-inflammatory properties due to their efficacy in reducing inflammation induced by both stimuli. Additionally, a third species was also considered a potential candidate due to its activity against the LPS-induced inflammation.

Conclusions: Significant anti-inflammatory activity was identified in several fractions, against both inflammatory pathways. The best-performing fractions can now be submitted to the next levels of anti-inflammatory experimentation: bioactive compound identification and purification; anti-inflammatory testing in human macrophages; and in vivo studies. This will allow to fully explore their therapeutic potential, strengthening the scientific basis for future applications, and positioning the project towards the development of innovative solutions with high socio-economic impact in the field of inflammation-related diseases.

ID540 | Biodegradable Additive-Manufactured Iron Implants: Balancing AntimicrobialPerformance and Cytocompatibility in Orthopaedic Applications

Author: Sofia Costa de Oliveira

Rise-Health, Department of Pathology, Faculty of Medicine of the University of Porto, Portugal

E-mail: sqco@med.up.pt

Other authors: João Pedro Monteiro1, Isabel Marcos Miranda2, Miguel Marta3, Pedro Nogueira4,5,6, Pedro Lopes7, Luís Oliveira7, Jorge Lino Alves7, M. Fátima Vaz4,5

1Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal 2Rise-Health, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Portugal 3Orthopaedics Department, São João University Hospital, 4200-319 Porto, Portugal 4.IDMEC—Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal 5.Departamento de Engenharia Mecânica, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal 6.CeFEMA— Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal 7.INEGI—Institute of Science and Innovation in Mechanical and Industrial Engineering, 4200-465 Porto, Portugal

Background: Implant-associated infections (IAIs) remain a significant challenge in orthopaedic surgery, hindering tissue regeneration and compromising long-term implant success. Conventional treatments often require implant removal surgery, increasing the risk of IAIs due to microbial adhesion and biofilm formation on implant surfaces, which resist antimicrobial therapies and may lead to chronic osteomyelitis. Additively manufactured biodegradable porous iron (Fe) implants offer a promising alternative by eliminating the need for removal surgery.

This study evaluates their potential to replace conventional non-biodegradable implants, leveraging their favourable mechanical properties, biocompatibility, antimicrobial activity, and controlled degradation.

Methods: Lattice structures composed of Rhombitruncated Cuboctahedron (RTCO) unit cells with a relative density of 10% made of porous Fe and 316L stainless steel were manufactured by laser powder bed fusion. All samples were ultrasonically cleaned in isopropanol for 30 minutes, dried at room temperature, and sterilised with ethylene oxide. To assess the cytotoxicity of Fe and its concentration-dependent effects, assays were performed using the human fibroblast-like cell line MG63. Microbial adhesion and biofilm formation assays evaluated iron's antibacterial efficacy against E. coli (ATCC 25932), MRSA (ATCC 43300), S. epidermidis (ATCC 35987), C. albicans (10231) and C. auris (NCPF 8971).

Results: A dose-dependent reduction in cell viability with acceptable cytocompatibility at Fe extract concentrations up to 25%, moderate cytotoxicity at 50%, and significant cytotoxicity at over 70% (35% viability relative to controls). A decrease in MRSA, E. coli, Streptococcus epidermidis and C. albicans biofilm formation was observed in Fe compared to 316L scaffolds. No statistically significant reduction in adhesion or biofilm formation was observed in C. auris.

Conclusions: These findings demonstrate that porous Fe implants exhibit promising cytocompatibility and intrinsic anti-biofilm properties, positioning them as viable candidates for orthopaedic implants to solve the issues with IAIs. The Fe antimicrobial mechanisms involved in microbial adhesion and biofilm inhibition deserve further exploration.

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ID583 | Novel bioactive compounds from cyanobacteria: from discovery to biotechnological applications

Author: Leonor Ferreira

1 CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, 4450-208 Matosinhos, Portugal 2 FCUP – Faculdade de Ciências da Universidade do Porto, 4169-00, Porto, Portugal

E-mail: lferreira@ciimar.up.pt

Other authors: Ralph Urbatzka1, Vítor Vasconcelos1,2, Mariana Reis1

1 CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, 4450-208 Matosinhos, Portugal 2 FCUP – Faculdade de Ciências da Universidade do Porto, 4169-00, Porto, Portugal

Background: Cyanobacteria, a diverse group of photosynthetic prokaryotes, are one of the most promising yet underexplored sources of bioactive metabolites. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) at CIIMAR houses over 900 cyanobacterial strains from distinct habitats and with vast pharmaceutical potential. To unlock this rich chemical diversity, we developed a biodiscovery pipeline that integrates a library of cyanobacterial fractions, physiologically relevant bioactivity models (cancer, obesity, Alzheimer´s), and advanced metabolomic analysis for fast dereplication. This work will exemplify how this approach allows efficient prioritization of promising strains and discovery of novel bioactive compounds.

Methods/Results: From Leptothoe sp. LEGE 181152, previously reported as producer of cytotoxic metallophores, we discovered a new analogue of phormidolide A - phormidolide E. Unlike phormidolide A, phormidolide E displays potent cytotoxicity (IC50 = 0.8 µM) against human colon carcinoma cell line HCT 116, inducing cell death and disrupting of the extracellular matrix of cancer spheroids, alongside anti-angiogenesis activity in a zebrafish model. Phormidolide E reveals a unique pharmacophore with potential for new anticancer drug development.

Genomic and metabolomics analysis of Phormidium sp. LEGE 15488 uncovered numerous nonribosomal peptides/polyketides biosynthetic gene clusters and many novel peptide-like compounds. Among these, we discovered a novel pseudospumigin-like compound that reduced appetite by 53% at 10 µM in a zebrafish feeding assay, revealing a promising pathway for obesity treatment. Other compounds from this strain are under evaluation for anti-Alzheimer activity.

Conclusions: These finds highlight the biotechnological potential of cyanobacteria and the value of integrated biodiscovery pipelines in accelerating the development of novel therapeutics.

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ID596 | Recombinant production of shk_20d1 from physalia physalis and its neurotoxic potential in drosophila melanogaster

Author: Mirian Rando Cabello

Centre of Biotechnology of Azores (CBA), University of Azores, 9500-321 Ponta Delgada, Portugal

E-mail: miriam.rando.cabello@gmail.com

Other authors: Zuzanna Tomkielska1,2, Ana Casas2, Nelson Simões1 and Duarte Toubarro1

1 Centre of Biotechnology of Azores (CBA), University of Azores, 9500-321 Ponta Delgada, Portugal 2 Mesosystem Investigação & Investimentos by Spinpark, Barco, 4805-017 Guimarães, Portugal.

Animal venoms from diverse species, including marine species like jellyfish, constitute a valuable reservoir of bioactive peptides. Advances in molecular biology and recombinant technologies have facilitated the characterization and functional assessment of these peptides, shedding light on their mechanisms of action and biomedical relevance.

In this study, we recombinantly expressed the peptide Shk_20D1, originally identified in the venom of Physalia physalis, using E. coli as a heterologous system. Oral administration of Shk_20D1 was performed in Drosophila melanogaster at concentrations ranging from 500 to 31.25 µg/mL. Behavioral and neurotoxic effects were assessed using negative geotaxis assays to evaluate locomotor alterations and climbing behavior, and real-time monitoring assays to analyze circadian rhythms and sleep duration. Phenotypic symptoms were carefully monitored in both assays.

Shk_20D1 induced a dose-dependent increase in mortality, from which the LDnn was determined. At sublethal doses, peptide administration induced two distinct phenotypic responses depending on dose and time of exposure: (i) drowsiness and reduced mobility, and (ii) hyperexcitability, spasms, and erratic jumping behavior, consistent with neurotoxic activity. Abnormal climbing patterns and impaired geotaxis were also observed, further supporting neuromotor disruption.

These findings confirm the neurotoxic effects of Shk_20D1, reinforcing its role in the toxicity associated with P. physalis venom and its capacity to modulate nervous system activity. The Drosophila model proved suitable for phenotypic screening of venom-derived peptides, and future work will extend this research to vertebrate systems to further explore therapeutic potential and mechanisms of action.

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ID609 | Towards Efficient Purification of Membrane-Bound Enzymes: The Study Case of Catechol-O-Methyltransferase

Author: Ana Margarida Gonçalves

1 RISE-Health, Health Sciences Department, University of Beira Interior, Covilhã, Portugal. 2 UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal 3 Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2819-516 Caparica, Portugal

E-mail: ggmargarida@gmail.com

Other authors: Cláudio Jorge Maia, Maria João Romão, Luís António Passarinha

1 RISE-Health, Health Sciences Department, University of Beira Interior, Covilhã, Portugal. 2 UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal 3 Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2819-516 Caparica, Portugal 4 Laboratório de Fármaco-Toxicologia, UBI Medical, Universidade da Beira Interior, Covilhã, Portugal

Background: Membrane-bound catechol-O-methyltransferase (MBCOMT) is an integral ubiquitous enzyme that regulates the inactivation of catechol neurotransmitters. Dysregulation of this pathway has been strongly linked to Parkinson's disease and other neurodegenerative disorders, positioning MBCOMT as a promising therapeutic target. However, the intrinsic instability and low solubility of membrane proteins present major challenges for recombinant production and purification [1].

Methods: Based on the AlphaFold structural model of MBCOMT (AF-P21964-F1), a 30-amino-acid deletion was introduced in the N-terminal anchor region of the recombinant protein, targeting the amphipathic features that contribute to instability. Protein expression was biosynthesized in Escherichia coli BL21, induced with 0.5 mM IPTG for 6 h, and recovered by a glass bead lysis. To further enhance solubility and expression, a dual tag including Maltose-Binding Protein (MBP) and a 6×His sequence was fused to the target protein. The recombinant protein was purified using a dual affinity approach, exploiting the major affinities through immobilized metal affinity chromatography (IMAC) and MBP affinity chromatography (MBP-AC).

Results The N-terminal deletion combined with the dual MBP-His6 tag, resulted in the overexpression of MBCOMT with high levels of enzymatic activity. Initial purification using an IMAC FF crude column with Ni²n-charged resin, was able to elute the target protein at 200 mM imidazole; however, co-elution of homologous proteins, including endogenous MBP, was observed. To achieve higher purity, a second purification step was performed using either MBP-AC or an IMAC HP column. Only IMAC HP was able to recover a pure fraction of MBCOMT, eluted at 100 mM of imidazole.

Conclusion: Overall, the combination of structural modification and dual-tag strategy enabled the efficient production and purification of biologically active MBCOMT. The reported strategy provides a robust platform for structural and functional studies and supports further biomedical research into Parkinson's disease and related neurodegenerative disorders.

[1] Gonçalves, A.M.; Sousa, Â.; Pedro, A.Q.; Romão, M.J.; Queiroz, J.A.; Gallardo, E.; Passarinha, L.A. Advances in Membrane-Bound Catechol-O-Methyltransferase Stability Achieved Using a New Ionic Liquid-Based Storage Formulation. Int J Mol Sci 2022, 23, doi:10.3390/ijms23137264.

ID634 | Impact of High-Pressure Processing and Pressure-Assisted Thermal Processing (PATP) on Antioxidant Properties of Dark and Light Honey-Based Water Preparations (aw ≈ 0.85)

Author: Hana Scepankova

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal and CIMO, Mountain Research Center, Polytechnic Institute of Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal

E-mail: hanka.scepankova@gmail.com

Other authors: Vanessa Paula (2), Carlos Alberto Cruz Pinto (1), Leticia Estevinho (2), Jorge Manuel Alexandre Saraiva (1)

(1) LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal; (2) CIMO, Mountain Research Center, Polytechnic Institute of Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal

Non/lower-thermal technologies such as high-pressure processing (HPP) and pressure-assisted thermal processing (PATP) have attracted attention as alternatives that can enhance microbial safety. Our previous study (Scepankova, Pinto, Estevinho, & Saraiva, 2022) demonstrated that PATP at 600 MPa/75 °C for 15 min reduced spores of Bacillus subtilis in honey-water preparations with adjusted water activity (aw ≈ 0.85) below the detection limit (1.30 CFU/g), confirming its potential for microbial safety. Building on this work, honey-based water preparations (HWP) of dark and light multifloral honey, adjusted to the same water activity (aw ≈ 0.85), were developed as model systems to investigate the stability of antioxidant properties under HPP (600 MPa/15 min, at room temperature) and PATP (600 MPa/15 min/75 °C).

Dark HWP showed high initial antioxidant values (FRAP = 660.1 ± 31.7 µmol TE/100 g; TPC = 100.2 ± 3.9 mg GAE/100 g; DPPH = 19.85 ± 1.08%), which remained unchanged after both HPP and PATP. Light-colored HWP displayed lower initial values (FRAP = 283.4 ± 35.0 µmol TE/100 g; TPC = 59.99 ± 0.03 mg GAE/100 g; DPPH = 11.5 ± 3.8%). HPP largely preserved FRAP and TPC, whereas PATP caused a significant FRAP decrease (221.1 ± 5.2 µmol TE/100 g) and a reduction in TPC (52.08 ± 3.33 mg GAE/100 g). No significant changes were observed in DPPH.

Overall, dark HWP (aw ≈ 0.85) proved more resilient to both HPP and PATP, retaining phenolics and antioxidant activity, while light-colored HWP was more susceptible to degradation, particularly under PATP. These results highlight the importance of the botanical origin of honey when applying processing technologies and support HPP as a promising technique for preserving antioxidant properties in honey-derived liquid formulations, whereas PATP, despite its relevance for microbial safety, may accelerate degradative changes, particularly in honey with lower phenolic content.

Scepankova, H., Pinto, C. A., Estevinho, M., & Saraiva, J. A. (2022). High-Pressure-Based Strategies for the Inactivation of Bacillus subtilis Endospores in Honey. Molecules, 27, 5918. https://doi.org/https://doi. org/10.3390/ molecules27185918

ID648 | Cats Naturally Infected with SARS-CoV-2 Generate Potent Broadly Neutralizing Antibodies against Viral Variants

Author: Isa Moutinho

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).

E-mail: isasoraiia_@hotmail.com

Other authors: Other authors: Mafalda Henriques1,2, Sara Cardoso1,2, Teresa da Penha Coutinho1,2, Carlos Penha-Gonçalves3, Jocelyne Demengeot3, Miguel Castanho4, João Gonçalves5, Luís Tavares1,2, Solange Gil1,2, Telmo Nunes1,2, Frederico Aires-da-Silva1,2

3 Instituto Gulbenkian de Ciência, Oeiras, Portugal. 4 Instituto de Medicina Molecular-João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal 5 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Background: The COVID-19 pandemic has highlighted the need for novel sources of neutralizing antibodies against SARS-CoV-2 and emerging variants. Domestic cats live in close contact with humans, are naturally susceptible to infection, and typically remain asymptomatic with rapid recovery, suggesting an efficient immune response. These features position cats as a model to study natural antibody responses against SARS-CoV-2. However, the prevalence of infection in cats and their antibody repertoire remains insufficiently characterized. This study assessed SARS-CoV-2 seroprevalence in cats from Lisbon, compared antibody prevalence between animals of unknown exposure and those from COVID-19 positive households, and evaluated cat antibodies' neutralization potency against SARS-CoV-2 variants of concern. Methods: Two populations were analyzed: 733 cats with unknown exposure status and 76 cats from COVID -19-positive households. Serum samples were screened by ELISA for IgG against the receptor-binding domain (RBD) of SARS-CoV-2. ELISA-positive sera were tested for neutralizing antibodies by surrogate virus neutralization tests (sVNT) and pseudotyped virus assays for Wuhan-Hu-1, Alpha, Delta, and Omicron variants. PBMCs from cats with promising antibody responses were used to construct a cat-derived single-domain antibody (sdAb) library. High-affinity binders to SARS-CoV-2 RBD were selected by phage display. Results: Among cats with unknown exposure, 14.7% were seropositive, with 20.4% showing neutralizing antibodies. In cats from COVID-19-positive households, seroprevalence was higher (30.3%), with 69.6% showing strong neutralization. Antibodies demonstrated broad cross-recognition and neutralization of multiple variants. Some cats neutralized variants not yet circulating in humans at sampling time, underscoring their broad antibody response. Candidates selected by phage display with high affinity to RBD are under characterization to determine neutralizing potency, binding properties, stability and expression yields.

Conclusions: Cats naturally infected with SARS-CoV-2 develop potent, broadly neutralizing antibody responses, including against variants of concern and even those not yet circulating in humans at the time of sampling. These findings position cats as a valuable model and highlight their antibody repertoire as a promising resource for antibody discovery, engineering, and therapeutic development against SARS-CoV-2 and future viral threats.

ID659 | From egg to an antimicrobial agent: Avian Immunoglobulin Y (IgY) antibodies to address the Antimicrobial Resistance burden

Author: Catarina Almeida

1 CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal Institution; 2 RYA Purification Technologies, 3830-352 Ílhavo, Portugal

E-mail: ac.almeida@ua.pt

Other authors: Márcia C. Neves 1,2; João A. P. Coutinho 1,2; Mara G. Freire 1,2

1 CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal Institution; 2 RYA Purification Technologies, 3830-352 Ílhavo, Portugal

Background: Antimicrobial Resistance (AMR) stands out as one of the utmost global health threats, with Methicillin-Resistant Staphylococcus aureus (MRSA) remaining one of the leading causes of severe and hard-to-treat infections. The antibiotic's efficacy loss reinforces the need for novel therapeutics, such as biopharmaceuticals. Avian Immunoglobulin Y (IgY) antibodies have emerged as a promising class of therapeutic biomolecules due to their polyclonal nature, which enables them to recognize several epitopes on an antigen, diminishing the risk of resistance. In this work, the potential of IgY specifically directed to Penicillin-Binding Protein 2a (PBP2a) as biopharmaceuticals for infections caused by MRSA was addressed.

Methods: These antibodies were isolated from the egg yolk of hyperimmunized eggs by a 2-step selective precipitation with a biopolymer (PCT/IB2019/057993). The obtained anti-PBP2a IgY-lyophilized or not-were formulated with or without conventional protein stabilizers under different conditions. The storage performance was monitored by assessing the protein's aggregation, structural integrity, and antigen-binding capacity with Size Exclusion-High Performance Liquid Chromatography (SE-HPLC), Circular Dichroism (CD), and immunoassays, such as dot-blot and Enzyme-Linked Immunosorbent (ELISA), respectively. The in vitro and in vivo toxicities were also studied using human colorectal adenocarcinoma cell lines and rodent models, respectively.

Results: The selected stabilizers reduced the IgY aggregation, while their structural integrity and PBP2a recognition were preserved over storage. These antibodies posed no cytotoxicity and no toxic effects on the animal's diet, motor performance, and renal, hepatic, and cardiac functions, apoptosis, as well as did not induce pro- and acute-inflammatory responses.

Conclusions: This evidence highlights IgY as an effective, stable, and safe potential biopharmaceutical to address the AMR burdens.

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ID660 | Novel excipients based on analogues of glycine-betaine deep eutectic solvents for RNA stabilization

Author: Ana F. Pereira

1 CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal

E-mail: anafilipa98@ua.pt

Other authors: Ana S. C. Marques1, Augusto Q. Pedro1, Ana P. M. Tavares1, Céline Guillermain2, Aminou Mohamadou2, Fani Sousa3, Mara G. Freire1

1 CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal; 2 University of Reims Champagne-Ardenne, Institute of Molecular Chemistry of Reims (ICMR- UMR7312), CNRS, Reims, France; 3 CICS-UBI – Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Covilhã, Portugal

It is increasingly evident the impact that RNA has in the biopharmaceutical field on the treatment and prophylaxis of a range of hard-to-treat diseases, either with the use of RNA as the therapeutic agent or the target itself. However, the clinical progress of RNA-based therapeutics is still hindered due to some challenges persisting in the RNA bioprocessing technologies. One of the main bottlenecks is the high propensity of this biomolecule to degradation due to its labile nature, making it difficult to ensure long-term storage.

Deep eutectic solvents (DES), an emerging class of “designer solvents”, are starting to gain traction as efficient excipients of biomolecules and may be composed of natural and low-toxic compounds, thus offering innovative and potentially more biocompatible approaches in this field. In this context, this study aims to investigate the potential of several biobased DES to act as excipients for the stabilization of RNA, even when exposed to stress conditions, such as in the presence of ribonucleases. Several analogues of glycine-betaine-based DES were selected for this purpose, due to the biocompatible and natural origin of glycine-betaine, and its relatively low cost and high availability. Moreover, these components have already been used in health-related formulations.

The structural integrity and stability of RNA in the selected DES was assessed by agarose gel electrophoresis, circular dichroism (CD) spectroscopic measurements and UV/Vis spectroscopy. Overall, this work highlights the potential of DES as biocompatible solvents to act as excipients for the preservation of RNA biopharmaceuticals.

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ID674 | Exploring the production of cell-derived nanovesicles with Neprilysin activity

Author: Sandra N Pinto

1 iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal. 2 Bioengineering Department, Instituto Superior Técnico, Lisbon, Portugal.

E-mail: sandrapinto@ist.utl.pt

Other authors: Tiago P. Dias1, Beatriz Lebre1, Joana Machado1, Bárbara Almeida1, Nuno Bernardes1,2, Fábio Fernandes1,2

1 iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Lisbon, Portugal. 2 Bioengineering Department, Instituto Superior Técnico, Lisbon, Portugal.

Background: Currently available therapeutic options in Alzheimer's disease (AD) are targeted toward reduction of amyloid beta (Aβ) peptide aggregation. The main obstacles to the development of effective therapies in AD and other neurological disorders are the limitations of most therapeutics to overcome the blood brain barrier (BBB). Neprilysin (NEP) is capable of degrading amyloid beta peptides, preventing oligomerization and plaque formation. Extracellular vesicles (EVs) have an inherent ability to overcome the BBB and can be used as a vehicle to deliver proteins to the brain.

Methods: Extracellular vesicles (EVs) from HEK293 were isolated using two different methods: size-exclusion chromatography (SEC) and polymer-based precipitation (PBP). The NEP-loaded EVs (NEP-EVs) were characterized for size, protein content, and proteolytic activity. Their ability to degrade a control peptide and inhibit Aβ fibrillation/oligomerization was assessed. In addition, we also explored nanovesicles produced directly from the plasma membrane of human-derived cell lines (NEP-nEBs) as putative EV-mimics with greatly increase extraction yields.

Results: The method of purification significantly impacted the function of NEP-EVs. Purification via PBP yielded samples with high levels of soluble NEP (sNEP), which were effective at inhibiting Aβ fibrillation. In contrast, NEP-EVs purified by SEC lacked sNEP and showed no ability to inhibit Aβ fibrillation. All EV samples, however, exhibited significant NEP activity by effectively degrading control peptides. Interestingly, while NEP-nEBs displayed higher overall proteolytic activity than the NEP-EVs against a control peptide, they accelerated rather than inhibited Aβ fibrillation, mirroring the effect seen with synthetic liposomes mirroring plasma membrane composition.

Conclusions: The NEP levels achieved through overexpression in HEK293-derived EVs were insufficient to significantly impair Aβ aggregation and fibrillation. While nanovesicles obtained directly from the HEK293 plasma membrane achieved higher NEP activity, their potential inhibitory effect on Aβ fibrillation was nullified by the positive contribution of the membrane surface itself to Aβ aggregation. This suggests that simply increasing NEP activity is not enough to efficiently negate Aβ fibrillation and that the characteristics of the delivery vehicle (in this case EVs or nEBs) is a critical factor.

ID679 | Where photosynthetic aquatic microorganisms meet thought: Pavlova gyrans RCC1553 and Synechocystis sp. LEGE07211 in neuroprotection

Author: Cláudia Coutinho

1 CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal 2 CIIMAR/CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

E-mail: pg53747@alunos.uminho.pt

Other authors: Pedro Geada 1 3, Mariana Reis 2, Rosário Martins 2 4

1 CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal 2 CIIMAR/CIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal 3 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal 4 E2S|P.PORTO - School of Health, Polytechnic Institute of Porto, Rua Dr. António Bernardino de Almeida, 400, 4200-072 Porto, Portugal

Background: Neurodegenerative diseases (NDs), such as Alzheimer, Parkinson, and Huntington, are severe disabling conditions defined by the progressive loss of nerve cells. Current treatments are often associated with uncomfortable side effects, limited long-term efficacy, and the absence of true disease-modifying agents that can halt or reverse progression. Microalgae and cyanobacteria comprise new sources of discovery of potential new treatments.

Methods: Pavlova gyrans RCC 1553 and Synechocystis sp. LEGE 07211 extracts were screened to assess neuroprotective activity. Organic (acetone, ethanol, methanol) and aqueous extracts were tested across cell lines (3T3-L1, Raw 264.7, SH-SY5Y, and hCMEC/D3) for cytotoxicity (MTT assay), antioxidant activity (DPPH assay), anti-inflammatory activity (Griess assay in LPS-induced macrophages), and acetylcholinesterase (AChE) and n-secretase inhibition. Untargeted metabolomics (LC-HR-MS/MS) was employed for metabolite annotation using GNPS, SIRIUS and MetaboAnalyst.

Results: All the extracts were non-cytotoxic across the panel of cells. The highest antioxidant activity was found in the aqueous extract of Synechocystis sp. (82,61 ± 4,10) %, followed by its methanolic extract, (63,97 ± 2,63) % scavenging activity, both at 0,56 mg/mL. The methanolic extract of P. gyrans exhibited a (48,72 ± 0,95) % scavenging activity. Organic extracts showed anti-inflammatory activity by restoring nitric oxide levels to the baseline. The strongest AChE inhibition was observed in acetone extracts at 6,25 µg/mL, with Synechocystis sp. showing (70,68 ± 2,15) % inhibition and P. gyrans (64,60 ± 1,47) %. The metabolomics analysis revealed fatty acids as the predominant metabolite class in active extracts (46,67%), followed by amino acids/peptides and terpenoids (20 % each).

Conclusions: Extracts from P. gyrans RCC 1553 and Synechocystis sp. LEGE 07211 exhibited antioxidant, anti-inflammatory, and AChE inhibitory activities without cytotoxic effects. Untargeted metabolomics analysis mainly identified fatty acids, as possible bioactive metabolites. These findings demonstrate the neuroprotective potential of aquatic photosynthetic microorganisms against NDs.

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ID700 | DoctorVida® pocket: the world's smallest real-time isothermal PCR for truly portable molecular testing

Author: Gonçalo Doria

1STAB VIDA Lda, Portugal

E-mail: goncalo.doria@stabvida.com

Other authors: Eduardo Coelho1, Carla Clemente1, Júlia Heisel1, Rafael Pestana1, Mariana Conceição1, Ana Calheta1, Paulo Fernando1, Madalena Sequeira1, Orfeu Flores1

1STAB VIDA Lda, Portugal

The growing demand for rapid, reliable, and decentralized molecular diagnostics has driven innovation toward compact, user-friendly systems capable of delivering laboratory-grade results at the point of need. DoctorVida® pocket, developed by STAB VIDA Lda, is at the forefront of this transformation.

As the world's smallest real-time isothermal PCR device, DoctorVida® pocket unites temperature control, real-time fluorescence detection and cloud-based analysis in a single handheld unit. Despite its compact size, it performs isothermal amplification with high sensitivity and reproducibility, producing results within 20~60 minutes. Its free mobile app (iOS/Android) provides intuitive control, real-time visualization and secure result sharing, bringing molecular testing to both professionals and non-specialists.

Initially conceived and validated during the COVID-19 pandemic, DoctorVida® pocket demonstrated reliable SARS-CoV-2 detection outside traditional laboratory settings[1]. Building on that success, STAB VIDA expanded its use to nutrigenomics with lactose intolerance genotyping[2], precision oncology with EGFR mutation analysis[3], and avian sexing. Beyond healthcare and veterinary uses, independent groups have further been using the platform for food authentication[4,5] and pathogens detection in agriculture, confirming its versatility across biological, environmental and industrial settings. Ongoing developments also include: Legionella testing in water, Salmonella detection in primary production samples, and sexually transmitted infections (STI), further extending its reach into environmental, food safety and clinical screening applications.

With its open, assay-agnostic architecture, true portability and low cost, DoctorVida® pocket sets a new benchmark for decentralized molecular testing. Here we will showcase its concept, applications, and future roadmap - a technology bridging laboratory accuracy with field convenience, empowering users to perform diagnostics anywhere, anytime.

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ABSTRACTS

TOPIC #4: Health Microbiology

ORAL COMMUNICATIONS

ID207 | Bacteriocin-mediated prevention of secondary pneumococcal pneumonia by a human commensal Streptococcus mitis strain

Author: Catarina Candeias

1Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal

E-mail: candeias@itqb.unl.pt

Other authors: João Lança 1, Marta Alenquer 2, Daniela Brás 2, Carina Valente 1,3, Maria João Amorim 2, Raquel Sá-Leão 1

1 Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal; 2 Cell Biology of Virus Infection Laboratory, Católica Biomedical Research Centre (CBR), Católica Medical School, Universidade Católica Portuguesa, Oeiras, Portugal; 3 Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal

Background: Streptococcus pneumoniae remains a major public health threat despite widespread use of vaccines and antibiotics. Its well-established synergy with influenza A virus (IAV) often results in secondary bacterial pneumonia, a condition associated with high morbidity and mortality. As pneumococcal pneumonia is invariably preceded by nasopharyngeal colonization, preventing colonization represents a promising intervention strategy. Current approaches - including pneumococcal conjugate vaccines and antibiotics - can drive serotype replacement and antimicrobial resistance, underscoring the need for novel, targeted, and serotype-independent alternatives.

Methods: We recently described seven human-derived commensal streptococcal strains (S. oralis A22 and S. mitis B22-G22) which potently inhibited pneumococcal growth and biofilm formation in vitro. In the current study, we used a mouse model to assess the in vivo efficacy of one candidate, S. mitis strain F22, in preventing S. pneumoniae colonization and secondary pneumococcal pneumonia following IAV infection. The role of bacteriocins in mediating this protection was also evaluated.

Results: Colonization with the commensal S. mitis strain F22 significantly reduced pneumococcal density in the nasopharynx by day 10 post-infection and prevented pneumococcal dissemination to the lungs following IAV co-infection. This protective effect was dependent on bacteriocins encoded at the blp1 locus. Among these, Bac2v1 - alone or in combination with Bac1 - significantly reduced pneumococcal colonization. Notably, Bac2v1 exerted serotype-independent inhibitory activity, impairing the growth of diverse pneumococcal strains.

Conclusions: Our findings provide the first in vivo proof-of-concept that commensal streptococci, and specifically their bacteriocins, can disrupt S. pneumoniae colonization and prevent progression to secondary bacterial pneumonia. This work introduces a previously unrecognized precision-based approach to pneumococcal disease prevention, offering a potential complement to current strategies.

ID252 | Marine-Derived Antimicrobial Peptides: A Novel Source of Molecules against Antimicrobial Resistance

Author: Ana Rita Bragança

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 3 Aquatic Research Network (ARNET) Associate Laboratory

E-mail: anaritabrag@hotmail.com

Other authors: Rui Silva 1,2,3, Margarida Casal 1,2,3, Isabel Soares-Silva 1,2,3, Raul Machado 1,2,3

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 3 Aquatic Research Network (ARNET) Associate Laboratory

Antimicrobial resistance is one of the most pressing global public health challenges, compromising the effectiveness of antibiotics and complicating the treatment of bacterial infections. The continuous emergence of multidrug-resistant strains has led to severe clinical complications, including increased morbidity and mortality. In this context, the discovery of novel antimicrobial compounds has become imperative, offering potential alternatives to currently available antibiotics. The vast and largely unexplored microbial diversity of the marine environment represents a rich source for discovering novel antimicrobial molecules. Among these, antimicrobial peptides (AMPs) produced by marine microorganisms are particularly promising drug candidates due to their broad-spectrum activity and stability under physiological salt conditions.

Our group established a biobank of marine microorganisms, which was investigated for the production of antimicrobial compounds. Antimicrobial activity was assessed according to EUCAST/CLSI guidelines against several clinically relevant bacteria belonging to the ESKAPEE group (namely, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae). Of the 84 bacteria tested, 75 exhibited activity against at least one target species, with 5 showing activity against all. Of the 75 marine species, 14 were selected for further analysis. Growth kinetics were assessed over a 24-hour period to evaluate both microbial growth profiles and the production of antimicrobial compounds in the extracellular medium. Samples were collected at key growth phases: early exponential (6h), late exponential (12h), and stationary (24h). Among these, the late exponential phase (12 h) produced cell-free supernatants with the highest antimicrobial activity. Preliminary data from proteomic analyses suggest that the observed antimicrobial activity is likely mediated by AMPs, representing a promising alternative to conventional antibiotics. Bioinformatics analyses will be conducted to identify and characterise the AMPs by comparing amino acid and nucleotide sequences with existing databases, followed by structural prediction and molecular dynamics simulations to evaluate peptide–membrane interactions.

The identification of novel antimicrobial molecules from marine microorganisms holds the promise of providing innovative solutions against antibiotic resistance, serving as a catalyst for the emergence of a new generation of antibiotics.

ID308 | Bacterial Vaginosis: it's not just who's there, but what they do

Author: Nuno Cerca

1-LIBRO, CEB, University of Minho, Braga, Portugal

E-mail: nunocerca@ceb.uminho.pt

Other authors: Lúcia Sousa 1, Jacob H. Elnaggar 2, John Lammons 2, Christopher M. Taylor 2, Christina A. Muzny3

2-Department of Microbiology, Immunology, and Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA, USA: 3- Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, USA; 4- LABELLS, University of Minho, Braga, Portugal

Background: Bacterial vaginosis (BV) is the most prevalent vaginal infection in women of reproductive age and is characterized by a shift from a Lactobacillus-dominated microbiota to a diverse anaerobic polymicrobial community. Among the most frequently associated bacteria are Gardnerella vaginalis, Fannyhessea vaginae, and Prevotella bivia. Although these species have been implicated in BV biofilm development, the molecular mechanisms underlying their interactions and in vivo activity remain poorly understood. Previous in vitro studies revealed that G. vaginalis exhibits distinct gene expression profiles when grown alone or in triple-species biofilms with F. vaginae and P. bivia, particularly in a genital tract secretion-simulating medium. This study aimed to assess whether the genes identified in vitro are also expressed in vivo and whether their expression is associated with the presence or abundance of BV-associated bacteria in vaginal specimens.

Methods: Four G. vaginalis genes previously highlighted by RNA-seq analysis of in vitro triple-species biofilms were selected for quantification by qPCR in vaginal swab specimens. Vaginal specimens were collected longitudinally from women who developed incident BV (iBV and from healthy controls. RNA was extracted from each specimen and cDNA synthesis was followed by qPCR targeting the selected genes. In parallel, the microbial composition of each specimen was assessed by 16S rRNA sequencing.

Results: Microbiome analysis by 16S rRNA sequencing revealed a marked difference in community structure: BV-positive specimens harbored approximately 90 distinct genera or species, whereas samples from healthy controls contained a much lower diversity, typically around 10 taxa, and were dominated by Lactobacillus spp. Gene expression analysis showed that three of the four G. vaginalis target genes were only detected in a single negative control, suggesting their potential utility in guiding future advancements in molecular diagnostics for bacterial vaginosis.

Conclusions: This study highlights that BV cannot be explained solely by the presence or abundance of specific species, but rather by microbial interactions that modulate gene expression and pathogenic potential. The results support a shift from taxonomy-based diagnostics to approaches considering microbial activity and dynamics, with implications for improving both diagnosis and treatment of BV.

ID362 | Unravelling P. aeruginosa-S. aureus interactions and their intracellular survival using a new in vitro model

Author: Maria Olivia Pereira

1 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 LABBELS – Associate Laboratory, Braga/Guimarães,

E-mail: mopereira@deb.uminho.pt

Other authors: Filipa Alexandra Cardoso Ribeiro; Ana Margarida Rodrigues de Sousa

1 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 LABBELS – Associate Laboratory, Braga/Guimarães,

Background: Cystic fibrosis (CF) is a recessive genetic disease, leading to the accumulation of thick mucus in the lungs and the development of chronic respiratory infections. Pseudomonas aeruginosa and Staphylococcus aureus are the main causative agents and significant contributors to high morbidity and mortality rate in CF due to their ability to form antibiotic resistant biofilms within mucus. This work is aimed at developing an in vitro model recapitulating the complexity of CF airway environment and polymicrobial biofilm physiology to understand how bacteria-bacteria interactions within biofilms favor antibiotic resistance and infection persistence and interplay with host cells response

Methods: The in vitro model was composed by A549 epithelial cells seeded and covered by CF artificial mucus. Different clinical CF isolates of P. aeruginosa and S. aureus were added to mucus composing three distinct polymicrobial consortia. Suspended biofilms were allowed to form for 6 h with or without antibiotic treatments. Further, bacterial growth, adhesion and internalization was determined. Cellular viability and morphology and production of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) were analyzed.

Results: Regardless the presence of antibiotics, bacterial growth occurred predominantly in polymicrobial biofilms suspended within mucus. Interestingly, in co-infection, P. aeruginosa protected S. aureus from ciprofloxacin and vancomycin action, highlighting the role of interspecies interactions in antibiotic tolerance. During the 6 h-infection, a small fraction of both species internalized into A549 cells without compromising epithelial viability and morphology. However, prolonged co-culturing showed that internalized bacteria likely induced host cell death and disseminated outwards since increased bacterial load was recorded in suspension. Moreover, robust activation of IL-8 was detected likely induced by lipopolysaccharide of P. aeruginosa, leading neutrophil recruitment and persistent inflammation.

Conclusions: This simple in vitro model successfully mimicked key features of CF infections, revealing how interspecies interactions were established in favor of antimicrobial tolerance. Moreover, it revealed that bacterial internalization in epithelial cells sustained infection chronicity and modulated host inflammation.

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ID368 | Atopic dermatitis patients in Portugal are reservoirs and key-drivers of ST398-MSSA evolution

Author: Diana Caieiro

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
E-mail: dcaieiro@itqb.unl.pt

Other authors: Nuno Alexandre Faria1, Mariana Araújo1, Ana Botelho1, Leonor Ramos2, Margarida Gonçalo2, Maria Miragaia1

1 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras Portugal; 2 Centro Hospitalar e Universitário de Coimbra, Coimbra Portugal

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects up to 3.5% of the Portuguese population. Staphylococcus aureus is recognised as a major trigger for disease severity. Despite S. aureus role in AD, the genetic lineages associated with AD in Portugal have never been characterised. We aimed to characterise the genetic background of S. aureus collected from AD patients in Portugal and enrich the global understanding of its molecular epidemiology and evolution in AD; Swabs were collected from the anterior nares, AD-lesion and non-lesion skin of 65 patients in 2024-2025 in a Portuguese hospital. S. aureus were isolated in selective media and species confirmed by mannitol fermentation and coagulase production. Isolates were characterised by spa typing, from where the sequence type (ST) was inferred; and by Immune Evasion Cluster (IEC) typing. The whole genome of 19 S. aureus isolates from the most prevalent ST were sequenced by Illumina. Genomes were compared by single nucleotide polymorphisms analysis (SNPs) and their content in antibiotic resistance and virulence genes were determined using bioinformatic tools. Pathogenwatch database was used for global perspective interpretation; S. aureus was detected in 83% of patients (54/65). Thirty-six spa types belonging to 12 STs were found among 106 isolates. The most prevalent clonal type was ST398-MSSA (44%, 46/106), most carrying IEC type C (91%, 42/46). Phylogenetic analysis showed that ST398 isolates from different sites within the same patient were more closely related to each other (2-46 SNPs) than to isolates from other patients (162-982 SNPs). Intra-patient isolates varied in antibiotic resistance (blaZ) and virulence (clfA, cna) genes, with IS21 mutations in 4/6 patients; inter-patient isolates differed in antibiotic resistance (ermT, AAC(6')-Ie-APH(2'')-Ia) and virulence (clfA, cna, sdrC, esaG, sak). When compared with 471 ST398 isolates from different countries and sources, most AD isolates from Portugal (84%, 16/19) were grouped into a distinctive cluster; AD patients are a reservoir of ST398-MSSA, a prevalent community lineage in Portugal emerging as a cause of severe hospital infections. The clustering of AD-associated S. aureus from Portugal suggests regional and/or disease driven clonal evolution. High within-patient S. aureus genetic similarity supports an endogenous source for lesion colonisation, while site-specific genetic changes indicate adaptation to distinct niches.

ID383 | hijacking host immunity: c4bp recruitment by moonlighting retropepsin-type proteases in rickettsiales

Author: Ana Luísa Matos

1 PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research, University of Coimbra, 3004-504 Coimbra, Portugal; 2 Institute for Interdisciplinary Research, University of Coimbra, 3004-504 Coimbra, Portugal; 3 CNC-UC - Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal; 4 CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra

E-mail: almatos@cnc.uc.pt

Other authors: tian luo 5, frida mohlin 6, elisabete ferreira 7, anna m. blom 6, jere w. mcbride 5,8,9,10,11, isaura simões 3,4,12

5 Department of Pathology, University of Texas Medical Branch, Galveston, 77555 Texas, USA; 6 Division of Medical Protein Chemistry, Department of Translational Medicine, Lund University, 214 28 Malmö, Sweden; 7 UCIBIO, Applied Molecular Biosciences Unit, NOVA School of Science and Technology, 2829-516 Caparica, Portugal; 8 Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, 77555 Texas, USA; 9 Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, 77555 Texas, USA; 10 Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, 77555 Texas, USA; 11 Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, 77555 Texas, USA; 12 Department of Biological Sciences, Old Dominion University, Norfolk, 23529 Virginia, USA

Background: Rickettsiales comprises a diverse group of obligate intracellular bacteria transmitted by arthropod vectors that cause mild to fatal diseases. Rickettsioses are gaining global momentum due to their resurgence patterns, lack of protective vaccines, and response to few antibiotics. Bacterial proteases are crucial players in pathogenesis by targeting and degrading host factors. Our lab described the highly conserved retropepsin-type protease named Aspartic protease from Rickettsia conorii – APRc. APRc interacts with immunoglobulins, mediates serum resistance, and targets additional human serum components acting as an evasin. Herein, we identified an APRc-homologue in Ehrlichia chaffeensis (APEhc) and demonstrated that APEhc and APRc are novel C4BP ligands.

Methods: APRc and APEhc interaction with C4BP was evaluated by pull-down assays. APRc- and APEhc-C4BP interaction domains were mapped by MST. C4BP cofactor activity was assessed in the presence of APRc/APEhc and purified C3b or C4b by WB. Proteases' impact on complement activation was assessed by measuring C5b-9 complex formation using a modified ELISA. C4BP binding at the surface of Ehrlichia was shown by whole-cell ELISA.

Results: We show that APEhc is highly conserved across Ehrlichia species and targets multiple human serum components. Both APEhc and APRc bind to C4BP, retaining its cofactor activity and enabling the cleavage of complement C3b and C4b. E. chaffeensis recruits and binds C4BP at its surface in a concentration-dependent manner, and C4BP maintains its regulatory cofactor activity when bound to the bacteria. Both proteases significantly inhibit the activation of all complement pathways. This complement-inhibitory activity appears to occur independently of APRc's catalytic function, whereas APEhc's catalytic activity enhances it.

Conclusions: We identified a novel retropepsin-type protease in E. chafeensis that targets human serum proteins and modulates the complement system. We confirmed that APEhc and APRc interact with the complement inhibitor C4BP and maintain its functionality. Our findings uncover a multifunctional role for APEhc and APRc in promoting evasion from complement-mediated killing and support that such strategies may be shared among bacterial retropepsin-type homologs, highlighting promising avenues for future research and therapeutics.

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ID414 | The Dangerous Duo: Resistance and Virulence in Bacteria

Author: Célia Domingues

CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal; INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal

E-mail: celiapfd@hotmail.com

Other authors: João S. Rebelo 1, Helena Darmancier 2, Francisco Dionisio 1, Teresa Nogueira 1,2

1 CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal; 2 INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal

Background: Antimicrobial resistance and bacterial virulence pose significant challenges to public health. The coexistence of resistance and virulence genes may increase bacterial pathogenicity, but their genomic relationships remain unclear. Moreover, a single bacterial genome may harbor multiple families of resistance genes, leading to multidrug resistance and further increasing the public health risk. Understanding whether these traits co-occur may help to anticipate the emergence of highly adaptive and pathogenic strains.

Methods: We performed a comprehensive bioinformatic analysis of publicly available bacterial genomes and plasmids. Resistance genes were identified using ResFinder, and virulence-associated genes using VFDB. Correlations were analysed both among resistance genes and between resistance and virulence determinants.

Results: Resistance and virulence genes were widespread across bacterial genomes. Strong correlations were observed among resistance genes, suggesting potential co-selection, while correlations between resistance and virulence genes varied across genomes. These correlations were stronger in plasmids, which is particularly concerning given their ubiquity and high mobility across bacteria, thereby enhancing the potential dissemination of both resistance and virulence traits.

Conclusions: This study highlights the potential for multidrug-resistant strains to emerge with enhanced survival or pathogenic traits. Such patterns are particularly relevant for pathogens of critical public health importance, including foodborne bacteria such as Listeria monocytogenes. Genome-wide analyses of resistance and virulence gene associations provide valuable insights for surveillance, risk assessment, and strategies to limit the spread of multidrug-resistant pathogens.

ID443 | Identifying and characterizing enterococcal persistence in veterinary and human settings

Author: Catarina Geraldes

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), Faculty of Veterinary Medicine, University of Lisbon, Portugal

E-mail: cgeraldes@fmv.ulisboa.pt

Other authors: Carolina Silva 1,2†, Filipa Vale 3, Miguel Ramos 3, Micaela Oliveira 3, Mónica Nunes 3, Ricardo Dias 3,4, Eva Cunha 1,2, Luís Tavares 1,2, Joana Guerreiro 1,2, 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), Faculty of Veterinary Medicine, University of Lisbon, Portugal; 3 BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; 4 cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Portugal; † Both authors equally contributed to the presented work.

Background: Although broadly studied, bacterial persistence still has many unsettled queries, especially regarding specific bacteria. Enterococcus, known for their genomic versatility and intrinsic and extrinsic resistances, have been related with infections promoted by persistent strains, which should be further characterized.

Methods: A total of 40 Enterococcus spp. were used in this study, including 10 strains each from: veterinary hospital surfaces; veterinary clinical samples; veterinary commensal samples; and human clinical samples. First, the minimal inhibitory concentration (MIC) of ciprofloxacin (CIP) was determined according to CLSI guidelines. Growth kinetics were also studied by measuring the optical density at 600 nm for 24h, at 37oC. Then, persistence of all isolates was induced through contact with 10 times the respective MIC of CIP for 48 hours, at 37oC, while bacterial concentration was estimated at 0, 24 and 48h to ascertain the formation of a biphasic killing curve. Presumed persisters were characterized by whole-genome sequencing (WGS) using Oxford Nanopore Technologies, and by transcriptomic analysis through RNA sequencing (RNA-seq) using the MGI-G99 platform. Their growth curve was also constructed to check for an elongated lag phase, associated with cell recuperation. Recuperated cells were submitted to susceptibility testing, to affirm that survival was due to persistence and not to acquisition of resistance determinants.

Results: The average MIC value for CIP was 162 µg/mL, with veterinary clinical isolates presenting the highest MIC values (311 µg/mL) and commensal isolates the lowest (2 µg/mL), which reflects the usage of fluoroquinolones in veterinary medicine. For all isolates, persistence was successfully induced only for 1 human isolate. WGS results revealed no mutations, while transcriptomic results indicated a possible involvement of stress response pathways, which may underlie adaptive mechanisms associated with bacterial persistence.

Conclusions: The results from this pilot study contribute for understanding and characterizing enterococcal persistence with the purpose of finding targets for prevention and treatment of these infections.

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ID449 | Human and retail meat Campylobacter isolates: emerging antimicrobial resistance, cluster dynamics and source attribution

Author: Mónica Azevedo

1 National Reference Laboratory of Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisboa, Portugal; 2 Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisboa, Portugal

E-mail: monica.azevedo@insa.min-saude.pt

Other authors: Maria-Leonor Lemos 1, 3; Matilde Cordas 2; Inês Cruz 2, 4; Sónia Ramos 4, 5; Isabel Santos 4, 5; Alexandra Nunes 2, 4, 5; Mónica Oleastro 1

3 ICBAS-Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal; 4 Research in Veterinary Medicine (I-MVET), Faculty of Veterinary Medicine, Lusófona University, University Center of Lisbon, Lisboa, Portugal; 5 Veterinary and Animal Research Center (CECAV), Faculty of Veterinary Medicine, Lusófona University, University Center of Lisbon, Lisboa, Portugal

Background: Campylobacter surveillance is hindered by antimicrobial resistance (AMR) and limited outbreak detection. Rising resistance to key antibiotics threatens treatment effectiveness, while outbreaks remain hard to trace due to sporadic cases, underdiagnosis, and low resolution of conventional typing. Integrating genomic tools and One Health strategies is crucial to enhance AMR monitoring, source attribution, and timely public health action. Through the implementation of WGS-based surveillance, we aimed to identify predominant sequence types (STs) and significant AMR markers, clarify genomic clusters, and assess poultry meat's role in clinical settings.

Methods: C. jejuni and C. coli genomes of phenotypically characterized isolates from human (N = ~1800; Portuguese National Surveillance Program) and retail poultry meat samples (N = 136), were de novo assembled with INNUca v4.2.2. Genetic relatedness was assessed through a gene-by-gene approach, and clusters identified with ReporTree v2.5.4. In silico MLST, AMR detection (ResFinder, CARD, AMRFinderPlus), and porA typing (PubMLST) were performed.

Results: In human and poultry samples, C. coli ST-10042 and C. jejuni ST-10846 were predominant. Genomic clustering revealed potential outbreaks, with poultry isolates clustering closely with clinical isolates. Known AMR determinants were confirmed, including gyrA T86I (Ciprofloxacin), tet(O)/tet(O/32/O) (Tetracycline), blaOXA-61 promoter G57T (Ampicillin), aph(2")-Ih, aph(3')-III (Gentamicin), and 23S rRNA A2075G (Erythromycin). Importantly, the emergence of apmA (Gentamicin) and ermN (Erythromycin), carried by mobile genetic elements, suggests a high potential for horizontal transfer. Increasing resistance to second-line Amoxicillin-clavulanic acid and decreased susceptibility to Ertapenem were also observed and newly associated with blaOXA-61 variants and specific porA alleles.

Conclusions: WGS-based surveillance enabled detection of previously undetected clusters, significant AMR markers, and characterization of novel β-lactam resistance mechanisms. The genomic overlap between poultry and clinical isolates underscores poultry as a major source of Campylobacter in Portugal and highlights the need for integrated One Health surveillance.

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ID452 | Phylogenomic Discordance and Niche-Linked Structure in Candida albicans

Author: Mariana Fernandes

RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; Faculty of Health Sciences, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal

E-mail: marianamzf@hotmail.com

Other authors: Emanuel Maldonado1,2, José Martinez-de-Oliveira1,2, Ana Palmeira-de-Oliveira1,2,3, Joana Rolo1,2

1 RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 2 Faculty of Health Sciences, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 3 Labfit–Health Products Research and Development Lda, UBImedical, Estrada Nacional 506, Covilhã, Portugal

Background: Candida albicans is a leading yeast pathogen and a frequent asymptomatic colonizer of multiple niches. Metabolic plasticity, in which mitochondrial function plays a key role, is directly associated with its ability to adapt and express various virulence factors. However, the evolutionary dynamics and possible lineage-specific niche specialization remain poorly characterized, as do the evolutionary patterns of mitochondrial genes.

Methods: We assembled mitochondrial and nuclear genomes of 591 C. albicans isolates from different anatomical sites and geographical origins, using their publicly available sequencing data. Two phylogenetic trees were estimated, one from 14 protein-coding mitochondrial genes and the other from 12 conserved nuclear genes. Population structure was determined by clustering, with niche associations statistically tested. Selective pressures were estimated using FEL, MEME, and BUSTED.

Results: Phylogenetic analyses revealed incongruent topologies, with discordant placements restricted to a subset of specific lineages, while most clusters remained stable across both trees. These inconsistencies suggest complex evolutionary trajectories, possibly involving recombination. Concurrently, clustering analysis established a significant association between phylogenetic groups and anatomical origin ($p < 0.001$), even after excluding known niche-associated groups (e.g., Candida africana). Selective pressure analysis across the mitochondrial genes revealed overall strong purifying selection and limited genetic diversity. However, COX1 showed signals of episodic diversifying selection ($p < 0.01$), suggesting possible functional adaptations in the respiratory chain of certain lineages.

Conclusions: The nuclear and mitochondrial phylogenetic discordances and the population structure associated with anatomical niche demonstrate the complex evolutionary dynamics of C. albicans. Our findings underscore the need to study this pathogen through integrated nuclear and mitochondrial data. Furthermore, the identification of episodic selection in COX1 raises specific new questions regarding the potential role of mitochondrial-driven metabolic adaptation in ecological fitness, virulence expression, and differential antifungal resistance across distinct fungal lineages.

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ID493 | Insert-Based Two-Layer Model Reproduces Spatial Coexistence of Staphylococcus aureus and Pseudomonas aeruginosa in Chronic Wounds

Author: Vânia Pinheiro

LEPABE, ALiCE, Faculty of Engineering of the University of Porto, Porto, Portugal

E-mail: up202308538@edu.fe.up.pt

Other authors: Anabela Borges1, Nuno Cerca2, Darla Goeres3

1 LEPABE, ALiCE, Faculty of Engineering of the University of Porto, Porto, Portugal; DEQB – Department of Chemical and Biological Engineering, Faculty of Engineering of the University of Porto, Porto, Portugal; 2 CEB – Centre of Biological Engineering, Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal; LABBELS – Associate Laboratory, Braga, Portugal; 4 CBE – Center for Biofilms Engineering, Montana State University, Bozeman, MT, USA

Background: Chronic wound infections, such as diabetic foot ulcers, are polymicrobial. Staphylococcus aureus and Pseudomonas aeruginosa commonly coexist in chronic wounds, with clinical samples showing P. aeruginosa populating the deeper wound layers. However, in vitro models often fail to reproduce this coexistence, as P. aeruginosa tends to outcompete the S. aureus. The aim of this study was to compare different in vitro conditions to better reproduce a polymicrobial chronic wound biofilm model, enabling the evaluation of the spatial organization of the two species.

Methods: Two-layer wound model was developed to mimic skin: a fat layer (Lubbock Chronic Wound Biofilm (LCWB) medium supplemented with agar and fat) and a dermis layer (LCWB medium supplemented with agar). The model was established in 6-well plates with and without inserts, that elevated the dermis and fat layers from the bottom to allow the nutrients to diffuse into the chronic wound from the bottom. Inoculation schemes tested included: (i) simultaneous inoculation of S. aureus and P. aeruginosa at equal densities (106 CFU/ml), (ii) simultaneous inoculation of S. aureus and P. aeruginosa at unequal densities (S. aureus 107 CFU/ml vs P. aeruginosa 105 CFU/ml), (iii) delayed inoculation of P. aeruginosa 24 h afterS. aureus at equal densities, and (iv) delayed inoculation of P. aeruginosa 24 h after S. aureus at unequal densities. Biofilms were maintained for 3 days with daily addition of fresh LCWB medium. Bacterial populations were quantified by CFU counts. Spatial organization and species distribution were visualized using fluorescence in situ hybridization (FISH) combined with confocal microscopy.

Results: Across all conditions, CFU counts and confocal imaging confirmed the persistence of both species over 3 days. S. aureus thrived when equal inoculum densities were used and P. aeruginosa inoculation was delayed by 24 h, better reflecting clinical chronic wounds. Spatial analysis showed adding P. aeruginosa 24 h later resulted in both bacteria present on the third day as individual species clusters.

Conclusions: This study demonstrates that modifying inoculation timing, inoculum ratios, and culture configurations can shift the balance of species within in vitro chronic wound models. The use of inserts combined with delayed P. aeruginosa inoculation enabled S. aureus to thrive and reproduced the spatial separation and coexistence of the two species observed in chronic wound infections.

ID499 | From old drugs to novel antifungal strategies: inhibiting efflux mechanisms in Candida albicans

Author: Liliana Rodrigues

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Rua da Junqueira 100, 1349-008, Lisboa, Portugal

E-mail: lrodrigues@ihmt.unl.pt

Other authors: Débora Marques, Sofia Santos Costa

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Rua da Junqueira 100, 1349-008, Lisboa, Portugal

Background: Candida albicans is a major cause of nosocomial fungal infections, associated with high morbidity and mortality. Resistance to antifungal drugs, particularly azoles, is on the rise, highlighting the urgent need for novel therapeutic strategies. Azole resistance can result from several mechanisms, including target modifications and overexpression of efflux pump genes. Efflux inhibitors can enhance intracellular drug accumulation and potentially reverse or prevent resistance. Drug repurposing provides a cost-effective and time-efficient strategy to identify new therapeutic agents, including efflux inhibitors.

Methods: An in silico repurposing approach using the DrugBank database was employed to identify approved drugs targeting C. albicans membrane transport proteins. A chemically diverse subset of compounds was selected for in vitro evaluation. Minimum inhibitory concentrations (MICs) against C. albicans ATCC 90028 were determined by microdilution. In addition, the potential adjuvant activity with fluconazole and ethidium bromide (EtBr), a common efflux pump substrate, was also assessed. Finally, efflux inhibition was further evaluated through real-time fluorometric measurement of EtBr accumulation.

Results: The in silico analysis identified 245 predicted C. albicans membrane transport targets associated with 607 approved drugs. A total of 59 representative drugs were selected for experimental screening. Miltefosine exhibited the lowest MIC (2 mg/L), while amlodipine and procainamide showed adjuvant activity, reducing the MIC of fluconazole by at least four-fold. Fluorometric accumulation assays revealed amlodipine, fluvoxamine and fluoxetine as potential efflux inhibitors in C. albicans.

Conclusions: The identification of repurposed drugs with adjuvant and efflux inhibitory activity supports their potential application in the development of novel therapeutic strategies aimed at enhancing antifungal efficacy and overcoming resistance in C. albicans.

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ID502 | Uncovering the Antifungal Potential of Bacteria Isolated from the Microbiota of Marine Microalgae

Author: Teresa Pissarro

Instituto de Tecnologia Química e Biológica António Xavier - ITQB NOVA, Oeiras, Portugal

E-mail: t.pissarro@itqb.unl.pt

Other authors: Francisco Xavier Nascimento 2, Rita Ventura 1 and Catarina Pimentel 1

Instituto de Biologia Experimental e Tecnológica - iBET, Oeiras, Portugal

Background: Invasive fungal infections are associated with high rates of mortality and morbidity among hospitalized patients. Advances in life-saving medical treatments, coupled with a limited repertoire of effective antifungals and the increasing resistance to current drugs, have worsened this scenario. There is a growing demand for new antifungal agents, a need recently highlighted by the World Health Organization (WHO). The marine environment represents an untapped source of valuable medicines and, in particular, the microbiota of marine organisms has attracted great interest because, if cultivable, it represents a sustainable and low-cost means of producing effective drugs.

Methods: A bacterial library isolated from the microbiome of the marine microalgae Phaeodactylum tricornutum and Nannochloropsis oceanica was screened for antifungal activity against Candida albicans using agar diffusion assays. Genomes of active bacterial strains were analyzed for the presence of relevant biosynthetic gene clusters (BGCs) and compound production by one strain was confirmed by HPLC-MS. Promising compounds were chemically synthetized and tested for antifungal activity against four fungi listed in the WHO critical priority group using standard broth-dilution assays, with cytotoxicity evaluated in fibroblasts.

Results: Among 39 bacteria with antifungal activity against C. albicans, the most active strain contained a BGC for pentabromopseudilin (PBP). PBP production was detected when the bacterium was cultured alone or in co-culture with C. albicans. Chemically synthetized PBP and its non-halogenated analog, pseudilin, were tested for antifungal activity. PBP inhibited the yeast form of C. albicans but not the filamentous form induced by temperature or serum. It also showed strong activity against Candida auris, Aspergillus fumigatus and Cryptococcus neoformans. Cytotoxicity testing revealed PBP toxicity at low concentrations, while pseudilin was non toxic.

Conclusions: Marine bacteria secrete compounds with antifungal potential such as PBP, which is highly active against clinically important fungi. To overcome its toxicity, we are developing PBP-inspired derivatives with similar potency but lower toxicity.

ID573 | First report of Candida auris candidemia in Portugal: Genomic characterisation and antifungal resistance-associated genes analysis

Author: Sofia Costa de Oliveira

Rise-Health, Department of Pathology, Faculty of Medicine of the University of Porto, Portugal

E-mail: sqco@med.up.pt

Other authors: Isabel M. Miranda1†, Micael F. M. Gonçalves2†, Dolores Pinheiro3, Sandra Hilário4, José Artur Paiva5, João Tiago Guimarães3, 6

1Rise-Health, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Portugal; 2Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Portugal; 3Department of Clinical Pathology, São João University Hospital, Porto, Portugal; 4GreenUPorto – Sustainable Agrifood Production Research Centre/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Portugal; 5Intensive Care Medicine Service, Local Health Unit Sao Joao; Faculty of Medicine, University of Porto, Portugal; 6Department of Biomedicine, Faculty of Medicine & EPIUnit, Institute of Public Health, University of Porto, Portugal

Background: Candida auris is a multidrug-resistant yeast associated with high mortality and rapid hospital transmission, and was classified as a critical priority pathogen by WHO in 2022. This study reports the first documented cases of C. auris candidemia in Portugal (2023) and provides genomic insights into antifungal resistance, including the identification of novel mutations.

Methods: Eight C. auris isolates were recovered from colonised (n=5) and candidemia (n=3) patients admitted to a major hospital in northern Portugal. In five patients, C. auris was isolated from routine surveillance screening swabs (axilla and groin) and urine, using Sabouraud dextrose agar. In three patients, C. auris was isolated from positive blood cultures. All isolates were identified by MALDI-TOF MS - matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (Vitek MS V3®). Whole-genome sequencing was performed to determine the phylogenetic relationships and to identify resistance-associated mutations. Antifungal susceptibility profiles were carried out according to EUCAST (European Committee on Antimicrobial Susceptibility Test) protocols and epidemiological cut-offs (ECOFFs).

Results: All isolates clustered within Clade I and exhibited resistance to fluconazole and amphotericin B. Most of the isolates showed a resistant phenotype to anidulafungin and micafungin. All isolates were resistant to caspofungin. Genomic analysis revealed the presence of known missense mutations such as Y132F in ERG11, E709D in CDR1, A583S in TAC1b, K52N and E1464K in SNQ2, K74E in CIS2, and

M192I in ERG4, alongside with a novel mutation S237T in CRZ1, and polymorphisms in GCN5, a gene involved in chromatin remodelling and stress-response regulation.

Conclusions: This study documents the first cases of C. auris candidemia in Portugal and reports novel mutations potentially implicated in antifungal resistance. Our findings highlight the ongoing evolution of C. auris, underscore the need for enhanced genomic surveillance and provide insights essential for guiding effective clinical management strategies.

ID577 | Pathoadaptive genomic traits of Staphylococcus aureus from atopic dermatitis

Author: Mariana Araújo

Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB-NOVA, Oeiras, Portugal

E-mail: mariana.araujo@itqb.unl.pt

Other authors: Nuno Alexandre Faria (1), Margarida Gonçalo (2), Joanna Nakonieczna (3), Carmen Torres (4), Floriana Campanile (5), Kátia Netto dos Santos (6), Rita Gonçalves Sobral (7), Maria Miragaia (1)

1 Laboratory of Bacterial Evolution and Molecular Epidemiology, ITQB-NOVA, Portugal, 2 Coimbra Local Health Unit and 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, UCIBIO, Applied Molecular Biosciences Unit, NOVA FCT, Portugal.

Background: Atopic dermatitis (AD) is a chronical inflammatory skin disease characterized by an impaired skin barrier, abnormal skin microbiome and a type 2 skewed immune response. Staphylococcus aureus skin lesions colonization is linked to more severe AD, but the mechanisms involved are unknown. We aim to identify S. aureus genetic markers associated with AD.

Methods: Whole genome sequencing data of 394 AD-related and 427 non-AD-related S. aureus collected from healthy people was analysed and their phylogenetic relatedness was assessed by SNPs analysis. To identify S. aureus markers potentially linked to AD, we performed a k-mer based genome wide association study (GWAS) using strict statistical parameters followed by a functional analysis. The linkage of the most promising genetic markers identified by GWAS with AD was validated by testing specific phenotypes, positive selection and the concordance between phylogenetic trees constructed from single genetic markers and the entire genome.

Results: According to the phylogenetic analysis, two main clusters were identified, one enriched in isolates from AD patients (AD cluster) and one enriched in isolates from non-AD patients (non-AD cluster) ($p<0.05$). The GWAS comparing AD and non-AD clusters identified a total of 682 genes associated with the AD. Functional analysis grouped these genes in 13 overrepresented biological processes ($p<0.05$). The ureC (a urease subunit) and lyrA (lysostaphin resistance) genes were selected for detailed analysis. S. aureus collected from AD patients had a higher resistance to lysostaphin and a higher growth rate comparing with non-AD S. aureus ($p<0.05$), but no difference was observed for urease activity. Positive selection was detected at five codons in ureC when comparing AD and non-AD clusters and at 13 sites in lyrA in some phylogenetic branches. The tree constructed from the entire genomes was concordant with those constructed from the ureC and lyrA but did not agree with that of gyrA – a S. aureus gene not associated with AD.

Conclusions: S. aureus isolates from AD patients exhibited distinct genomic signatures and enhanced growth potential. Enrichment of specific ureC and lyrA alleles may facilitate lesion colonization via more efficient degradation of urea - a component of the skin's moisturizing factor - and increased resistance to cell wall–targeting antimicrobials produced by the host and commensals. These genetic markers may help identify high-risk S. aureus in AD.

ID595 | Interplay Between Ciprofloxacin Resistance, Biofilm Development, and Virulence in Acinetobacter baumannii

Author: Gabriela Jorge Da Silva

1 Faculty of Pharmacy, University of Coimbra, Coimbra 3000-548, Portugal 2 Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra 3000-548, Portugal

E-mail: gjsilva@ci.uc.pt

Other authors: Sérgio G. Mendes 1,2,3, Sofia I. Combo 1,2,3, Thibault Allain 3,4, Inês Mó 1, Sara Domingues 1,2, Andre G. Buret 3, Gabriela J. Da Silva 1,2*

1 Faculty of Pharmacy, University of Coimbra, Coimbra 3000-548, Portugal 2 Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra 3000-548, Portugal 3 Departments of Biological Sciences, Inflammation Research Network, University of Calgary, 2500 University Dr. N.W, Calgary T2N 1N4, Canada 4 Department of Biology, California State University Bakersfield, Bakersfield, USA

Background: Acinetobacter baumannii is a high-priority pathogen due to its ability to rapidly acquire resistance to antibiotics and form biofilms, both of which contribute to therapeutic failure. The mechanisms linking resistance and biofilm development, however, remain insufficiently understood. The objective of this work was to unravel a potential co-regulation between antimicrobial resistance and biofilm formation.

Methods: Ciprofloxacin resistance was induced in A. baumannii ATCC 19606 by stepwise exposure, generating an isogenic ciprofloxacin-resistant derivative (CipR). MICs were determined by susceptibility testing, and biofilm assays quantified biomass and MBECs. WGS identified resistance-associated mutations. Transcriptomic analysis assessed biofilm gene expression. Functional assays included inhibition of quorum sensing (QS) with streptomycin sub-MIC, efflux pump inhibition with CCCP, metabolic activity (XTT assays), growth kinetics, and adhesion/invasion of human lung Calu-3 epithelial cells.

Results: CipR showed a MIC of 128 µg/mL. CipR formed significantly more biofilm than the parental strain ($P \leq 0.0001$). A strong positive correlation was observed between MIC values and biofilm biomass (Spearman's $r = 0.9818$). MBEC values were higher in the CipR biofilms. WGS revealed mutations in gyrB, a multidrug efflux transporter, and a hypothetical protein. Transcriptome profiling showed overexpression of the AbaI/AbaR QS system in CipR biofilm cells. QS inhibition reduced biofilm formation but did not alter ciprofloxacin MIC, indicating the involvement of additional co-regulatory mechanisms. Efflux pump inhibition with CCCP did not impact biofilm biomass or resistance. The acquisition of resistance was accompanied by metabolic fitness costs (XTT), supported by the slower growth rate observed when compared to the susceptible parent. Also, CipR showed downregulation of genes associated with adhesion (csuE, bap, bfmS, ompA) and reduced ability to adhere and invade lung epithelial cells.

Conclusions: This study demonstrates that ciprofloxacin resistance in A. baumannii correlates with increased biofilm formation and reduced virulence traits, suggesting a complex regulatory interplay. Although efflux systems and QS are known to influence resistance and biofilms, their partial dissociation here suggests additional co-regulators. Further studies may clarify additional regulatory networks that could serve as promising targets for therapeutic intervention.

ID598 | Broad-Host-Range Phages Targeting Multidrug-Resistant Pseudomonas aeruginosa - Expanding Phage Therapy Options

Author: Maria João Costa

CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal.

E-mail: mjcaetanocosta@gmail.com

Other authors: Sílvio Santos

1. CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal; 2. LABBELS – Associate Laboratory, Braga, Guimarães, Portugal.

Pseudomonas aeruginosa is a highly adaptable Gram-negative pathogen and a leading cause of healthcare-associated infections, including pneumonia, wound infections, and bacteremia. Its intrinsic resistance to multiple antibiotics, combined with the rapid acquisition of additional resistance determinants, poses a major clinical challenge. Reflecting this threat, the World Health Organization has listed P. aeruginosa among the “critical priority” pathogens requiring urgent development of novel therapeutics. Innovative strategies are therefore essential to control multidrug-resistant strains. Bacteriophages represent a promising alternative, offering strong lytic activity and the possibility of cocktail-based applications to broaden strain coverage and limit resistance development.

The host range of the isolated phages was evaluated across a panel of clinical isolates, including multidrug-resistant strains, using spot assays. Morphological characterization was performed by transmission electron microscopy (TEM), and genomic analyses were conducted to determine taxonomy and identify relevant features. The ability of the phage to control this problematic pathogen was assessed through infection dynamic assays in vitro.

Two lytic bacteriophages were isolated and characterized. The TEM analysis shows a rigid and contractile tail in both phages, consistent with the myovirus morphotype. Interestingly, the phages displayed a broad host range, an uncommon feature among P. aeruginosa phages, and their host ranges were complementary. Whole-genome sequencing revealed genomes of 60,907 bp and 65,842 bp, and annotation confirmed the absence of genes associated with lysogeny or virulence. Infection dynamics assays demonstrated the potential to control this problematic pathogen.

The broad and complementary host ranges of these myovirus phages, combined with their potent bactericidal activity, underscore their value as strong candidates for inclusion in phage cocktails targeting multidrug-resistant P. aeruginosa. Comprehensive characterisation further supports their safety for therapeutic application. By directly addressing one of the WHO’s highest-priority pathogens, these findings represent a significant step towards advancing phage-based therapies and contribute to innovative strategies for infection control and strengthened antimicrobial stewardship.

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ID640 | Epidemiology of Staphylococcus aureus at the Livestock-Wildlife Interface: Phylogenetic, Resistome, and Virulome Insights

Author: Mónica V. Cunha

1Center for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal.

E-mail: mscunha@fc.ul.pt

Other authors: Inês Afonso1, Daniela Pinto1§, José Lourenço2

1Center for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal. 2Universidade Católica Portuguesa, Católica Medical School, Católica Biomedical research Centre, Oeiras, Portugal §Current affiliation: Phage Biology Research and Infection Control (PhaBRIC), Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Background: Staphylococcus aureus poses a growing threat to human and animal health. While its transmission in human-livestock systems is well-characterized, little is known about its dynamics at the livestock–wildlife interface. Understanding the molecular diversity, antimicrobial resistance (AMR), and transmission routes of S. aureus in such multi-host systems is crucial to guide effective control strategies under a One Health framework.

Methods: A total of 338 animals were sampled across various regions of mainland Portugal, including cattle, wild boar, and red deer. S. aureus isolates first underwent phenotypic identification and molecular analyses based on spa-typing and MLST. Antimicrobial susceptibility was assessed via disk diffusion against a panel of human and veterinary drugs. Whole-genome sequencing (WGS) of selected isolates enabled in-depth analysis of resistome, virulome, and mobile genetic elements (MGEs), with phylogenetic reconstruction also incorporating publicly available and previously sequenced genomes from multiple hosts generated by our lab to explore transmission pathways.

Results: S. aureus was detected in 17.8% of animals (49 cattle, 6 wild boar, 5 red deer). Dominant genotypes included ST352 and ST6832 (t098) in cattle, alongside human-associated ST1, ST6, and ST72. Wildlife isolates harbored both epidemic human clones (ST1, ST5) and wildlife-predominant lineages (ST2678, ST425). Although phenotypic resistance was low, genomic analysis revealed AMR determinants, including multidrug efflux pumps and AMR-related enzymes. Virulence genes (e.g., leukotoxins, adhesins) and plasmids associated with AMR were also identified. Phylogenetics indicated clonal spread within herds and potential cross-species transmission at shared environments.

Conclusions: This study reveals diverse S. aureus lineages circulating across livestock and wildlife in Portugal, including genotypes linked to human infections and AMR. The findings highlight the ecological complexity of S. aureus colonization and transmission patterns, and underscore the need for integrated, genomic-based surveillance to inform AMR mitigation at the animal–environment–human interface.

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ID650 | A One Health Perspective on Backyard Production Systems in Portugal: Risk Factors and Antimicrobial Resistance

Author: Ana Esteves

1 Unidade de Genómica e Bioinformática, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Portugal. 2 Centro de Ciência Animal

E-mail: ana.esteves@insa.min-saude.pt

Other authors: Rita Ferreira1,Daniel Sobral1,Bruno Baroni1,Sónia Ramos2,4,João Dourado Santos1,Carlos Aniceto3 , Ausenda Machado3 , João Paulo Gomes 1,2,4; Alexandra Nunes1,2,4

1 Unidade de Genómica e Bioinformática, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Portugal. 2 Centro de Ciência Animal e Veterinária (CECAV), Faculdade de Medicina Veterinária, Universidade Lusófona,Portugal. 3 Departamento de Epidemiologia, Instituto Nacional de Saúde Doutor Ricardo Jorge, Portugal. 4 I-MVET- Investigação em Medicina Veterinária, Faculdade de Medicina Veterinária, Universidade Lusófona - Centro Universitário de Lisboa, Portugal.

Background: Antimicrobial-resistant (AMR) zoonotic bacteria are a global health concern, transmitted through food, animal contact, and environmental pathways. Backyard Production Systems (BPS) are particularly at risk due to informal practices, limited veterinary oversight, and unregulated medicament use. In Portugal, BPS registration is low and on-farm slaughter without inspection is permitted, complicating risk assessment. No studies have previously addressed AMR and zoonotic risks in Portuguese BPS.

Methods: Samples from poultry, pigs, small ruminants, and environmental compartments (soils with and without animal access, vegetable gardens) were collected in ten farms. An epidemiological questionnaire characterized farm management, animal husbandry, and medicament use to identify potential risk factors. An innovative probe-based metagenomics approach, using the Urinary Pathogen ID/AMR Enrichment Kit (UPIP), was applied for the detection of AMR markers. As a proof of concept for AMR detection, we analyzed 12 distinct bacterial strains with known AMR phenotypes in parallel. UPIP results for AMR marker detection were then compared with both genotypic and phenotypic resistance profiles to assess the method's reliability.

Results: The proof of concept demonstrated high concordance between AMR marker detection, AMR genotypes identified using WGS-based tools, and AMR phenotypic resistance, thereby confirming the feasibility of the approach. Farm samples analyses revealed widespread AMR markers across all sample types. Pathogen diversity was highest in pigs and vegetable gardens, while AMR marker diversity peaked in pigs and soils with animal access. Questionnaire data indicated frequent consumption of home-produced poultry and occasional medicament use without veterinary prescription.

Conclusion: BPS may act as hotspots for the circulation of AMR bacteria and genes. The combination of probe-based metagenomic screening with epidemiological data provides a robust framework to understand transmission dynamics. This first study on Portuguese BPS validates probe-based metagenomics as a powerful tool for AMR marker detection and highlights its potential to enhance monitoring and risk-mitigation measures within a One Health framework.

Projecto Estratégico 2023-25: Backyard_FSR - Food safety risks from backyard animals raised for non-commercial purpose – what risks are associated with their consumption? AE is supported by Fundação para a Ciência e a Tecnologia (FCT)

POSTERS

ID208 | Antimicrobial resistance of Salmonella Typhi and Paratyphi isolates in Portugal, January 1994 - June 2025

Author: Ângela Pista

National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

E-mail: angela.pista@insa.min-saude.pt

Other authors: Ana Margarida Henriques; Raquel Caeiro; Alexandra Tomás; Leonor Silveira

National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

Background: Typhi and Paratyphi serovars can cause severe systemic infections that require antimicrobial treatment. The increasing prevalence of multidrug resistance (MDR), particularly in S. Typhi, poses significant therapeutic challenges. This study aimed to characterize the antibiotic resistance profiles of S. Typhi and Paratyphi A, B, and C isolates received at the National Institute of Health Doutor Ricardo Jorge between January 1994 and June 2025, and to assess their genetic relatedness.

Methods: Isolates were sent by public and private hospitals/laboratories across Portugal and were serotyped using the Kauffman-White-Le Minor scheme. Antimicrobial susceptibility testing (AST) was conducted by the disc diffusion method for 18 antibiotics, according to EUCAST recommendations. Whole-genome sequencing (WGS) was performed on either a Miseq or Nextseq Illumina platform. Raw reads were submitted and analysed using Enterobase, CGE and VFDB tools.

Results: A total of 191 isolates were received and serotyped as S. Typhi (n=167) or Paratyphi A (n=13), B (n=7) or C (n=4). No isolates were recovered from 1997-2000, 2002-2008, 2010-2011, and 2013-2016. As such, 57.1% (109/191) isolates underwent AST and WGS (85 S. Typhi and all Paratyphi isolates). The number of isolates were higher in 2018, followed by 2024 and 2025 (until June). Overall, 64.2% (70/109) isolates were resistant to at least one antibiotic, and 17.1% (12/70) of these were MDR. Prior to 2017, all isolates were susceptible. However, resistance increased thereafter, particularly in S. Typhi. Resistance to pefloxacin was the most common, followed by nalidixic acid, ampicillin, trimethoprim, sulphamethoxazole, and chloramphenicol. The predominant resistance profile was NAL-PEF (64.3%; 45/70). Two S. Typhi isolates (from 2020 and 2025) were resistant to ≥10 antibiotics (across five classes) and harboured the blaCTX-M-15 gene. Fluoroquinolone resistance was mainly associated with point mutations in gyrA (p. S83F, p.S83Y, pD87N, p.D87G) and parC (p.T57S, p.S80I) genes. Genomic analysis showed that the four Paratyphi C isolates were classified as Choleraesuis. Small genetic clusters were identified (2 - 5 isolates each).

Conclusions: Although typhoid and paratyphoid serotypes are not endemic or frequent in Portugal, continued laboratory surveillance remains essential for monitoring circulating isolates, to support the implementation of control measures and to enable early detection of potential outbreaks.

ID212 | Effect of Plasmodium falciparum Infection on Host Glycolysis: Focus on Rapoport-Luebering Shunt

Author: Ana Balau

Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), 1349-008 Lisbon, Portugal.

E-mail: a21001549@ihmt.unl.pt

Other authors: Emmanuela Rodrigues1, Gustavo Capatti Cassiano1, Ana Paula Arez1,2

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), 1349-008 Lisbon, Portugal; 2 Research Centre for Antropology and Health (CIAS), Department of Life Sciences, University of Coimbra, Portugal

Background:Malaria remains a major public health challenge, especially in Sub-Saharan Africa, where Plasmodium falciparum causes most infections(1).Resistance to antimalarials and insecticides highlights the need for new therapeutic strategies, with host-targeted therapies offering a promising approach by interfering with parasite survival through modulating host cell pathways. One promising lead comes from pyruvate kinase deficiency (PKD) in red blood cells (RBC) which is associated with protection against malaria(2-4).This condition leads to the accumulation of 2,3-diphosphoglycerate (2,3-DPG), potentially creating an intracellular environment unfavourable for parasite development(5).In normal conditions, infected red blood cells (iRBC) exhibit increased ATP levels and decreased 2,3-DPG levels(6).The parasite lacks the enzyme bisphosphoglycerate mutase (BPGM) and seems to suppress its activity in iRBC and possibly in nearby non-infected red blood cells (niRBC)(7).Understanding BPGM´s role during P. falciparum infection may reveal important host-parasite metabolic interactions.We aim to study BPGM activity by measuring ATP and 2,3-DPG levels in normal RBC and in RBC with an induced PKD phenotype, both infected and non-infected.

Methods:Intracellular ATP levels, 2,3-DPG concentration have been evaluated as surrogates to BPGM activity have been evaluated in four groups: 1)RBC maintained at 4°C; 2)RBC maintained at 37°C in culture medium; 3)niRBC and iRBC from the same culture assessed separately after magnetic separation and 4) niRBC+iRBC from the same culture assessed together.Commercial kits are used to quantify 2,3-DPG, ATP and BPGM concentrations.The same measurements are repeated after inducing the PKD phenotype through enolase inhibition with sodium fluoride.

Results:Significant differences in 2,3-DPG and ATP levels are observed between RBC, iRBC and niRBC and similar BPGM concentrations across the four groups analysed.Those results suggesting a modulation of glycolytic activity in iRBC and neighbouring niRBC.

Conclusions:Preliminary results suggest a regulation of glycolytic activity both in iRBC and nearby niRBC, with potential mechanisms and/or parasite-secreted molecules under investigation. Understanding host-parasite interactions and their impact on host metabolic pathways could help to identify new antimalarial targets.

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3. PMID:19743919
4. PMID:23082140
5. PMID:35372095
6. PMID:12841355
7. PMID:16246333

ID217 | Green healthcare disinfection strategies using phytochemicals as enhancers of conventional biocides

Author: Mariana Sousa

1 LEPABE — Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, Department of Chemical and Biological Engineering, University of Porto, s/n, 4200-465 Porto, Portugal; 2 AliCE — Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, s/n, 4200-465 Porto, Portugal

E-mail: up201709542@edu.fe.up.pt

Other authors: Anabela Borges; Manuel Simões

1 LEPABE — Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, Department of Chemical and Biological Engineering, University of Porto, s/n, 4200-465 Porto, Portugal; 2 AliCE — Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, s/n, 4200-465 Porto, Portugal; 3 DEQB — Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, s/n, 4200-465 Porto, Portugal

Background: Healthcare-associated infections (HAIs) are a persistent public health issue due to the spread of multidrug-resistant bacteria, including on the surface of hospital settings, forming biofilms [1,2]. Biofilms enhance bacterial resistance and limit the effectiveness of biocides [3]. Phytochemicals, secondary metabolites from plants, have gained attention as eco-friendly antimicrobial agents, either used alone or to boost the efficacy of biocides [4]. This study evaluated the antimicrobial activity of five phytochemicals — eugenol (EUG), salicylic acid (SAL), shikimic acid (SHI), trans-m-coumaric acid (COU), and choline chloride (COL) — alongside two common biocides, benzalkonium chloride (BAC) and peracetic acid (PAA), against two major pathogens causing hospital infections: Staphylococcus aureus and Escherichia coli.

Methods: The antimicrobial activity of phytochemicals and biocides was tested against S. aureus and E. coli using the European Standard EN 1276 to determine dose-response and time-response effects, including minimum bactericidal concentrations (MBC). The checkerboard assay, analyzed with Combenefit software, assessed synergism potential in dual and triple combinations through the fractional bactericidal concentration index (FBCI). Membrane damage was assessed via epifluorescence microscopy (Live/Dead BacLight™ staining), at ¼×MBC and MBC.

Results: Among the phytochemicals tested, SAL (with an MBC of 500 mg/L for both bacteria) and EUG (with an MBC of 1000 mg/L for E. coli and 1700 mg/L for S. aureus) showed the highest antimicrobial efficacy. MBCs of 3 mg/L and 1 mg/L and high disinfection rates were obtained for BAC and PAA, respectively, and for both bacteria. BAC combined with EUG (750 mg/L) showed synergistic effects against

S. aureus (FBCI = 0.309) and E. coli (FBCI = 0.350). The other dual combinations exhibited additive effects. Triple combinations demonstrated promising bactericidal activity, reducing bacterial culturability effectively. Maximum log reductions of 5.78 ± 0.07 for E. coli and 5.82 ± 0.04 for S. aureus within 30 minutes were obtained. These findings were corroborated by epifluorescence microscopy, revealing a predominant red fluorescence, indicative of compromised membrane integrity and non-viable cells.

Conclusions: This study demonstrates that phytochemicals can significantly enhance the efficacy of conventional biocides, enabling effective and greener disinfection approaches at lower concentrations.

ID235 | Inhibition of Bilophila wadsworthia targeting the production of toxic and inflammatory sulfide

Author: Beatriz Moniz

Instituto de Tecnologia Química e Biológica António Xavier (ITQB), 2700-157, Portugal

E-mail: beatriz.moniz@itqb.unl.pt

Other authors: Inês A. C. Pereira 1, Andreia I. Pimenta 1

Instituto de Tecnologia Química e Biológica António Xavier (ITQB), 2700-157, Portugal

Background: Sulfidogenic bacteria (SB) utilize sulfur compounds as terminal electron acceptors for their energy metabolism, producing hydrogen sulfide (H2S) as a product. Bilophila wadsworthia (Bw), a member of the gut microbiota and prominent member of this group , uses taurine as a sulfite source, which is converted into H2S via the dissimilatory sulfite reduction (Dsr) pathway. The Western diet is characterized by a high intake of processed foods, red meat, sweets and unhealthy fats and these dietary habits promote the increase of taurine levels in the gut and, consequently, prompt the proliferation of Bw. The increased abundance of this bacterium in the gut disrupts the microbiota equilibrium causing dysbiosis (an unbalanced microbiota) and enhances H2S production. Even though H2S plays a beneficial role under physiological concentrations, an excessive concentration of sulfide in the colon is associated with changes in colonic architecture, heightened inflammation, mucous layer disruption, and epithelial cell damage, all of which contribute to intestinal tissue imbalance and are strongly linked to inflammatory bowel diseases and colorectal cancer.

Methods: Our approach focuses on targeting a critical membrane complex, DsrMKJOP, which is responsible for dissimilatory sulfide production, to inhibit Bw growth through its respiration pathway and H2S production. This study assesses the effectiveness of a range of inhibitors of the DsrMKJOP complex. Minimal inhibitory concentrations (MIC) and minimum bacterial concentrations (MBC) were determined for a series of compounds for Bw in monoculture and in synthetic gut communities, mimicking physiological and dysbiotic conditions. Additionally, toxicity assessment was performed using human colonic epithelial cell lines (Caco-2 and HT29-MTX) and Galleria mellonella larvae as animal model.

Results and conclusions: Some compounds were found to be both inhibitory and bactericidal against Bw, without damaging human colonocytes cell lines. These results highlight DsrMKJOP as a promising therapeutic target to modulate SB pathogenic activity in the gut and potentially control the onset of H2S-induced diseases.

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ID258 | Illuminating the Invisible: GFP as a Beacon for Antibiotic-Induced Phage Activity in Escherichia coli

Author: Maria João Silva

Division of Infection Medicine, Department of Clinical Sciences, Faculty of Medicine, Lund University, SE-22184 Lund, Sweden

E-mail: maria_joao.silva@med.lu.se

Other authors: Tim Van Den Bossche2, 3, Mattias Collin1, and Rolf Lood1

1. Division of Infection Medicine, Department of Clinical Sciences, Faculty of Medicine, Lund University, SE-22184 Lund, Sweden; 2. VIB-UGent Center for Medical Biotechnology, VIB, 9052 Ghent, Belgium; 3. Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University, 9052 Ghent, Belgium

Background/Objectives: Antibiotic resistance presents an urgent public health threat. By developing a streamlined and effective method for studying bacteriophage induction, this research marks a step further in understanding how antibiotic-resistant genes might spread across different environments. This knowledge is essential for creating strategies to reduce the spread of antimicrobial resistance (AMR), particularly from a One Health perspective. In this study, we develop and validate a Green Fluorescent Protein (GFP)-based method as a proxy for bacteriophage induction. This method screens compounds for their potential to promote bacteriophage induction. Methods: This study utilized a recA-GFP construct in Escherichia coli to measure fluorescence as an indicator of SOS response activation. The experiments involved treating E. coli cultures with varying concentrations of the DNA-damaging chemical mitomycin C and measuring fluorescence over time. Additionally, droplet digital PCR (ddPCR) quantified bacteriophage induction in a lambda phage-carrying E. coli strain, allowing for correlation analysis between the two methods. Results: The recA-driven SOS response depended on both dose and time, with increasing concentrations of mitomycin C leading to higher fluorescence. ddPCR analysis confirmed that mitomycin C induced prophage activation, with gene ratios increasing at higher drug concentrations over time. A strong Spearman correlation (>0.7) was noted between fluorescence and ddPCR results at elevated concentrations and relevant time points, indicating the validity of the GFP-based model as a proxy for bacteriophage induction. Conclusions: The findings demonstrate a strong association between the two methods of measuring phage induction, suggesting that the GFP-based E. coli model is a reliable, cost-effective, and efficient tool for studying phage induction and its potential role in AMR spread. This method could facilitate the screening of environmental samples and specific drugs to evaluate their impact on bacteriophage induction, which opens the door for applications such as screening for antibiotic resistance dissemination.

ID259 | Triggering the Hidden Players: Antibiotic Classes and Their Role in Phage-Induction

Author: Maria João Silva

Division of Infection Medicine, Department of Clinical Sciences, Faculty of Medicine, Lund University, SE-22184 Lund, Sweden

E-mail: maria_joao.silva@med.lu.se

Other authors: Mattias Collin1, and Rolf Lood1

1. Division of Infection Medicine, Department of Clinical Sciences, Faculty of Medicine, Lund University, SE-22184 Lund, Sweden

Background/Objectives: Antibiotic resistance is a pressing public health threat. By studying what triggers bacteriophage induction, especially which antibiotics or classes of antibiotics cause it, we fill a gap in understanding how antibiotic-resistant genes might spread across different environments. This knowledge is crucial for developing strategies to curb the spread of antimicrobial resistance (AMR), particularly from a One Health perspective. In this study, we utilize the previously developed Green Fluorescent Protein (GFP)-based method as a proxy for bacteriophage induction. Using this method, we screened antibiotics to assess their potential to promote bacteriophage induction. Methods: This study used a recA-GFP construct in Escherichia coli to measure fluorescence as an indicator of SOS response activation. The experiments involved treating E. coli cultures with different antibiotic concentrations and monitoring fluorescence over time. Additionally, droplet digital PCR (ddPCR) was used to quantify bacteriophage induction in a lambda phage-carrying E. coli strain at specific concentrations to enable correlation analysis between the two methods and to validate the results. Results: Fluoroquinolones, as DNA-damaging antibiotics, show a strong fluorescence response due to the activation of the SOS response, consistent with ddPCR results. Other antibiotic classes do not trigger GFP production in the proxy method; however, ddPCR results indicate phage induction, though not as strongly.

Conclusions: The findings indicate that fluoroquinolones are more effective at inducing phages than other antibiotic classes. This knowledge facilitates the adaptation of clinical treatments in both human and veterinary medicine, once again highlighting the significance of this research for the One Health approach to science. Finally, the results open opportunities for applications such as screening for antibiotic resistance spread and treatment options.

ID292 | Understanding Early Bacterial Colonization during Bacterial Vaginosis

Author: Ângela Lima

LIBRO, CEB, University of Minho, Braga, Portugal

E-mail: angela.lima695@gmail.com

Other authors: Joana Castro 2, Christina Muzny 3, Nuno Cerca 1 e 4

1 LIBRO, CEB, University of Minho, Braga, Portugal; 2 INIAV, Vila do Conde, Portugal ; 3 Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, USA; 4 LABELLS, University of Minho, Braga, Portugal

Background: Bacterial vaginosis (BV) is the most common vaginal infection among women of childbearing age, with a global prevalence of 23–29% and annual healthcare costs of approximately 4.8 billion dollars. It is characterized by a dysbiotic state, marked by the depletion of vaginal lactobacilli and overgrowth of anaerobic bacteria. Despite extensive research, the etiology of BV remains unclear, and the sequence of events leading to the displacement of lactobacilli and the proliferation of anaerobes is not fully understood. Furthermore, recent data have shown that multiple Gardnerella subgroups frequently coexist in the vaginal microbiota of women with BV. A recent conceptual model suggests that Gardnerella and Prevotella spp. are early colonizers of the vaginal epithelium; however, experimental data supporting this model are lacking. In this context, we aimed to test the hypothesis that combinations of Gardnerella spp. have an enhanced capacity to displace Lactobacillus crispatus. We also sought to assess whether Prevotella spp. have an innate ability to displace L. crispatus, thereby promoting early BV development.

Methods: Adhesion interference assays using either (i) single-species of Gardnerella or Prevotella and (ii) dual combinations of Gardnerella spp. were conducted on an in vitro HeLa cell model, both in the presence and absence of pre-established L. crispatus. Quantification of the bacterial species involved in the assays was subsequently carried out using quantitative polymerase chain reaction (qPCR).

Results: Our data revealed synergy between different Gardnerella spp., highlighting their capacity to overcome the presumed protective role of L. crispatus and to promote conditions conducive to the formation of a polymicrobial biofilm characteristic of BV. Notably, the combination of G. vaginalis and G. leopoldii exhibited the strongest synergistic effect on initial adhesion to HeLa cells, whereas the pairing of G. leopoldii and G. swidsinskii showed the highest capacity to reduce L. crispatus colonization. Curiously, only 2 out of 6 Prevotella spp. tested, P. amnii and P. copri, demonstrated a significant ability to displace L. crispatus.

Conclusions: Although this in vitro study does not provide definitive proof that BV is initiated by the disruption of the normal vaginal microbiota by Gardnerella or Prevotella spp., it offers strong support for this hypothesis.

ID295 | Reduced Efficacy of Artemether–Lumefantrine for Uncomplicated Plasmodium falciparum Malaria in Luanda, Angola

Author: Maria das Neves Imaculada Pereira (Maria Pereira)

2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal 3 Clínica Multiperfil, Luanda, Angola 5 CESPU/Gandra Porto-Polytechnic and University Higher Education Cooperative

E-mail: mnipjardim@yahoo.com.br

Other authors: Ana Margarida Gonçalves 1,2(*), Beatriz Fonseca 1,2, Ana Pereira 1,2, Claudia Fançony 4, Nuno S. Osório 1,2, Maria Isabel Veiga 1,2

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal 4 Health Research Centerof Angola (CISA), Caxito, Angola

Introduction. Despite remarkable progress in recent years, malaria, primarily caused by Plasmodium falciparum, remains a significant public health challenge. It continues to be one of the leading causes of morbidity and mortality, with a prevalence of 17% in Angola in 2024. The current treatment for uncomplicated P. falciparum malaria involves using artemisinin-based combination therapies (ACTs), with Angola adopting artemether-lumefantrine (AL) as a first-line therapy for uncomplicated P. falciparum. However, therapeutic efficacy trials have reported AL effectiveness below the 90% threshold set by WHO in several locations across the continent, including Angola. Artemisinin resistance and delayed parasite clearance has emerged as a serious concern and poses a growing threat to the effectiveness of ACTs. This development could reverse the substantial progress achieved in malaria control and elimination.

Methods. A clinical trial was carried out to evaluate the efficacy of AL in hospitalized patients in Luanda diagnosed with P. falciparum malaria, all presenting with parasitemia levels exceeding 1500 parasites/mm³ at the time of admission (Trial Ref.: PACTR202405595426468). A total of 95 subjects were enrolled in the study. At the baseline visit, biochemical analyses were performed, and demographic data were collected. During follow-up visits, blood samples were taken to assess parasitemia, gametocyte presence and nucleic acid extraction, posteriorly used to monitor treatment response, including early treatment failure and adequate clinical and parasitological response.

Results. This study assessed the cumulative incidence of success by day 28 of AL therapy in patients with uncomplicated malaria. An efficacy of 77% was found, which is below the defined WHO threshold, reinforcing the urgency of regular surveillance of the effectiveness and efficacy of AL in the country.

Conclusions. This work aims to support and encourage future research on the urgent need to monitor and update information on anti-malarial drug efficacy, a critical factor in achieving malaria elimination goals by 2030. The next steps include the whole genome sequencing of both human and parasite samples to identify key variables for developing a machine learning model to predict therapeutic failure in malaria, thereby improving treatment personalization strategies.

ID301 | Time-dependent Antibacterial Effects of Cold Atmospheric Plasma on Periodontal Pathogens

Author: Neusa Silva

Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal

E-mail: neusa.silva@edu.ulisboa.pt

Other authors: moana marques, mariana brito da cruz, ana marques, henrique luís, antónio mata, susana sério

(1) Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal ; (2) Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal ; (3) Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal ; (4)Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Rede de Higienistas Orais para o Desenvolvimento da Ciência (RHODes) Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal; (5) Universidade de Lisboa, Faculdade de Medicina Dentária, Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), LIBPhys-FTCUID/FIS/04559/2013, Rua Professora Teresa Ambrósio, 1600-277 Lisboa, Portugal; (6) Laboratory of Instrumentation, Biomedical Engineering and Radiation Physics (LIBPhys-UNL), Department of Physics, NOVA School of Science and Technology, NOVA University Lisbon, 2829- 516 Caparica, Portugal.

Background: Cold atmospheric plasma (CAP) has emerged as a promising antimicrobial strategy due to its ability to disrupt biofilm architecture and inactivate microbial cells, primarily through the generation of reactive oxygen and nitrogen species (RONS).1,2 This study evaluated CAP's time-dependent effects against six periodontal pathogens: Streptococcus oralis (SO), Actinomyces naeslundii (AN), Veillonella parvula (VP), Fusobacterium nucleatum (FN), Aggregatibacter Actinomycetemcomitans (AA), Porphyromonas gingivalis (PG).

Methods: Bacterial strains were revived from cryopreservation, cultured on blood agar for 3 days, and subcultured for 3 additional days. Cultures were grown in brain heart infusion medium (BHI-2) for 30 hours, diluted to 10-2, and plated in quadruplicated. After air-drying for 10 minutes, plates were treated with CAP at 2 mm distance for 15 - 300 seconds at treatment distance of 2 mm. Control included air flow (negative control) and 2% chlorhexidine (positive control). Inhibition zones were measured using a digital ruler. Statistical analysis was performed using Kruskal-Wallis test with post-hoc pairwise comparisons, and Bonferroni correction was applied for multiple testing (p 0.05). At 180 seconds, all strains exhibited inhibition zones, with the largest for FN and PG ($p > 0.05$). Maximum inhibition occurred at 300 seconds, with SO, FN, and PG showing the largest zones ($p > 0.05$). Negative controls showed no inhibition, while positive controls displayed variable effects.

Conclusions: These findings demonstrate CAP's time-dependent antibacterial activity against periodontal pathogens, with effectiveness increasing at 180 – 300 seconds of exposure.

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ID307 | Gut2Brain: Exploring microbiota-gut-brain axis in obesity

Author: Abigail González

1 CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

E-mail: aloabigc@gmail.com

Other authors: Clara Guimarães1, Catarina Gómes1, Andreia F. Salvador1,2, Catarina Teixeira1,2; Natividade de Sá Couto-Pereira3, José A. Teixeira1,2, Adriana Sampaio3, Eva Conceição4; Clarisse Nobre1,2

1 CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal; 2 LABBELS - Associate Laboratory, Braga, Guimarães, Portugal; 3 CIPsi – Psychology Research Centre, University of Minho, Campus de Gualtar, Braga, Portugal; 4 Faculty of Psychology and Education Sciences, University of Porto, Porto, Portugal

Background: Obesity and associated comorbidities remain major public health challenges. Emerging evidence suggests that gut microbiota imbalances may influence eating behavior, offering a novel perspective into the mechanisms underlying obesity and potential strategies for its management.

Methods: A randomized clinical trial was conducted with subjects with obesity and binge eating disorder (BED), who were assigned to receive either a prebiotic inulin (16 g/day) or a placebo for 12 weeks. Body weight, blood and stool samples, psycho-behavioral, and neurological data were collected before (T0) and after (T1) the intervention. Stool samples were also used for in vitro fermentation with the prebiotic to assess the production of short-chain fatty acids (SCFAs), microbial shifts, and effects on intestinal epithelium.

Results: After 12 weeks, no significant changes in Body Mass Index were observed; however, the prebiotic group showed significant shifts in gut microbiota composition. Both in vivo and in vitro, prebiotic supplementation consistently increased Bifidobacterium and decreased Bacteroidota. Despite some inter-individual variability, fermentation kinetics generally showed increased optical density and decreased pH, alongside elevated SCFA production, particularly lactate, acetate, and propionate. The bacterial metabolites did not show cytotoxic effects when in contact with intestinal epithelial cells. Psycho-behavioral questionnaires indicated a trend toward reduced scores following prebiotic supplementation, suggesting a potential in regulating eating behavior.

Conclusions: Prebiotic supplementation induced favorable shifts in the gut microbiota, including the enrichment of beneficial genera and suppression of potentially harmful bacteria. In vitro findings supported increased microbial activity and SCFA production. Although BMI remained unchanged, the observed trends in microbiota composition and behavioral scores suggest that prebiotics may positively influence the gut-brain axis and eating behavior.

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ID328 | Preliminary evaluation of the antibacterial potential of Laurus azorica and Morella faya extracts

Author: Mariana Moniz Viveiros

University of the Azores, Faculty of Sciences and Technology, Centre for Ecology, Evolution and Environmental Changes (cE3c), Azorean Biodiversity Group & Global Change and Sustainability Institute (CHANGE), 9500-321 Ponta Delgada, Portugal.

E-mail: marianamonizv@outlook.com

Other authors: 1- Laura Alarcón, 2,3 - António Machado, 4 - Violante Pacheco de Medeiros, 5,6 - Ana Maria Loureiro da Seca, 5 - Maria do Carmo Barreto

1- ÍES Benjamín Rua, 28933 Móstoles, Madrid, Spain; 2 - University of the Azores, Faculty of Science and Technology, Department of Biology, Azores Biotechnology Center (CBA), 9500-321 Ponta Delgada, Portugal; 3 - Universidad San Francisco de Quito USFQ, Colegio de Ciencias Biológicas y Ambientales COCIBA, Instituto de Microbiología, Laboratorio de Bacteriología, Calle Diego de Robles y Pampite, Quito 170901, Ecuador; 4 - Phytosphera Azorica, 9500-794 Ponta Delgada, Portugal; 5 - University of the Azores, Faculty of Sciences and Technology, Centre for Ecology, Evolution and Environmental Changes (cE3c), Azorean Biodiversity Group & Global Change and Sustainability Institute (CHANGE), 9500-321 Ponta Delgada, Portugal; 6 - LAQV-REQUIMTE, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.

Background: Due to the excessive prescription and misuse of antibiotics, bacteria are increasingly exhibiting resistance mechanisms, posing a serious threat to public health.1 The demand for herbal medicines has been increasing with the aim of reducing bacterial resistance to antibiotics, due to the presence of bioactive compounds in plant extracts.1,2 Laurus azorica (Seub.) Franco and Morella faya (Aiton) Wilbur are two plant species belonging to the Laurissilva forests, largely understudied regarding their antibacterial activity. In this study, extracts of L. azorica and M. faya leaves were evaluated for their antibacterial potential against Gram-positive bacteria, more exactly Staphylococcus aureus ATCC 25923/DSM 20044 (coagulase-positive) and Staphylococcus epidermidis ATCC 14990/DSM 1104 (coagulase-negative) due to their importance in nosocomial-associated infections. Methods: The ethanolic and aqueous extracts of the dried leaves of L. azorica and M. faya were prepared by two methods: maceration and ultrasound-assisted extraction. The antibacterial potential of the selected extracts was then evaluated by microdilution assay in 96-well plates to quantify the biomass inhibition of both Gram-positive staphylococci. The extracts were tested between 0.49 and 250 µg/mL and compared with two well-known antibiotics, namely penicillin (0.02 – 8 µg/mL) and streptomycin (0.06 – 32 µg/mL). All microplates were incubated for 24h at 37°C and the biomass growth was measured at 620 nm. Results: All plant extracts demonstrated biomass inhibition against both staphylococci, but the results demonstrated that the extracts by ultrasound of both species were more effective in growth inhibition of both Gram-positive staphylococci. The aqueous extract of L. azorica showed growth inhibition rate between 7-54% and 15-57% for S. aureus and S. epidermidis, respectively. Meanwhile, the M. faya ethanolic extract showed inhibition rate against S. aureus between 0% and 57%, and for S. epidermidis, the aqueous extract showed inhibition rates between 15% and 57 %. Conclusions: In general, the extracts prepared by ultrasound-assisted extraction of both plants were effective against staphylococci.

ID334 | Berberine–Gentamicin Synergy Enhances Photodynamic Inactivation of Staphylococcus aureus Biofilms from a Diabetic Foot Ulcer Isolate

Author: Anabela Borges

1 LEPABE, ALICE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; 3 DEQB—Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

E-mail: apborges@fe.up.pt

Other authors: Ariana Soraia Carreira Gonçalves 1,2; Manuel Simões 1,3

1 LEPABE, ALICE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; 2 Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal; 3 DEQB—Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

Background: Chronic wound infections pose a significant clinical challenge due to colonization by polymicrobial communities, with Staphylococcus aureus being one of the predominant pathogen [1]. Antimicrobial photodynamic inactivation (aPDI) has emerged as a promising alternative to conventional therapies; however, its efficacy against biofilms is often limited [2]. This study investigates, for the first time, the potential of combining the phytochemical berberine (Ber) and antibiotic gentamicin (Gen) to enhance aPDI against S. aureus biofilms.

Methods: A clinical strain of S. aureus isolated from a diabetic foot ulcer was used. Synergistic concentrations of Ber-Gen were identified by checkerboard assay, and the effect of blue light photoactivation (420 nm, 30 mW/cm2, 10 min) was evaluated for both biofilm prevention and eradication. For pre-formed biofilms, treatments consisted of one or three irradiation cycles (applied every 24 h), with biofilm regrowth monitored at 48 and 72 h. Biofilm mass, metabolic activity, and culturability were assessed using crystal violet, resazurin, and colony-forming unit (CFU) counts (log CFU/cm2), respectively. To elucidate the mode of action, reactive oxygen species (ROS) generation, membrane damage, and biofilm structural disruption were examined through fluorometry, flow cytometry/confocal microscopy, and optical coherence tomography, respectively.

Results: Photoactivated Ber-Gen successfully prevented biofilm formation, reducing biomass and metabolic activity by over 90 % and achieving around 6 log CFU/cm2 reduction. In pre-formed biofilms, the treatment induced around 7 log CFU/cm2 reduction in culturability and over 90 % decrease in biomass and metabolic activity. While regrowth occurred within 24 h after a single irradiation cycle, three cycles prevented recovery for up to 72 h. Mechanistically, Ber-Gen-mediated aPDI triggered strong ROS production, membrane disruption, and collapse of biofilm structure.

Conclusions: These findings highlight the potential of Ber-Gen-mediated aPDI as a promising strategy for tackling persistent S. aureus biofilms in chronic wound infections.

ID335 | Antibiofilm and Cytoprotective Potential of Natural Iron Chelators Kojic Acid and Maltol Against Staphylococcus aureus

Author: Anabela Borges

1 LEPABE, ALICE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; 3 DEQB—Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

E-mail: apborges@fe.up.pt

Other authors: Miguel M. Leitão 1,2; Manuel Simões 1,3

1 LEPABE, ALICE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Associate Laboratory for Innovation in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; 2 CIQUP-IMS—Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal; 3 DEQB—Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

Background: Iron is essential for the formation, maturation and spread of biofilms, playing a central role in bacterial metabolism and the regulation of virulence. Iron deficiency can disrupt motility, surface hydrophobicity and protein composition, and impair biofilm stability [1]. This study investigates the antibiofilm potential of two natural iron chelating agents, kojic acid and maltol, against Staphylococcus aureus individually and in combination with antibiotic tobramycin.

Methods: The chelating capacity of kojic acid and maltol was determined based on their affinity for Fe(III) and compared with ethylenediaminetetraacetic acid (EDTA). Antibiofilm activity against established S. aureus (CECT 976) biofilms was evaluated by crystal violet staining (biomass), alamar blue (metabolic activity) and culturability (log (CFU cm−2)). The integrity of the cell membrane was analysed by flow cytometry and epifluorescence microscopy using propidium iodide dye. Moreover, the effect of the natural compounds and their combination with tobramycin on the production of virulence factors (proteases, lipases, gelatinases and siderophores) was determined using agar plate assays. Cytotoxicity and protection against Fe(III)-induced stress were evaluated in HepG2 cells by measuring metabolic activity, cell mass and ROS production.

Results: Kojic acid and maltol showed a strong Fe(III) affinity (~90%), comparable to EDTA. The selected compounds alone reduced the metabolic activity by over 75% in pre-established biofilms. Moreover, the combination of maltol and tobramycin significantly reduced biofilm cells culturability by 3-log (CFU cm−2). The compounds alone and in combination with tobramycin also induced membrane damage and completely inhibited protease production. In HepG2 cells, both compounds prevented Fe(III)-induced cytotoxicity and showed a good safety profile, although maltol slightly increased ROS formation.

Conclusions: Kojic acid and maltol showed strong iron chelation and significant antibiofilm activity against S. aureus. Their ability to potentiate tobramycin, inhibit virulence factors and protect mammalian cells from Fe (III) damage emphasises their potential as safe adjuvants for the treatment of S. aureus biofilm-associated infections.

ID338 | Targeting Plumbing Reservoirs: A Novel Antimicrobial for Hospital Biofilms

Author: Débora Castro

CEB, Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; Success Gadget - Nanotecnologia e Novos Materiais, Lda, Rua Filipa Borges, 1245, 4750-823 Barcelos, Braga, Portugal

E-mail: id10569@alunos.uminho.pt

Other authors: Marta Ferreira 1; Sara Cardoso 4; Isabel Ferreri 2; Mariana Henriques 1,3; Isabel Carvalho 1,3

1 CEB, Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; 2 Success Gadget - Nanotecnologia e Novos Materiais, Lda, Rua Filipa Borges, 1245, 4750-823 Barcelos, Braga, Portugal; 3 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal; 4 ULSAAVE – ULS do Alto Ave, Guimarães, Portugal

Background: Hospital sink surfaces are increasingly recognised as reservoirs for biofilm-forming pathogens and sources of outbreaks. Often formed on the stainless steel and PVC components of sink drainage systems, such biofilms are difficult to eradicate with conventional disinfectants, particularly in moist environments. This study validates in situ the antimicrobial PipeUCare, designed for disinfecting non-porous surfaces in greywater systems, including hospital sink drains.

Methods: PipeUCare was applied to sinks in eighteen hospital rooms. Three zones were analysed: S – sink drain, D– adjacent dry surface, and W – adjacent wet surface. Post-application sampling included microbial quantification (CFU·mL-¹) and viability assessment (OD690). This study considered three common plumbing materials: stainless steel, rigid PVC, and flexible PVC.

Results: The results showed that PipeUCare achieved a >5-log reduction in microbial load at most sites, showing efficacy in both dry and wet zones. Zone D (dry) displayed near-complete biofilm eradication across all materials, while Zone W (wet) also showed strong, though slightly lower, reductions, confirming activity under humid conditions. Zone S, showed variable responses, but in most cases PipeUCare outperformed conventional products in disinfection and recolonization prevention. The most frequent isolates were Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter asburiae.

Conclusions: These findings validate PipeUCare as an effective tool for biofilm control in high-risk hospital environments. By identifying realistic contamination zones and addressing them in situ, the study supports surface-specific antimicrobial strategies in infection prevention protocols and highlights the importance of targeting plumbing-related in clinical microbiology.

ID340 | Exploring the efficacy of different honeys against a triple-species biofilm isolated from pressure injuries

Author: Alexandra Catarina Pinto

1 CEB - Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; 2 Fibrenamics, Institute of Innovation on Fiber-Based Materials and Composites, University of Minho, 4800-058 Guimarães, Portugal; 3 The Health Sciences Research Unit:Nursing (UICISA:E), Nursing School of Coimbra (ESEnfC), Coimbra, Portugal

E-mail: alexandracatarina@ceb.uminho.pt

Other authors: Ricardo Barros1; Joana C. Antunes2; Mariana Henriques1,4; Raul Fangueiro2; Anabela Salgueiro-Oliveira3; Fernanda Gomes1,4

1 CEB - Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; 2 Fibrenamics, Institute of Innovation on Fiber-Based Materials and Composites, University of Minho, 4800-058 Guimarães, Portugal; 3 The Health Sciences Research Unit:Nursing (UICISA:E), Nursing School of Coimbra (ESEnfC), Coimbra, Portugal; 4 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Pressure injuries (PIs) remain a major healthcare challenge due to chronic wound colonization and biofilm formation, which complicates treatment and delay healing. This study identified main colonizing bacteria from PI patients, assessed their biofilm-forming potential, with mimicked the wound environment in vitro, and tested two Portuguese honeys as natural antimicrobial alternatives.

Methods: 43 PIs samples from 34 patients were analysed using selective/differential medium and confirmed by MALDI-ToF. Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus faecalis were isolated to develop a three-species biofilm model. Heather and eucalyptus honeys were tested through Minimum Inhibitory Concentration (MIC) assays. The most effective was further evaluated against pre-formed and developing triple-species biofilm.

Results: The most predominate microorganisms isolated were S. aureus, Proteus mirabilis, P. aeruginosa and E. faecalis. Some species of Candida were isolated too. Eucalyptus honey showed lower MIC values than heather honey. It inhibited S. aureus and P. aeruginosa and reduced both the formation and the viability of pre-formed triple-species biofilms involving E. faecalis.

Conclusions: Portuguese eucalyptus honey shows potential as a natural antimicrobial for biofilm control in PIs. Further studies will explore its integration into wound care strategies for improved infection control and healing.

ID347 | Evolutionary insights by subclinical ciprofloxacin exposure in Arcobacter butzleri through genomics and transcriptomics

Author: Inês Martins

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal.

E-mail: ines.margarida.martins@ubi.pt

Other authors: Daniel Sobral 2, Alexandra Nunes 2, Mónica Oleastro 3, Susana Ferreira 1

1 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, 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.

Background: Arcobacter butzleri is an emerging enteropathogen increasingly recognized for its antimicrobial resistance potential. While resistance is typically linked to therapeutic antibiotic concentrations, subclinical levels can also drive the selection of multidrug-resistant mutants, potentially altering bacterial physiology and virulence. In A. butzleri, little is known about the evolutionary dynamics driven by subclinical antibiotic concentration exposure and their associated trade-offs.

Methods: We performed 12-day adaptive laboratory evolution experiments in the presence of ciprofloxacin, a critically important antimicrobial classified in the World Health Organization's Watch group, combined with whole genome sequencing and transcriptomics. Evolved populations were analyzed for cross-resistance to antibiotics, biocides, heavy metals, and ethidium bromide, as well as the underlying resistance mechanisms. For that, the emergence of differential phenotype profiles was comprehensively investigated via genomic and transcriptomic approaches and correlated with phenotypic data.

Results: Exposure at 0.5×, 1×, and 2× the minimum inhibitory concentration (MIC) of the parental strain (0.03 µg/mL) selected for resistant mutants with reduced susceptibility to multiple antibiotics and biocides. Whole-population sequencing revealed several key genetic alterations at 1× and 2×MIC, with ciprofloxacin resistance associated with mutations in gyrA gene, and mutations in the transcriptional regulator of an efflux system correlated with decreased susceptibility to ethidium bromide. Furthermore, mutations in genes encoding flagellum-associated proteins were correlated with impaired motility. To address transcriptional adaptation, RNA-sequencing was performed using differential expression analysis. Amongst the genes most strongly modulated by ciprofloxacin exposure, we could find genes involved in virulence, such as efflux systems, transporters, and motility. Additionally, we also identified differentially expressed transcripts involved in oxidative stress response, metal homeostasis, chemotaxis and signaling, and DNA recombination and repair.

Conclusions: Together, these results highlight the complex interplay between genetic and transcriptional adaptations during resistance development, underscoring the need for improved surveillance frameworks and therapeutic strategies to curb the spread of resistance in ubiquitous pathogens like A. butzleri.

ID359 | Exploring the antibacterial potential of Azorean macroalgae extracts against opportunistic pathogens

Author: Mariana Moniz Viveiros

University of the Azores, Faculty of Sciences and Technology, Centre for Ecology, Evolution and Environmental Changes (cE3c), Azorean Biodiversity Group & Global Change and Sustainability Institute (CHANGE), 9500-321 Ponta Delgada, Portugal

E-mail: marianamonizv@outlook.com

Other authors: 1 - Andreia Costa, 2 - Gonçalo Pereira da Rosa, 1,3 - António Machado, 2,4 - Ana Maria Loureiro da Seca, 2 - Maria do Carmo Barreto

1 - University of the Azores, Faculty of Science and Technology, Department of Biology, Azores Biotechnology Center (CBA), 9500-321 Ponta Delgada, Portugal; 2 - University of the Azores, Faculty of Sciences and Technology, Centre for Ecology, Evolution and Environmental Changes (cE3c), Azorean Biodiversity Group & Global Change and Sustainability Institute (CHANGE), 9500-321 Ponta Delgada, Portugal; 3 - Universidad San Francisco de Quito USFQ, Colegio de Ciencias Biológicas y Ambientales COCIBA, Instituto de Microbiología, Laboratorio de Bacteriología, Calle Diego de Robles y Pampite, Quito 170901, Ecuador; 4 - LAQV-REQUIMTE, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.

Background: Antimicrobial resistance (AMR) is one of the greatest global health challenges, limiting the effectiveness of conventional antibiotics. Marine macroalgae are a promising source of bioactive metabolites with antimicrobial potential. In this study, extracts from Caulerpa prolifera (Chlorophyta) and Gongolaria abies-marina (Phaeophyceae), two macroalgae present in the Azores, were investigated for their ability to inhibit opportunistic bacteria associated with biofilm-related infections.

Methods: Sequential extracts of C. prolifera (dichloromethane, acetone, ethanol) and an aqueous fraction of G. abies-marina were tested against Staphylococcus epidermidis DSM 20044 (Gram-positive) and Escherichia coli DSM 498 (Gram-negative). The antibacterial activity was assessed through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays using the microdilution method in 96-well plates, with growth inhibition evaluated by resazurin colorimetric assay. As positive controls, three clinically relevant antibiotics were used: penicillin, streptomycin, and amoxicillin/clavulanic acid.

Results: Overall, the extracts, tested in the range of 0.49–250 µg/mL, exhibited limited antibacterial activity and no bactericidal effect. Partial inhibition was observed with specific extracts: the acetone extract of C. prolifera inhibited S. epidermidis growth up to 58% at 250 µg/mL, while the dichloromethane extract inhibited E. coli growth up to 28% at 125 µg/mL. The remaining extracts showed negligible effects across the tested concentrations. In contrast, the reference antibiotics demonstrated full inhibition at expected MIC values (≤0.5 µg/mL for penicillin against S. epidermidis, 1–2 µg/mL for streptomycin, and ≤2 µg/mL for amoxicillin/clavulanic acid).

Conclusions: Further studies are needed to optimize extraction methods, alternative solvents and to assess seasonal variations in order to maximize antimicrobial efficacy and to highlight antibacterial potential of Azorean macroalgae.

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ID364 | Lipossomal Antisense Oligomer for Controlling Fluconazole Resistance in Candida glabrata

Author: Patrícia Serra

1 CEB - Centre of Biological Engineering, 4710-057 Braga, Portugal; 3 INIAV - National Institute for Agrarian and Veterinarian Research, Rua dos Lagidos, 4485-655 Vairão, Vila do Conde, Portugal

E-mail: pmserra02@gmail.com

Other authors: Daniela Araújo 3; Bruna Gonçalves 1,2; Mariana Henriques 1,2; Joana Castro 1,3; João Santos 4; Nuno Guimarães 4; Nuno F. Azevedo 4; Sónia Silva 1,2

1 CEB - Centre of Biological Engineering, 4710-057 Braga, Portugal; 2 LABBELS – Associate Laboratory, 4710-057 Braga/Guimarães, Portugal; 3 INIAV - National Institute for Agrarian and Veterinarian Research, Rua dos Lagidos, 4485-655 Vairão, Vila do Conde, Portugal; 4 LEPABE, ALiCE — Laboratory for Process Engineering, Environment, Biotechnology and Energy, Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

Background: Candida infections affect the global population, contributing to high morbidity and mortality rates of up to 60%. These infections not only prolong hospitalizations but also impose an economic burden on healthcare systems worldwide. Among pathogenic species, Candida glabrata has emerged as a major clinical concern due to its growing resistance against antifungal agents, particularly azoles. In this context, antisense therapy (AST) has emerged as a promising approach to treat infectious diseases. This work focused on developing antisense oligonucleotides (ASOs) targeting PDR1, a transcription factor involved in antifungal resistance in C. glabrata, with the aim of restoring fluconazole susceptibility.

Methods: An ASO was designed to target the PDR1 mRNA, and its capacity to downregulate gene expression and enhance fluconazole efficacy was tested in vitro. To improve the in vivo delivery, was encapsulated in lipid-based nanoparticles (LipoASOs), a strategy that has been validated in other models. The safety and efficacy of these nanosystems were evaluated using the in vivo model Galleria mellonella, with larval survival and health index being measured.

Results: In vitro analysis confirmed that ASO reduced the target gene expression by around 30%, even under fluconazole-induced conditions. The ASO also enhanced the growth reduction of C. glabrata by at least 20%, either alone or combined with fluconazole. Although fluconazole alone failed to improve infection outcomes in vivo; the co-treatment with LipoASOs significantly increased larval survival, at 72 h, by 32% and 26% for the DOTAP:DOPC:PEG and DOTAP:DOPE:PEG formulations, respectively. The health indices of treated larvae remained higher than the untreated group.

Conclusions: Overall, these results demonstrate that the ASO can control C. glabrata growth and restore its sensitivity to fluconazole, especially when delivered via liposomal carriers. The strong in vivo performance of LipoASO–fluconazole combinations highlight their potential as a novel strategy to overcome resistance and improve treatment outcomes in infections caused by multidrug-resistant fungal pathogens.

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ID366 | Escherichia coli ATCC 25922 as a novel coaggregating strain and its implications for biofilm dynamics

Author: Maria José Saavedra

CITAB, University of Trás-os-Montes e Alto Douro, Quinta de Prados 5000-801, Vila Real, Portugal

E-mail: saavedra@utad.pt

Other authors: Daniela Dias1, Maria José Saavedra2*, Manuel Simões1, Ana Cristina Afonso1

1LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr Roberto Frias, 4200-465 Porto, Portugal 2CITAB, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Quinta de Prados 5000-801, Vila Real, Portugal

Bacterial coaggregation, the specific recognition and adhesion between genetically distinct cells, is a key mechanism in the establishment and architecture of multispecies biofilms [1]. While well studied in oral communities, its occurrence and ecological roles in other environments remain largely unexplored [2]. In this work, pairwise aggregation assays identified for the first time Escherichia coli ATCC 25922 as a coaggregating strain. Inhibition assays with sugars, heat, and proteases indicated that protein–protein interactions are the main drivers of this phenomenon. Dual-species biofilms involving E. coli ATCC 25922 displayed marked structural changes, including increased heterogeneity and robustness, suggesting a potential bridging role. The identification of this clinically relevant strain as coaggregating has several advantages: (i) it expands the diversity of known bridging species beyond the oral microbiome; (ii) it provides an accessible and well-characterized model for studying interspecies interactions; and (iii) it offers new insights into how clinical strains may persist and gain resilience within multispecies biofilms. These findings expand the diversity of known bridging bacteria beyond the oral microbiome and provide an accessible model for investigating interspecies interactions.

Keywords: Bridging bacteria, cell-cell interaction, model organism, multispecies biofilms.

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ID380 | Enterococcus thailandicus: Genomic evidence of a potential new player in enterococcal virulence and antimicrobial resistance

Author: Ana Raquel Freitas

3-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 4-Laboratório Associado i4HB, Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 5-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Instituto Universitário de Ciências da Saúde (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal

E-mail: anarpf@gmail.com

Other authors: 1,2,3,4-Juan C. Vázquez-Ucha; 3,4-Ana C. Almeida-Santos; 1-David Velasco; 1,2-Salud Rodríguez-Pallares; 1-Michelle Outeda-García, 1-Arianna Rodríguez-Coello, 1-Ana Fernández, 1-Pablo Aja-Macaya, 3,4-Carla Novais; 1,2-Alejandro Beceiro; 3,4-Luisa Peixe; 1,2,6-Germán Bou

1-Servicio de Microbiología and Instituto de Investigación Biomédica A Coruña (INIBIC), Complexo Hospitalario Universitario A Coruña (CHUAC), A Coruña; Spain 2-Ciber de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain 3-UCIBIO; Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 4-Laboratório Associado i4HB, Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal 5-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Instituto Universitário de Ciências da Saúde (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal; 6-Departamento de Fisioterapia, Medicina y Ciencias Biomédicas. Universidad de A Coruña

Background: Enterococcus thailandicus has been isolated from animals, fermented foods, wastewater, and humans, but its clinical relevance remains unclear. We investigated the pathogenic potential of E. thailandicus by phenotypic and genomic characterization of two human isolates from acute abdominal infections, complemented by a species- wide comparative genomic analysis of all publicly available E. thailandicus genomes.

Methods: Two E. thailandicus isolates were recovered from patients with acute cholecystitis and small bowel perforation. Species identification was performed by MALDI-TOF MS and ANI analysis, and antimicrobial susceptibility testing was conducted. Whole-genome sequencing (WGS) was performed, followed by in silico analysis of antimicrobial resistance (AMR) genes, virulence factors, bacteriocins, and plasmid replicons. All available E. thailandicus genomes (n=46) were included for phylogenetic comparison. Results: Both clinical isolates were broadly susceptible to antimicrobials. WGS revealed the presence of the ptsD gene (enhancing metabolism and typically linked to hospital-adapted E. faecium) and the bacteriocin gene enkB in both isolates. One isolate additionally carried the tet(M) gene and a plasmid replicase typically associated with E. faecium plasmids. Among the 46 genomes analyzed, 61% carried ptsD, all carried enkB, and 48% harbored acquired AMR genes, including functional optrA (26%) and poxtA (11%), both conferring linezolid resistance. Phylogenetic analysis showed wide genetic diversity with clustering by host or geography, consistent with ecological versatility.

Conclusions: This study reports the first genomic characterization of clinical E. thailandicus isolates, demonstrating its ecological adaptability and opportunistic pathogenic potential. The detection of emerging acquired resistance genes to the last-resort antibiotic linezolid in a significant number of E. thailandicus genomes is particularly concerning, as it highlights the role of this underexplored species as a reservoir of clinically relevant adaptive traits. Expanded genomic surveillance is warranted to clarify its evolutionary trajectory and potential impact on human health.

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ID392 | Genomic and Phenotypic Bases of Virulence in Aspergillus Section Fumigati

Author: Alexandre Mendonça

CBMA – Center of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal

E-mail: alexandremendonca.1997@gmail.com

Other authors: Raquel Sabino, Ricardo Franco-Duarte, Paula Sampaio

Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; Faculdade de Medicina, Instituto de Saúde Ambiental, Universidade de Lisboa, Lisbon, Portugal; Laboratório Associado TERRA - Laboratório para o Uso Sustentável da Terra e dos Serviços dos Ecossistemas, Instituto Superior de Agronomia, Lisbon, Portugal; CBMA – Center of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal

Background: Cryptic species within section Fumigati present variable pathogenic potential with differences in its clinical relevance. However, the genomic basis and phenotypic differences underlying these variations remain poorly understood. Our aim was to analyze genomic and in vitro differences among section Fumigati, focusing on virulence traits, including thermotolerance, cell wall features, osmotic and oxidative stress, and alternative carbon sources utilization.

Methods: A set of species comprising Aspergillus fumigatus, A. lentulus, A. udagawae, A. felis and A. hiratsukae were analyzed. All available genomes on databases were screened to compare copy number variation (CNV) in genes linked to thermotolerance, cell wall constitution, osmotic, oxidative stress, and metabolic flexibility. The same categories were evaluated in vitro with 16 isolates. For thermotolerance, conidia were grown in glucose minimal media (GMM) at 37, 40 and 42 ºC. Stress assays used GMM supplemented with calcofluor white, NaCl and H2O2, and glucose was replaced by mannitol or lactose for carbon source tests. Differences were quantified by metabolic activity, and microscopy used to assess mycelial development.

Results: Genomic analysis revealed no relevant CNV differences, but phenotypic assays showed variability.

A. fumigatus displayed the highest metabolic efficiency and mycelial development at all temperatures. Under osmotic stress, metabolic inhibition was lower in A. fumigatus (50%), though most species germinated after 72 hours. For cell wall stress, inhibition was lower in A. fumigatus (~60%) compared to cryptic species (>75%), whereas cryptic species exhibited difficulty rearranging cell wall to initiate growth. Under oxidative stress, A. parafelis outperformed A. fumigatus and others, showing clear germination. For glucose, A. fumigatus showed the highest metabolic activity, whereas A. lentulus was more efficient in metabolizing mannitol. As for lactose metabolism, it was more efficient in A. lentulus, A. hiratsukae and A. felis.

Conclusions: Despite no major CNV differences, phenotypic assays revealed interspecies variability. A. fumigatus demonstrated superior thermotolerance, cell wall adaptability and overall efficiency, supporting its pathogenic dominance. Yet, cryptic species showed distinct adaptations. Broader genomic, transcriptomic and in vivo studies are needed to clarify their clinical relevance.

ID393 | In silico prediction of microRNA-candidiasis associations

Author: Bruna Fernandes Gonçalves

Centre of Biological Engineering, University of Minho, Gualtar Campus, 4710-057, Braga, Portugal

E-mail: bruna.goncalves@ceb.uminho.pt

Other authors: Tiago Miranda (1,2), Beatriz Rodrigues(1,2), Sílvia Gonçalves(2), Mariana Henriques(2,3), Sónia Silva (2,3), Filipa Ferraz(1)

1. 1ALGORITMI Research Centre/LASI, University of Minho, Braga, Portugal 2Centre of Biological Engineering, University of Minho, Gualtar Campus, Portugal 3LABBELS — Associate Laboratory, Braga/Guimarães, Portugal

Background: Infections caused by Candida species present significant clinical challenges, contributing to high morbidity and mortality rates. Emerging evidence suggests that microRNAs (miRNAs) may serve as valuable biomarkers for diagnosing and treating candidiasis, although the field remains under development. The limited number of reported miRNAs associated with candidiasis stems from the complexity and high cost of screening numerous potential biomarkers, making in silico prediction essential for identifying candidates for experimental validation. Thus, this study aimed to predict miRNA–candidiasis associations using a bioinformatics approach.

Methods: A systematic literature review was conducted to retrieve all reported miRNA–candidiasis associations. Additionally, multiple online databases including miRBase, miRDB, CDT, and Human Diseases, were mined to assemble the data required for the predictive model, including miRNA target genes, candidiasis-related target genes (overall and type-specific) and their prediction scores. The prediction model relied on the classic target-gene hypothesis (i.e. overlap between miRNA targets and candidiasis targets).

Results: Analysis of the retrieved data revealed that the most appropriate features for the prediction model were found to be the proportion of overlapping target genes, their statistical significance, and the mean prediction scores of overlapping genes. Model development and selection of the best approach were performed by comparing prediction scores with the set of reported associations. The best model identified a set of 425 miRNAs with prediction scores exceeding 50%, including 9 top candidates with scores exceeding 75%, strongly providing a ranked list of potential candidates for laboratory validation. We are currently adapting the model to predict miRNA associations for each type of candidiasis and developing a machine learning model to automate updates to our prediction scores as databases and literature evolve.

Conclusions: By delivering a robust framework for predicting candidiasis-related miRNAs, this work has the potential to make meaningful contributions to biomedicine and to help mitigate the devastating consequences of candidiasis through improved diagnostics and therapeutics.

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ID396 | Conjugative plasmids as biological weapons

Author: João S. Rebelo

CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal

E-mail: joaorebelo_4@hotmail.com

Other authors: Célia P. F. Domingues1,2, Francisca Monteiro1, Teresa Nogueira1,2, Francisco Dionisio1

1 CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal; 2 INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal

Background: Bacteria typically incur a fitness cost when carrying plasmids. Given this burden, it is difficult to explain why plasmids are so widespread in nature. Occasionally, chromosomal mutations arise that compensate for these costs. However, such compensatory mutations generally require hundreds of generations to emerge after plasmid acquisition, by which time plasmid-bearing cells may already have been outcompeted by faster-growing plasmid-free populations. This raises a key question: why would plasmids depend on the eventual appearance of compensatory mutations to persist within bacterial communities? Another explanation for the ubiquity of plasmids is that plasmids acquire and disseminate host-beneficial genes, such as those conferring antibiotic resistance. Yet, these also take time to occur and provide advantages only under specific environmental conditions (e.g., if antibiotics are present). We hypothesised that plasmids may function as biological weapons. In other words, donor bacteria may use plasmids to impose a fitness cost on recipient bacteria, thereby exploiting the nutrients that recipients are less able to consume.

Methods: We developed mathematical models and performed computer simulations to study the competitive evolution of bacterial populations in both liquid and structured environments. Populations evolved for 500 generations. Our models accounted for the emergence of compensatory mutations in both plasmids and chromosomes, as well as the effect of plasmid retransmission by transconjugants.

Results: Our results suggest that plasmids can indeed act as biological weapons, particularly in structured environments. Cells already adapted to plasmids increase their inclusive fitness through plasmid transfer, imposing costs on nearby plasmid-free cells, while enhancing the replication opportunities of neighboring relatives. Consequently, donor bacteria increase their relative abundance in most simulated conditions. This advantage does not depend on the occurrence of compensatory mutations in plasmids or chromosomes, nor on plasmid retransmission by transconjugants.

Conclusions: We demonstrate that plasmids can be used as biological weapons. The results support our hypothesis independently of plasmid genetic content, suggesting that this mechanism is broadly applicable to transferable plasmids in general.

ID397 | Efflux-Driven Antimicrobial Resistance in Staphylococcus pseudintermedius

Author: Marta Leal

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal

E-mail: a21001253@ihmt.unl.pt

Other authors: Sofia Santos Costa 1, Dennis Hanke 2,3 , Catarina Morais 1, Constança Pomba 4 , Andrea T. Feßler 2,3, Stefan Schwarz 2,3, Isabel Couto 1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, 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 CIISA, Centre of Interdisciplinary Research in Animal Health, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal

Background: Staphylococcus pseudintermedius is an opportunistic bacterium responsible for the majority of canine skin infections. The emergence of methicillin-resistant (MRSP) strains, often linked to multidrug-resistance phenotypes, limits treatment options in veterinary settings. The role of efflux in antimicrobial resistance for this species remains largely overlooked. In this study, we assessed the efflux-mediated response of S. pseudintermedius to ethidium bromide (EtBr) and the biocide tetraphenylphosphonium bromide (TPP), two known substrates of efflux pumps (EPs).

Methods: Three S. pseudintermedius clinical strains (two MRSP, one MSSP) and the type strain (DSM21284T, MSSP) were submitted to stepwise adaptation to EtBr and TPP. The adapted strains were tested for their susceptibility to eight antibiotics, five biocides and EtBr, in the presence/absence of efflux inhibitors (EIs). Fluorometric EtBr accumulation assays were conducted in the presence/absence of glucose and/or EIs. Whole-genome sequencing (Illumina MiSeq) was carried out for parental and adapted strains.

Results: EtBr-adapted strains showed reduced susceptibility to fluoroquinolones and biocides (TPP, benzalkonium chloride, chlorhexidine), whereas TPP-adapted strains revealed, in most cases, only reduced susceptibility to the same biocides. EIs promoted a reduction (>4-fold) in MICs of fluoroquinolones and biocides in the adapted strains, indicating increased efflux activity, later confirmed by EtBr accumulation assays. WGS analysis revealed changes in the promoter region

of the norA gene encoding the main staphylococcal EP NorA, mainly upon EtBr exposure. Conclusions: This study demonstrated that exposure of S. pseudintermedius to EtBr and TPP triggers variable efflux-mediated responses, that may result in reduced susceptibility to several biocides and resistance to fluoroquinolones, which are included in therapeutic options for S. pseudintermedius infections.

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ID399 | Breaking the Biofilm Barrier: Cannabis sativa THC Extracts as Revolutionary Anti-Candida Therapeutics

Author: Célia Fortuna Rodrigues

Associate Laboratory i4HB—Institute for Health and Bioeconomy, University Institute of Health Sciences—CESPU (IUCS-CESPU), 4585-116 Gandra, Portugal; UCIBIO—Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal; LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, ALiCE-Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

E-mail: celia.fortuna@iucs.cespu.pt

Other authors: Vanessa O. Paulo, Ana F. Morais, Eduarda P. M. Silva, Carlos J. A. Ribeiro

1 Department of Pharmaceutical Sciences, University Institute of Health Sciences—CESPU (IUCS-CESPU), 458-116 Gandra, Portugal; a26946@alunos.cespu.pt 2 Associate Laboratory i4HB—Institute for Health and Bioeconomy, University Institute of Health Sciences—CESPU (IUCS-CESPU), 4585-116 Gandra, Portugal 3 UCIBIO—Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (1H-TOXRUN, IUCS-CESPU), 4585-116 Gandra, Portugal 4 Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal 5 Avextra Portugal S. A., 1070-060 Lisboa, Portugal

Background: Candida infections represent a critical global health threat, with biofilm-forming species causing life-threatening invasive candidiasis in immunocompromised patients and persistent oropharyngeal infections across all populations. The alarming rise of non-Candida albicans Candida species, including WHO critical-priority pathogen C. auris, coupled with widespread antifungal resistance, demands urgent therapeutic innovation. Biofilms amplify this crisis by creating impenetrable microbial fortresses that resist conventional treatments, requiring drug concentrations up to 1000-fold higher than planktonic cells. We investigated Cannabis sativa tetrahydrocannabinol (THC)-rich extracts as novel anti-biofilm agents against three clinically relevant Candida species, targeting both established biofilms and preventing new formation, the dual-action strategy essential for therapeutic success.

Methods: A THC-rich Cannabis sativa extract was tested against reference strains of C. albicans, C. glabrata, and C. parapsilosis using standardized protocols: minimum inhibitory/fungicidal concentrations (MIC/MFC), minimum biofilm eradication concentration (MBEC), biomass quantification, confocal laser scanning microscopy, and antioxidant activity assessment.

Results: C. sativa extracts demonstrated extraordinary efficacy across all species:

-Fungicidal activity: 97.68-99.94% cell death across all tested Candida spp.

-Biofilm eradication: 82-98% destruction of established biofilms, with C. albicans showing highest susceptibility (98.12%)

-Biofilm formation: 27-38% reduction in biofilm biomass production, disrupting the formation process

-Antioxidant bonus: 54.52% radical-scavenging activity, suggesting additional therapeutic benefits

In addition, confocal microscopy confirmed high biofilm architectural disruption, transforming dense, multilayered structures into sparse, fragmented remnants.

Conclusion: These findings unveil C. sativa THC extracts as potent dual-action antifungals capable of both killing Candida cells and dismantling their biofilm defenses. With activity against WHO priority pathogens and potential to overcome resistance mechanisms, Cannabis-derived therapeutics could revolutionize candidiasis treatment, offering hope where conventional antifungals fail. This research

opens new frontiers in natural product-based antimicrobial therapy, positioning C. sativa as a promising source for next-generation antifungal agents greatly needed in clinical practice.

ID409 | Deciphering efflux-mediated resistance in Staphylococcus coagulans

Author: Mariana Andrade

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal.

E-mail: a21001252@ihmt.unl.pt

Other authors: Dennis Hanke2,3, Catarina Morais1, Andrea T. Feßler2,3, Stefan Schwarz2,3 Isabel Couto1, Sofia Santos Costa1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, 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

Background: Staphylococcus coagulans is a common cause of skin and soft tissue infections in companion animals, requiring antimicrobial therapy. While efflux is known as the first-line response to antimicrobial agents in other staphylococci, there is limited data on its role in S. coagulans. This study aims to investigate efflux-mediated antimicrobial and biocide resistance in S. coagulans.

Methods: S. coagulans DSM6628T was adapted to ethidium bromide (EtBr, a substrate of a wide range of multidrug resistance efflux pumps in staphylococci) through a step-wise process with increasing EtBr concentrations (0.125-32 mg/L). The parental and EtBr-adapted strain, DSM6628_EtBr, were evaluated for their antimicrobial and biocide susceptibility by minimal inhibitory concentration (MIC) determination. Efflux activity was analyzed by (i) re-determination of antimicrobial and biocide MICs in the presence of known efflux inhibitors, verapamil (VER) and thioridazine (TZ), at ¼ of their MIC; (ii) EtBr efflux and accumulation real-time fluorometric assays in the presence and absence of glucose. In addition, both strains were sequenced using a hybrid approach.

Results: S. coagulans DSM6628_EtBr was obtained after nine passages in EtBr, showing an EtBr MIC of 32 mg/L, a 128-fold increase from the parental strain. It exhibited reduced susceptibility to the hydrophilic fluoroquinolone norfloxacin and several biocides, with 2-8 fold MIC increases. The efflux inhibitors restored the norfloxacin and biocide MICs to the levels shown by the parental strain. The fluorometry assays confirmed the increased efflux activity of the EtBr-adapted strain. Current work focuses on gene expression analysis to identify the efflux pump(s) related to the efflux-mediated phenotype, and to complement WGS data that already revealed an amino acid exchange in NorA, a main efflux pump in staphylococci.

Conclusions: This work demonstrates an efflux-mediated response to exposure to efflux pump substrates, in reduced susceptibility phenotypes to antimicrobial agents and biocides in S. coagulans, highlighting the need for further research of this resistance mechanism in this relevant animal pathogen.

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ID413 | Salmonella spp. isolates in Portugal, 2024

Author: Leonor Silveira

National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

E-mail: leonor.silveira@insa.min-saude.pt

Other authors: Raquel Caeiro1; Ana Margarida Henriques1; Alexandra Tomás1; Ângela Pista1

1 National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

Background: Salmonella spp. remains one of the main causes of gastrointestinal infections in the European Union, only preceded by Campylobacter spp. The most common serovars of Salmonella spp. causing illness are Salmonella enterica enterica serovar Enteritidis, Typhimurium and the monophasic variant of Typhimurium (4,[5]:i:-). The aim of this study was to evaluate the Salmonella spp. isolates circulating in Portugal in 2024, accounting the number of cases, evaluating the available epidemiological data, and analysing the phenotypic and genotypic data gathered.

Methods: The National Reference Laboratory received isolates from public and private hospitals and laboratories across Portugal for serotyping using the Kauffman-White-Le Minor scheme. Isolates were also subjected to antimicrobial susceptibility testing (AST) according to EUCAST recommendations, by the disc diffusion method for 18 antibiotics. Genomes of Salmonella isolates of serovars of interest were sequenced, either on a Miseq or Nextseq Illumina platform, and raw reads were submitted to Enterobase and to several CGE tools.

Results: In 2024, a total of 873 isolates were received corresponding to an increase of approximately 40%, comparing to the previous year. Most cases belonged to children (n=409; 46.8%) and in residents of Lisbon and Tagus Valley (25.3%). The most common serotypes were S. monophasic Typhimurium (32.2%; n=281), S. Enteritidis (27.4%; n=281), and S. Typhiumurium (17.3%; n=151). In total, 510 isolates (58.4%) were resistant to at least one antibiotic tested. Multi-drug resistance was observed in 20.3% (177/873) of isolates, with the most common MDR serovars being S. Typhimurium (n=98; 55.4%) and its monophasic variant (n=55; 31.1%). Four isolates were ESBL producers, presenting blaDHA-1, blaCTX-M-65, blaCTX-M -9, and blaOXA-1, and one was also a carbapenemase producer, presenting both blaOXA-1 and blaOXA -48. WGS revealed several clusters, namely among the top three serovars.

Conclusions: The considerable increase in cases was intrinsically connected to several clusters of the most frequent serovars, most specially of MDR isolates. To our knowledge, this was the first time a carbapenemase producer Salmonella, isolated from a human clinical sample, was detected in Portugal. Salmonella continued laboratory surveillance and antimicrobial resistance monitoring are essential for disease prevention and control.

ID422 | Towards a paratransgenesis tool: Whole Genome Sequencing of bacteria isolated from Anopheles mosquitoes midgut microbiota

Author: Margarida Marques

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

E-mail: a21002435@ihmt.unl.pt

Other authors: Vânia Pobre2, Sofia G. Seabra1, Sofia Santos Costa1, Sandra N. Pinto3, Henrique Silveira1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal; 2 Instituto de Tecnologia Química e Biológica António Xavier, ITQB, Universidade NOVA de Lisboa, UNL, Lisbon, Portugal; 3 iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, IST, Lisbon, Portugal

Background: Malaria is the most prevalent mosquito-borne disease worldwide. Paratransgenesis involves the genetic modification of symbiotic microorganisms of disease vectors, to reduce pathogen transmission. Ideal traits for a paratransgenesis tool include minimal risk of spreading antimicrobial resistance, robust biofilm formation and flexibility for genetic manipulation. The genomic characterization of bacteria from the Anopheles microbiota is crucial for understanding the bacterial mechanisms with potential to be explored for paratransgenesis. This work highlights the importance of whole genome sequencing (WGS) in identifying/characterizing ideal bacterial candidates for a paratransgenesis strategy.

Methods: WGS was performed for Pseudomonas mendocina Ag_C1 and Serratia marcescens As_A1-C1, bacteria isolated from Anopheles midguts, with the aim of acting as potential candidates for paratransgenesis, specifically targeting biofilm formation. Sequencing was performed using Illumina paired-end technology. FastQC and Trimmomatic were used for quality control and preprocessing of the raw reads. The sequences were de novo assembled using Unicycler, and assembly quality was assessed with Quast. Genome annotation was carried out using Prokka.

Results: Genome annotation identified 4984 coding sequences for P. mendocina and 5179 for S. marcescens, including genes linked to biofilm formation. For P. mendocina, genes essential for flagella and type IV pili production were identified. Genes from the alg operon, along with several of its regulators (e.g. kinB, mucA) were identified, which are crucial for alginate synthesis, responsible for the mucoid phenotype of Pseudomonas biofilms. For S. marcescens, genes associated with motility (fli, flg, flh operons) were identified. Genes bsmA and wecA were detected, which are essential to produce exopolysaccharides for the extracellular matrix. Genes involved in two-component regulatory systems and adhesion processes (rssA) were identified, but also genes associated with reduced biofilm production (bssS, tabA). Additionally, the swr genes, essential for quorum sensing regulation in this species, were not detected.

Conclusion: These findings reveal genomic differences in bacteria within Anopheles microbiota, with P. mendocina presenting a higher number of identified genes associated with biofilm formation. This bioinformatic approach lays the groundwork for future interventions aimed at reducing malaria transmission.

ID423 | Efflux pump contribution to antimicrobial resistance in Neisseria gonorrhoeae: phenotypic and molecular characterization

Author: Amélia Marinheiro

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa; Rua da Junqueira nº 100, 1349-008 Lisboa, Portugal

E-mail: amelia.marinheiro@outlook.com

Other authors: Sofia Santos Costa; Miguel Viveiros; Liliana Rodrigues

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa; Rua da Junqueira nº 100, 1349-008 Lisboa, Portugal

Background: The emergence of antimicrobial resistance (AMR) in Neisseria gonorrhoeae has become an increasing concern worldwide. Among the key mechanisms involved, efflux pumps (EPs) play a central role by reducing the intracellular antimicrobial concentration, potentially facilitating the development of additional resistance mechanisms. This study aims to investigate the role of EPs in AMR development in N. gonorrhoeae through in vitro adaptation and subsequent phenotypic and molecular characterization of adapted strains.

Methods: The reference strain N. gonorrhoeae ATCC 49226 was gradually exposed to increasing concentrations of azithromycin - an efflux-prone antibiotic used in first-line treatment - and ethidium bromide (EtBr), a model efflux pump substrate. Afterwards, the resulting adapted strains were assessed for changes in antimicrobial susceptibility by determining minimum inhibitory concentrations (MIC) for azithromycin, EtBr, and other agents, in the presence and absence of efflux inhibitors. Efflux activity of the adapted strains was further evaluated by real time fluorometry, measuring EtBr accumulation at different conditions, w/wo selected efflux inhibitors. Lastly, gene expression analysis of EP components and associated regulators is currently underway, using RT-qPCR.

Results: Two adapted strains were obtained, derived from the parental reference strain. The azithromycin-adapted strain exhibited a 32-fold increase in the MIC for azithromycin compared to the parental strain, while the EtBr-adapted strain showed a two-fold increase. In fluorometric assays, the EtBr-adapted strain showed increased EtBr accumulation when in the presence of efflux inhibitors, suggesting enhanced efflux activity. In contrast, the azithromycin-adapted strain showed no significant difference in EtBr accumulation, compared to the parental strain, pointing to the possible involvement of alternative resistance mechanism beyond efflux. Ongoing molecular analysis may help clarify the contributions of efflux systems and regulatory genes in resistance evolution.

Conclusions: These results underscore the multifactorial nature of AMR in N. gonorrhoeae and reinforce the need for comprehensive surveillance and targeted therapeutic strategies.

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ID424 | Exploring new drugs for Neisseria gonorrhoeae through *in silico* repurposing targeting efflux mechanisms

Author: Inês Pereira

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa; R. da Junqueira 100, 1349-008 Lisboa; Portugal

E-mail: inespereira00@gmail.com

Other authors: André Pina 1; Inês Vitor 1; Beatriz Santos 1; Ana Henriques 1; Isabel Couto 1; Filomena Pereira 1; Miguel Viveiros 1; Ana Armada 1; Sofia Santos Costa 1; Liliana Rodrigues 1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa; R. da Junqueira 100, 1349-008 Lisboa; Portugal

Background: The rising antimicrobial resistance in Neisseria gonorrhoeae highlights the urgent need to develop novel therapeutic strategies. This pathogen can utilise efflux systems capable of extruding several antibiotics, including first-line agents such as ceftriaxone and azithromycin. This mechanism results in lower intracellular antibiotic concentrations and, consequently, reduced susceptibility towards these agents. Targeting these systems may restore antibiotic efficacy against resistant strains. Drug repurposing is an alternative to de novo drug discovery, identifying new therapeutic applications for drugs approved for other indications. In this study, we aim to identify and evaluate candidate drugs for repurposing as adjuvants for the treatment of N. gonorrhoeae infection, specifically targeting efflux mechanisms.

Methods: Public databases DrugBank and STITCH were used to identify approved drugs with homologs of target proteins involved with membrane transport or energy metabolism in N. gonorrhoeae. Selected drugs were further tested for their antimicrobial properties against N. gonorrhoeae ATCC 49226 by minimum inhibitory concentration (MIC) determination using a microdilution assay. This method was also employed to evaluate potential adjuvant effects by combining the candidate drugs at one-fourth of their MIC with a panel of antibiotics and the efflux pump substrates ethidium bromide (EtBr) and tetraphenylphosphonium bromide (TPP-Br). Potential efflux inhibitory activity was also studied using real-time fluorometric detection of EtBr accumulation.

Results: In silico analysis identified 32 potential targets, predicted to interact with 57 approved drugs, from which 20 were selected for further studies. Two drugs presented high antimicrobial activity (MIC ≤ 4 mg/L). Atovaquone was able to significantly reduce the MICs of azithromycin and kanamycin. Ketotifen and verapamil showed potential efflux inhibitory activity, as evidenced by a reduction in EtBr MIC and increased intracellular accumulation.

Conclusions: This work demonstrates the potential of repurposing drugs targeting efflux-systems for the development of new approaches to overcome the alarming increase of antimicrobial resistance in N. gonorrhoeae.

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ID425 | Gut Microbiome Signatures in Rural and Urban Elderly from the Algarve region

Author: José Matos

1 Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve Campus Gambelas, Faro 8005-139, Portugal; 3 Algarve Biomedical Center—Research Institute, 8005-139 Faro, Portugal;

E-mail: jjmatos@ualg.pt

Other authors: Pedro Castelo-Branco1,3, ALFAScore Consortium, Maria Leonor Faleiro2,3

1 Faculty of Medicine and Biomedical Sciences (FMCB), University of Algarve Campus Gambelas, Faro 8005-139, Portugal; 2 Algarve Biomedical Center—Research Institute, 8005-139 Faro, Portugal; 3 Faculty of Sciences and Technology, University of Algarve Campus Gambelas, Faro 8005-139, Portugal;

Background: Aging is accompanied by marked alterations in the human gut microbiome and several factors, including diet, lifestyle, and geographic context, have been shown to modulate microbial diversity. In elderly individuals, the gut bacterial community structure may provide valuable insights into both resilience and vulnerability to disease. Understanding these patterns is particularly relevant in regions with distinct rural and urban settings, where differences in lifestyle and environment may allow recognizable microbial signatures. This study aimed to characterise and compare the intestinal microbiome of older adults living in rural and urban areas from South of Portugal, Algarve, with a focus on diversity indices and functional microbial outputs.

Methods: Fecal samples were collected from elderly participants and processed through a meta-taxonomic approach using the 16S rRNA Barcode Kit (SQK-RAB204). Sequencing was performed with the Oxford Nanopore system. The microbial community structure was established by Principal Component Analysis (PCA) based on Bray–Curtis dissimilarity. α and β diversity were assessed to capture richness, evenness, and inter-individual variability. At functional level total bile acid and the short-chain fatty acids (SCFAs) were determined.

Results: Notable differences were observed between geographic groups. Elderly participants from rural areas exhibited higher abundances of Bacillota. α-diversity analysis indicated greater species richness in the rural group, while β-diversity analysis revealed higher heterogeneity among urban participants. These findings suggest that lifestyle and environmental conditions strongly shape microbiome resilience, with urban participants displaying lower richness and evenness. Importantly, beneficial bacteria, such as Agathobacter rectalis and Faecalibacterium prausnitzii, were enriched in rural individuals. Total bile acid levels were similar between rural and urban individuals, while acetate production was higher in urban elderly participants.

Conclusions: This study highlights distinct bacterial signatures in the gut microbiome of rural and urban elderly populations from Algarve. By demonstrating how demographic and environmental factors interact to shape microbial ecosystems in aging. Ultimately, this knowledge may support the development of microbiome-based strategies and interventions aimed at promoting resilience, preventing disease, and improving quality of life in elderly.

ID427 | Fluconazole-resistant yeasts with virulence profiles from the vulva: evidence of a potential reservoir

Author: Joana Rolo

RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal

E-mail: joanarolo@fcsaude.ubi.pt

Other authors: Maria Margarida Silva, Mariana Zagalo Fernandes, Sofia Moura, Ana Sofia Oliveira, Carlos Gaspar, José Martinez-de-Oliveira, Ana Palmeira-de-Oliveira

1RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 2Faculty of Health Sciences, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 3Department of Clinical Pathology, Santarém District Hospital, Lezíria Local Healthcare Unit, Santarém, Portugal; 4Labfit–Health Products Research and Development Lda, UBImedical, Estrada Nacional 506, Covilhã, Portugal

Background: Vulvovaginal candidosis is an affliction caused by Candida yeasts, that affects millions of women worldwide. Most episodes of infection are associated with Candida albicans, but non-Candida albicans (NCA) species have been increasingly reported in the female genital tract. To better understand the role of NCA in infection, in this study we aim to fully identify and characterize vulvar NCA isolates.

Methods: 46 yeast isolates yielding non-green colonies on CHROMagar Candida, indicative of NCA species, were obtained from 23 vulvar swabs of women attending a gynecological consultation. Species identification was performed by sequencing D1/D2 domain of the large subunit of 28S rRNA gene. Fluconazole susceptibility was assessed by the microdilution broth method. Biofilm biomass was quantified using crystal violet staining. Phospholipase and hemolysin production was assessed by plating a calibrated suspension in egg-yolk Sabouraud agar, and blood agar, respectively. Finally, adherence to cervical cells was assessed by incubating yeasts with a monolayer of HeLa cells.

Results: Among the 46 NCA vulvar isolates, 11 different species were identified. We found that the most frequently recovered species among vulvar samples was Candida parapsilosis (25%), followed by Nakaseomyces glabratus (15%) and Meyerozyma guilliermondii (15%). Overall, we found that 45% (21/46) of the vulvar isolates were resistant to fluconazole. Regarding biofilm production, we found that Clavispora lusitaniae was the species that produced a higher amount of biomass. Pichia kudriavzevii was the only species that produced phospholipase and we were able to detect hemolysin production in isolates belonging to six different yeast species. Finally, we found that all species had the ability to adhere to HeLa cells, with the exception of Candida parapsilosis.

Conclusions: We found a high species diversity among NCA vulvar isolates. Of note, we also recovered a high frequency of fluconazole-resistant isolates. The expression of virulence factors, such as biofilm formation, extracellular enzymes production and adherence to cervical cells were frequently found among vulvar isolates. These results indicate that the vulva could be acting as a reservoir for fluconazole-resistant yeasts, that are also potentially virulent.

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ID431 | Predictive Model for Artemether–Lumefantrine Treatment Failure for Uncomplicated Plasmodium falciparum Malaria

Author: Ana Margarida Gonçalves

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: b14842@med.uminho.pt

Other authors: Ana Santos-Pereira 1,2, Maria Jardim 3,4, Beatriz Fonseca 1,2, Claudia Fançony 1,2,4, Nuno S. Osório 1,2, Maria Isabel Veiga 1,2

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3 Clínica Multiperfil, Luanda, Angola; 4 Health Research Center of Angola (CISA), Caxito, Angola

Background. Substantial progress has been made in reducing the global malaria burden; however, the disease remains endemic in many regions. Sub-Saharan Africa, in particular, accounted for 94% of malaria cases and 95% of malaria deaths in 2022. That year, an estimated 249 million cases and 608,000 deaths occurred worldwide. Therapeutic failure in malaria cases poses a major obstacle to achieving eradication goals. In this context, drug efficacy clinical trials hold a crucial role in national surveillance systems by monitoring therapeutic effectiveness and identifying predictive factors which could help uncover the underlying causes of treatment failure. These insights are essential to update malaria treatment strategies, ensuring the success of treatment, and maintain progress in malaria research and disease control efforts.

Methods. A drug efficacy clinical trial involving artemether-lumefantrine (AL) was conducted in Angola, an endemic country where AL is the first-line malaria treatment (Trial registration: PACTR202405595426468), to develop three distinct predictive models for malaria treatment failure. Data from 108 patients with a 28-day follow-up were used to identify predictive factors, including demographic (e.g., age, sex), clinical (e.g., adverse symptoms and biochemical analyses), outcome (e.g., Early Treatment Failure, Adequate Clinical and Parasitological Response), and parasitological data (e.g., parasite density, fever clearance time).

Results. We aim to develop and validate three predictive models, each tailored to different levels of data complexity: (1) an initial model to leverage readily available data to be directly applicable in clinics with resource-limited settings to predict therapeutic failure using demographic, clinical, treatment outcome, and parasitological data; (2) a second model to enhance the initial model with features from microscopic blood smear analyses, taking into account the parasite morphology throughout the trial; and (3) a final model which will integrate genetic data from both the malaria parasite and the human host, exploring their potential role in understanding resistance to AL treatment.

Conclusions. Preliminary results highlight the importance of age and hemoglobin levels as potential predictors of the therapeutic outcome. Future tailoring of the model could deepen our understanding of the disease and the underlying factors for therapeutic failure in patients, potentially unravelling new research avenues.

ID439 | Potential of Cistus salviifolius and Helichrysum stoechas Extracts as Natural Adjuvants to Antibiotic Therapy Against Staphylococcus aureus

Author: Alexandra Coimbra

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

E-mail: alexandra.coimbra@ubi.pt

Other authors: Ângelo Luís 1, Pedro Dinis Gaspar 2, Susana Ferreira 1, Ana Paula Duarte 1

1 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal; 2 C-MAST—Center for Mechanical and Aerospace Science and Technologies, University of Beira Interior, 6201-001 Covilhã, Portugal

Staphylococcus aureus is a common commensal of the skin and mucous membranes that can become opportunistically pathogenic, causing mild to life-threatening infections. The worldwide rise of antimicrobial resistance is compromising the effectiveness of standard therapies and requires alternative strategies that are both safe and effective. Plant-derived phytomedicines have renewed interest because they can act through multiple mechanisms. By simultaneously targeting bacterial survival and virulence, these multi-target approaches may reduce treatment failure and slow the development of resistance. Accordingly, this study aimed to evaluate the extracts of Cistus salviifolius and Helichrysum stoechas against S. aureus, including methicillin-resistant strains (MRSA), highlighting their potential to enhance antibiotic efficacy while mitigating bacterial virulence.

Hydroethanolic extracts of C. salviifolius and H. stoechas were prepared and evaluated for their antibacterial activity against a reference strain of S. aureus and two clinical MRSA isolates, including assessment through time-kill assays. Their combined effects with the antibiotic's ampicillin, ciprofloxacin, and vancomycin were analysed using the checkerboard assay, along with the modulatory influence in ethidium bromide. The potential to attenuate virulence was assessed by testing quorum-sensing inhibition in Chromobacterium violaceum and the ability to prevent biofilm formation in S. aureus.

The H. stoechas extract exhibited the strongest activity, with MICs ranging from 7.8 to 62.5 µg/mL. When combined with the antibiotic's ampicillin, ciprofloxacin, or vancomycin, C. salviifolius and H. stoechas extracts showed predominantly synergistic or additive interactions, with several combinations restoring susceptibility in resistant strains. In support of a resistance-modulating mechanism, aerial parts extract of C. salviifolius altered ethidium bromide MICs in a manner consistent with efflux pump inhibition and exhibits slight inhibition of quorum-sensing in C. violaceum. Additionally, both extracts inhibited S. aureus biofilm formation at subinhibitory concentrations, demonstrating antivirulence activity that could reduce pathogenicity and improve antibiotic penetration.

Together, these findings indicate that the extracts not only possess intrinsic antibacterial activity but also act as potential adjuvants that attenuate virulence and counteract resistance mechanisms.

ID444 | Chlorohexidine's bactericidal activity and possible role in cross-resistance to antibiotics in enterococci

Author: Catarina Geraldes

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), Faculty of Veterinary Medicine, University of Lisbon, Portugal

E-mail: cgeraldes@fmv.ulisboa.pt

Other authors: Carina Matias 3†, Manuela Oliveira 1,2,4, Carina Vingsbo Lundberg 3

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), Faculty of Veterinary Medicine, University of Lisbon, Portugal; 3 Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark; 4 cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Portugal; † Both authors equally contributed to the presented work.

Background: Enterococci are bacteria inherent to the hospital environment, being a leading cause of hospital acquired infections, with prevention associated with the use of biocides such as chlorohexidine. For this reason, the study of bacterial-biocide interactions is of significant value to guarantee maximum efficiency by these compounds, but also to prevent any consequences of misuse, such as a potential cross-resistance to antibiotics.

Methods: A total of 8 isolates were used: 2 veterinary clinical isolates, 2 veterinary commensal isolates, 2 veterinary environmental isolates and 2 human clinical isolates. Isolates underwent a time kill assay in the presence of several concentrations of chlorohexidine - 8, 4, 2, 1 and 1/2 times the Minimal Bactericidal Concentration. Then, samples were collected at 0, 15, 30, 60, 130 minutes and 24-hour marks, serially diluted and plated onto Columbia agar supplemented with sheep blood in order to ascertain bacterial concentration. Two colonies were collected from the plate corresponding to the highest concentration of chlorohexidine that presented growth and the Minimum Inhibitory Concentration of six different antibiotics – ampicillin, vancomycin, linezolid, daptomycin, ciprofloxacin and gentamicin – was determined, according to CLSI guidelines, to determine modification on their antibiotic susceptibility patterns.

Results: Isolates presented dissimilar degrees of susceptibility to chlorohexidine, with the highest concentration tested, being capable of eliminating all bacteria in 15 minutes or less. Lower concentrations had a more prolonged response. No biphasic killing curves, associated with possible persister development were observed. A total of 6 isolates were submitted to MIC testing; however, no rises in these values were observed.

Conclusions: Although no antibiotic cross resistance was detected, the testing of a broader panel of isolates and antibiotics, expanding the time for resistance to develop, and the application of genotypic methodology would be important to further investigate this hypothesis.

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The work was conducted at the Antimicrobial Resistance & Pre-clinical Research laboratory (Contract Research), Bacteria, Parasites & Fungi at Statens Serum Institut.

ID453 | Vulvovaginal Mycobiome Dynamics during Pregnancy: A Preliminary Study

Author: Mariana Fernandes

RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; Faculty of Health Sciences, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal

E-mail: marianamzf@hotmail.com

Other authors: Emanuel Maldonado1,2, Carlos Gaspar1,2, José Martinez-de-Oliveira1,2, Ana Palmeira-de-Oliveira1,2,3, Joana Rolo1,2

1 RISE-Health, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 2 Faculty of Health Sciences, University of Beira Interior (UBI), Av. Infante D. Henrique, Covilhã, Portugal; 3 Labfit–Health Products Research and Development Lda, UBImedical, Estrada Nacional 506, Covilhã, Portugal

Background: The fungal component (mycobiota) of the human genital microbiota is largely unexplored in asymptomatic individuals, especially during pregnancy. Profound hormonal and immunological changes occur throughout gestation, which may influence fungal colonisation patterns and potentially impact maternal health and neonatal outcomes. The MycoV project aims to characterise the vulvar and vaginal mycobiota of pregnant and non-pregnant women via fungal-specific DNA extraction and sequencing of the 26S rRNA gene (D1–D2 region). Here, we report the fungal DNA load differences from the quantification phase.

Methods: A total of 70 paired swab samples were collected from both genital niches of asymptomatic pregnant (n = 22) and non-pregnant (n = 13) women. The vulvar samples were specifically collected from the anterior commissure. Fungal DNA was recovered using an optimised extraction protocol, and DNA yield was assessed through spectrophotometry.

Results: In non-pregnant women, DNA loads were consistently higher in the vagina (median 138.4 ng/µL, average 184.3 ng/µL) than in the vulva (median 1.2 ng/µL, average 2.3 ng/µL). In pregnant women, however, this niche difference was markedly attenuated, with vulvar DNA yields substantially elevated (median 67.6 ng/µL, average 84.3 ng/µL), approaching vaginal levels (median 147.4 ng/µL, average 148.7 ng/µL).

Conclusions: We found a notable and consistent difference in DNA yield between the vulvar samples of pregnant and non-pregnant women. These findings suggest pregnancy may favour increased fungal biomass or colonisation at particular genital niches, potentially due to pregnancy-associated physiological, immune and hormonal shifts.

The next phase of the project will employ high-throughput sequencing to characterise the taxonomic composition of the mycobiota across all samples. By comparing fungal diversity and abundance in both niches and physiological states, we aim to identify potential biomarkers of fungal dysbiosis or resilience.

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ID456 | Galleria mellonella Research Hub: a sustainable in vivo infection model at GHTM/IHMT-NOVA

Author: Sofia Santos Costa

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal.

E-mail: scosta@ihmt.unl.pt

Other authors: Mariana Andrade1, Joana Neves1, Joana Marques1, Pedro Rita1, Ana Armada1, Sofia G. Seabra1, Sofia Cortes1, Filipa Vale2, Liliana Rodrigues1, Henrike Krüger-Haker3,4, Andrea T. Fessler3,4, Stefan Schwarz3,4, Marcelino Garrine1,5, Inácio Mandomando1,5,6,7, Henrique Silveira1, Miguel Viveiros1, Isabel Couto1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal; 2 Pathogen Genomics and Translational Microbiology Group, BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; 3 Institute of Microbiology and Epizootics, Centre for Infection Medicine, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 4 Veterinary Centre for Resistance Research (TZR), School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 5 Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique; 6 Instituto Nacional de Saúde (INS), Marracuene, Maputo, Mozambique; 7 ISGlobal-Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

Background: Galleria mellonella larvae represent a sustainable invertebrate infection model that is gaining interest for assays on pathogenicity and infection, host-pathogen interaction or pharmaco-toxicological studies. The advantages of this model include its low cost, easy manipulation of the larvae, a short life cycle that ensures a constant availability of larvae, and most importantly, an innate immune system that shares several similarities with the mammal innate immune response. Herein, we present the set of assays implemented at G. mellonella Research Hub, a platform at GHTM/IHMT-NOVA for the scientific community.

Methods: The G. mellonella Research Hub comprises (i) a dedicated room for the maintenance of the colony at 28ºC in the dark, where the larvae are reared under a standardized high-nutrition diet, and (ii) a laboratory dedicated to assays with G. mellonella. We have optimized infection assays for various human and animal bacterial pathogens, as well as assays for assessing drug toxicity and efficacy, with measurement of different parameters, including larva mortality, standardized health score, bacterial burden, hemocyte count and viability by fluorescence microscopy.

Results: Our G. mellonella colony yields ~20,000 larvae/generation (~35 days). Studies from our team and collaborators, allowed to implement infection assays for reference strains of human and animal bacterial pathogens, shown here in decreasing order of virulence potential: Pseudomonas aeruginosa > A. baumannii > Escherichia coli > Staphylococcus spp. > Enterococcus faecalis > Neisseria gonorrhoeae > Helicobacter pylori > Pasteurella multocida >>> Mannheimia haemolytica. Other assays implemented include the establishment of LD50 values for several drugs and solvents, and drug efficacy assays in S. aureus, S. epidermidis and E. faecalis. We are currently expanding our set of assays to establish a hemocyte cell line and infection assays for the intracellular protozoan Leishmania spp.

Conclusions: The G. mellonella Research Hub represents a build-up on the in vivo infection models available at GHTM/IHMT-NOVA, paving the way for a more rapid and high-throughput testing of pathogenicity and drug discovery, increasing the sustainability of our research in compliance with the 3R principles.

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ID462 | In vivo selection fails to extend circulation time of phage 3043 against Acinetobacter baumannii

Author: Susana Sousa e Cunha

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: susana.cunha7@hotmail.com

Other authors: Alexandra Gabriel Fraga 1,2; Henrique Machado 1,2; Alexandre Lima 3; Joana Azeredo 3; and Jorge Pedrosa 1,2

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3 Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal

Background: Antimicrobial-resistant infections represent a serious public health threat, for which effective alternatives are urgently needed. Bacteriophage (phage) therapy has reemerged as a promising option, but its clinical use remains limited by challenges such rapid clearance by the mononuclear-phagocyte system. This reduces circulation time, bioavailability, and, ultimately therapeutic efficacy. In this study, we aimed to develop a mutant with prolonged systemic persistence, by using a previously described serial-passage technique in mice [1], to select a "long-circulating" phage active against Acinetobacter baumannii, one of the most dangerous and drug-resistant pathogens.

Methods: To improve the pharmacokinetic properties of phage 3043, in vivo selection was performed through serial intraperitoneal injections in BALB/c mice. The resulting phage population, referred to as 3043 LC (long-circulating), was then compared to the wild-type (WT) 3043 phage.

Results: In vitro characterization included a one-step growth curve, adsorption assay, and resistance assay to evaluate differences in phage replication dynamics, adsorption efficiency, and emergence of bacterial resistance, with no differences observed regarding these parameters. We next performed a biodistribution assay following intraperitoneal injection and observed no significant differences in the blood, spleen, or liver. Finally, we assessed the antibacterial activity of both phage variants in vivo using a murine systemic infection model with A. baumannii Ab45. Regardless of the infectious dose administered, phage 3043 LC exhibited comparable efficacy to the WT phage.

Conclusions: In conclusion, although this in vivo selection strategy improved the pharmacokinetic properties of phages Argo1 and Argo2 against E. coli [1], no similar benefits were observed for phage 3043. The lack of enhanced circulation or therapeutic efficacy suggests that the approach may be phage-specific, with some phages—such as 3043—exhibiting intrinsic stability or resistance to adaptive mutations, that limits further optimization by serial in vivo passaging.

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ID465 | Differential Effects of Fatty Acid Synthase and Acetyl-CoA Carboxylase Inhibition on Macrophage Immunity to Mycobacterium ulcerans infection

Author: Susana Sousa e Cunha

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

E-mail: susana.cunha7@hotmail.com

Other authors: Ana Margarida Barbosa 1,2; Henrique Machado 1,2; Jorge Pedrosa 1,2; Alexandra Gabriel 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

Background: Buruli Ulcer (BU) is a neglected tropical disease caused by Mycobacterium ulcerans, which produces mycolactone, the key virulence factor responsible for the painless necrotizing ulcers characteristic of BU. Despite the introduction of effective antibiotic treatment in 2004, delays in seeking medical care often led to permanent disability and substantial financial burdens. These challenges underscore the urgent need for innovative therapeutic strategies to improve clinical outcomes and reduce long-term impact. Renewed interest in immunometabolism has revealed how metabolic changes during infections may offer new therapeutic targets. Understanding these metabolic shifts could pave the way to novel therapeutic strategies in BU.

Methods: In this study, we aimed to explore host metabolic alterations during M. ulcerans infection. For that, two distinct strains of M. ulcerans were used to infect macrophages: mycolactone-producing strain (mycoMu) and non-mycolactone-producing strain (ΔmycoMu).

Results: Our RNA sequencing data revealed an upregulation of lipid metabolism pathways in mycoMu-infected macrophages, while inflammatory-related genes were upregulated in ΔmycoMu-infected macrophages. To further explore this data, we inhibited fatty acid synthesis using two different inhibitors: C75 or SorA. Interestingly, while no differences were found for mycoMu-infected macrophages upon treatment with C75, we did find an increased bacterial burden in ΔmycoMu-infected macrophages, associated with an increase in IL-β production. The addition of SorA did not result in any alterations in bacterial burden or in reactive oxygen species and cytokine production.

Conclusions: These findings suggest that modulation of host lipid metabolism may influence the outcome of M. ulcerans infection. Further studies are needed to better understand the role of this metabolic pathway to provide valuable insights for potential future therapeutic development.

ID479 | Enterococcus in Companion Animals and Humans: Comparative Analysis of Antimicrobial Resistance and Virulence Traits

Author: Joana Monteiro Marques

1 Centre 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 National Institute of Agricultural and Veterinary Research, IP (INIAV), Oeiras, Portugal

E-mail: jmarques@fmv.ulisboa.pt

Other authors: Beatriz Pita 4, Daniel Pinto 1,2, Maria Teresa Barreto-Crespo 5,6, Rosario Mato 7, Teresa Semedo-Lemsaddek 1,2,8

1 Centre 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; 4 NOVA School of Science and Technology (NOVA FCT), NOVA University of Lisbon, Caparica, Portugal; 5 Institute of Experimental Biology and Technology (iBET), Oeiras, Portugal; 6 Institute of Chemical and Biological Technology António Xavier (ITQB), Oeiras, Portugal; 7 Research Unit on Applied Molecular Biosciences (UCIBIO) and Associate Laboratory Institute for Health and Bioeconomy (i4HB), Caparica, Portugal; 8 Biosystems & Integrative Sciences Institute (BioISI), Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Background: Antimicrobial resistance (AMR) is a global health issue, requiring a One Health approach to track transmission across humans, animals, and the environment. Enterococcus spp., common commensals and opportunistic pathogens in both animals and humans, serve as indicators of interspecies bacterial exchange.

Methods: Enterococcus from companion animals and their tutors were analyzed to compare AMR profiles and virulence factors between hosts and within shared households. Fecal samples (n=45) were collected from 17 animals and 11 humans across five households. Samples were cultured on selective media, and DNA was extracted for RAPD-PCR and species identification. A total of 31 genetically distinct animal isolates and 48 human isolates were tested for susceptibility to 16 antimicrobials used in veterinary and human medicine. Hemolytic activity was assessed, and isolates were screened by multiplex PCR for resistance and virulence genes.

Results: In animals, 61% of isolates were E. faecalis, 29% E. faecium, and 10% E. hirae. Human isolates comprised 52% E. faecalis, 35% E. faecium, 8% E. hirae, and 4% other Enterococcus sp. Distinct resistance profiles were observed between groups, although both showed equal rates of erythromycin resistance (29%). Ampicillin resistance was universal in animals (100%) but absent in humans, while tetracycline resistance was moderate in animals (29%) and low in tutors (10%). Conversely, rifampicin resistance was moderate in tutors (27%) but low in animals (13%). Resistance to other antimicrobials, including penicillins, fluoroquinolones, glycopeptides and oxazolidinones, was rare or absent. Significant associations were found between host origin and resistance to ampicillin and rifampicin. Species identity was significantly associated with resistance to erythromycin and tetracycline. Hemolytic activity was detected in 16% of animal and 31% of human isolates, all carrying the cylA gene. Similar genetic and pathogenesis-associated profiles were identified in human enterococci within households.

Conclusions: The findings highlight enterococci as indicators for monitoring antimicrobial resistance and virulence in animals and humans, reinforcing the need for continued One Health surveillance.

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ID496 | Integrating Traditional Antifungal Tests and Flow Cytometry to Evaluate the Activity of Essential Oils Against Malassezia furfur

Author: Ana I. Lopes

Centro de Biotecnologia e Química Fina — Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

E-mail: anlopes@ucp.pt

Other authors: Cláudia S. Oliveira, Manuela E. Pintado, Freni K. Tavaria

Centro de Biotecnologia e Química Fina — Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Background: Malassezia spp. are lipid-dependent yeasts that are an important part of the normal skin mycobiome in humans and other warm-blooded animals. Although generally harmless, they can, under certain conditions, contribute to skin disorders, with Malassezia furfur (M. furfur) being the main species linked to invasive infections. Current treatment mainly relies on topical or systemic antifungals, which, despite their effectiveness, are limited by side effects, drug interactions, species-specific resistance, and frequent recurrences. In this context, essential oils (EOs) are emerging as promising alternatives, as they show broad-spectrum antimicrobial activity and multi-target mechanisms that lower the risk of resistance. Thus, this study aimed to evaluate the antifungal activity of six EOs—rosemary, eucalyptus, lavender, basil, sage, and thyme—against M. furfur.

Methods: Antifungal activity was assessed through: (i) agar diffusion assay; (ii) determination of minimum inhibitory (MIC) and fungicidal concentrations (MFC); (iii) growth inhibition curves at MIC; (iv) anti-biofilm activity at MIC, ½MIC, and ¼MIC; (v) membrane integrity analysis by flow cytometry using propidium iodide (PI) and thiazole orange (TO) for the most active oils (eucalyptus, lavender, thyme); and (vi) fluorescence microscopy of yeasts exposed to eucalyptus EO at ¼MIC.

Results: Inhibition zones ranged from 20 ± 0.00 mm (rosemary) to 33 ± 2.00 mm (eucalyptus), with basil and thyme achieving complete inhibition. MIC and MFC values ranged from 0.08% to 2.5% (v/v), with thyme showing the lowest values. Growth inhibition curves confirmed MIC data. Thyme also displayed the strongest antibiofilm effect. Flow cytometry suggested the absence of viable cells due to a lack of TO staining, even in untreated controls. However, fluorescence microscopy revealed that cells remained viable but failed to show TO uptake, likely because the lipid-rich membrane interfered with staining.

Conclusions: This work demonstrates the strong antifungal activity of EOs, particularly thyme, eucalyptus, and lavender, against M. furfur, affecting both planktonic and biofilm forms. Importantly, it represents one of the few studies applying flow cytometry to this yeast, exposing critical limitations in conventional staining methods. The novelty of this study advances knowledge on EO efficacy and highlights the need for further research to refine flow cytometry protocols for reliable assessment of M. furfur viability

ID497 | Assessment of Virulence and Pathogenicity in Staphylococcus spp. isolated from companion animals

Author: Daniela Araújo

1 National Institute for Agrarian and Veterinariay Research (INIAV), Rua dos Lagidos, 4485-655 Vila do Conde, Portugal; 2 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: daniela.araujo@iniav.pt

Other authors: Ruben Fernandes3, Sónia Silva 1,2,4, Carina Almeida 1,2,5, Ricardo Oliveira 1, Joana Castro 1,2

3 Agrarian School, Polytechnic Institute of Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Álvares, 4900 -347 Viana do Castelo, Portugal; 4 LABBELS–Associate Laboratory, Braga/Guimarães, 4710-057 Braga, Portugal; 5 LEPABE, ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Background: Infections caused by Staphylococcus have become a major public health concern, primarily due to increased antibiotic resistance and the spread of methicillin-resistant strains. While many Staphylococcus spp. are part of the normal microbiota, they can act as opportunistic pathogens, affecting not only humans but also companion animals. Their pathogenicity is linked to several virulence factors, including biofilm formation. This study aimed to compare the virulence traits and pathogenic potential of different Staphylococcus spp. isolated from infected dogs and cats.

Methods: A total of 11 isolates were examined through in vitro and in vivo assays. The presence of resistance (mecA and mecC) and virulence (tsst-1 and pvl) genes was investigated using PCR. In addition, the ability to form biofilms was assessed by measuring total biomass and cultivability after 24 h. To further evaluate pathogenicity, all isolates were tested in the Galleria mellonella infection model, monitoring survival and health indices over 72 h.

Results: Results showed that four isolates were methicillin-resistant (MRSA), all carrying the mecA gene. No isolate was found to harbour the emerging mecC variant. Concerning virulence determinants, the pvl gene was only detected in one isolate. Biofilm assays showed that S. hominis strain A11, S. haemolyticus A83-1, S. schleiferi J23-B, and S. aureus A81-1 produced the most robust biofilms, exhibiting the highest biomass levels. Pathogenicity testing in the G. mellonella model revealed significant variation among the isolates. Notably, S. aureus A81-1 emerged as the strongest biofilm producer and the most virulent strain, causing 100% mortality within 48 hours post-infection.

Conclusions: This study highlights the virulence characteristics and pathogenic potential of Staphylococcus isolates obtained from infected companion animals. The findings highlight why disease severity may differ among animals and emphasize the need for further research, including larger number of isolates using advanced techniques, such as whole-genome sequencing, to uncover the molecular mechanisms underlying Staphylococcus virulence.

ID500 | Uncovering Gastric Microbiota Biogeography Using both Spectral Imaging (SI) and Nucleic Acid Mimics–Fluorescence in situ Hybridization (NAMs-FISH)

Author: Sónia Miranda

1 LEPABE, ALiCE, Faculdade de Engenharia da Universidade do Porto, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; 2 i3S - Institute for Research and Innovation in Health, University of Porto, 4200-135 Porto, Portugal; 3 IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto, 4200-135 Porto, Portugal

E-mail: soniabatistamiranda@outlook.com

Other authors: Andreia S. Azevedo 1, Nuno F. Azevedo 1

1 LEPABE, ALiCE, Faculdade de Engenharia da Universidade do Porto, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;

Background: The three-dimensional organization of the gastric microbiota, which may play a role in an individual's health status, remains poorly understood. In this study, a novel approach was developed by combining Spectral Imaging (SI) with Nucleic Acid Mimics Fluorescence in situ Hybridization (NAMs-FISH) to investigate the biogeography of key gastric pathogens.

Methods: Seven different genus/species-specific LNA/2'-OMe probes targeting Campylobacter ssp., Clostridium spp., Citrobacter spp., Fusobacterium spp., Lactobacillus spp., Streptococcus spp. and Helicobacter pylori were designed. Hybridization conditions for each probe—including hybridization buffer composition, temperature, and incubation time—were individually optimized. The specificity and sensitivity of each probe were also evaluated. Once all probes were optimized, a set of hybridization conditions suitable for all probes was selected. The method was then validated by analyzing microbial mixtures containing varying known concentrations of each genus/species, using spectral imaging with a confocal microscope.

Results: The optimal hybridization condition selected for the probe mixture included a hybridization solution composed of 2 M urea, 4 M NaCl, 50 mM Tris-HCl, 5 mM disodium EDTA, 10% (w/v) dextran sulfate, 0.1% (w/v) sodium pyrophosphate, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, and 0.1% (v/v) Triton X-100. Hybridization was carried out for 60 minutes at a temperature of 62–64 °C. Subsequently, the method was successfully validated, showing a good correlation between the initial composition of gastric bacteria in the artificial mixtures (input) and the results obtained through FISH analysis (output).

Conclusions: This validated protocol enables in situ detection and spatial mapping of gastric pathogens. It will be applied to gastric samples from adult patients with gastric diseases and healthy individuals, aiming to characterize microbial spatial patterns associated with health and disease, and improve understanding of gastric microbiota organization and its clinical relevance.

ID510 | NAM-FISH – A Pivotal Technique to Study the Oral Biofilm' Biogeography

Author: Sónia Miranda

1 LEPABE, ALiCE, Faculty of Engineering of University of Porto, University of Porto, 4200-465 Porto, Portugal; 2 i3S - Institute for Research and Innovation in Health, University of Porto, 4200-135 Porto, Portugal; 3 IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto, 4200-135 Porto, Portugal

E-mail: soniabatistamiranda@outlook.com

Other authors: Maria Rodrigues 2, Nuno F. Azevedo 1, Luzia Mendes 2, Andreia S. Azevedo 1

1 LEPABE, ALiCE, Faculty of Engineering of University of Porto, University of Porto, 4200-465 Porto, Portugal; 2 FMDUP, Faculty of Dental Medicine of University of Porto, 4200-393 Porto, Portugal

Background: Periodontal biofilms are microbial communities that form around the tooth and are typically in balance with the host; when this balance is disrupted, it can lead to disease. Understanding the onset of periodontal disease requires studying the bacterial spatial distribution and their interactions within the biofilm. Streptococcus spp. are primary colonizers of these biofilms, while Fusobacterium spp. are also frequently found in dental plaque, being associated with periodontitis. On the other hand, although less prevalent, Lactobacillus spp., may play a beneficial role in the overall oral health status. Therefore, it is crucial to understand the biogeography of these microorganisms within dental plaque. For that, confocal fluorescence microscopy (CLSM) was combined with Nucleic Acid Mimics – Fluorescence in situ hybridization (NAM-FISH) to provide deeper insight into biofilm organization.

Methods: Tooths and supragingival and subgingival plaque samples were carefully collected to minimize disruption of the biofilm microstructure. Samples were fixed with 4% (vol/vol) paraformaldehyde and 50% (vol/vol) ethanol. After that, tooths underwent paraffin embedding and decalcification with EDTA for over a month, whereas supragingival and subgingival plaque samples underwent agar and paraffin embedding. For NAM-FISH, samples were sectioned and hybridized with a mixture of Streptococcus spp.-specific, Fusobacterium spp.-specific and Lactobacillus spp.-specific NAM probe. Visualization was performed using fluorescence microscopy and CLSM.

Results: Sample collection and fixation preserved biofilm microstructure, enabling detailed imaging. The NAM probes identified the respective specific genus, with Streptococcus spp. being detected in all samples, consistent with previous findings. Microscopy also showed that these microorganisms form well-defined structures like cauliflower and corncob-like structures, indicating preserved biofilm architecture.

Conclusions: NAM probes proved to be a reliable tool for detecting microorganisms, either in tooth surface and in supra- and subgingival biofilms. The preservation of biofilm structure during collection and processing confirms the method's suitability for studying microbial biogeography. In fact, this approach when combined with CLSM allowed detailed visualization of bacterial distribution and interactions providing a deeper insight into oral biofilm dynamics and its role in periodontal disease.

ID517 | Validation of rifabutin liposomes against Staphylococcus aureus clinical isolates

Author: Magda Ferreira

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

E-mail: magda.ferreira@campus.ul.pt

Other authors: Margarida Pinto 3, Frederico Aires-da-Silva 1,2, Ana Bettencourt 4, Maria Manuela Gaspar 4,5, Sandra Aguiar 1

1 Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Portugal; 2 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal; 3 Laboratório de Microbiologia do Serviço de Patologia Clínica do Centro Hospitalar Universitário de Lisboa Central, Portugal; 4 Research Institute for Medicines (iMed.ULisboa), Portugal; 5 Institute of Biophysics and Biomedical Engineering, Faculty of Sciences, University of Lisbon, Portugal.

Background: Antimicrobial resistance remains a critical issue in healthcare, particularly in infections caused by Staphylococcus aureus. This pathogen is difficult to eradicate using conventional therapies, and exhibits enhanced resistance mechanisms in biofilm form, which makes infection treatment even more challenging. Considering the limitations of current therapies, there is an urgent need for innovative approaches to effectively treat both planktonic and biofilm-associated infections. Our group has previously reported on the development and evaluation of liposomal formulations for antimicrobial delivery, and the validation of rifabutin (RFB) as a potential repurposed antibiotic. Building on these findings, this study focuses on validating rifabutin-loaded fusogenic liposomes against clinical isolates of S. aureus in both planktonic and biofilm forms.

Methods: To address this need, we evaluated the activity of rifabutin-loaded fusogenic liposomes (RFB LIP) against 40 staphylococcal clinical isolates. Antimicrobial activity was assessed through minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration of 50 % bacterial growth (MBIC50) determination, through the broth microdilution method and MTT reduction assay, respectively. The efficacy of RFB LIP was compared with free RFB in both planktonic and biofilm growth conditions.

Results: RFB maintains potent activity against clinical isolates when incorporated into fusogenic liposomes. In planktonic assays, MIC mean values indicated a modest decrease in susceptibility to RFB LIP compared with free RFB (p=0.0007). Regarding biofilm forms, RFB LIP demonstrated significant inhibitory effects, with only five isolates presenting MBIC50 values above the maximum concentration tested (>25.000 µg/mL).

Conclusions: These findings validate the potential of RFB LIP as an alternative therapeutic approach against S. aureus infections, including those associated with biofilms. Validating these results using clinical isolates highlights their importance in informing subsequent in vivo studies and bridging the gap between in vitro activity and clinical applicability.

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ID527 | Iron reduces the efficacy of fluconazole against *Candida glabrata*

Author: Carolina Vitorino Mariano

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: cv.mariano@itqb.unl.pt

Other authors: Soraia Caetano, Sofia Pontes 1, Catarina Amaral 1, Catarina Pimentel 1

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Background: Invasive fungal infections (IFIs) represent a significant global health concern, as recently acknowledged by the World Health Organization with the release of the first priority list of fungal pathogens. Fluconazole is the most frequently prescribed antifungal for treating IFIs, due to its affordability, oral bioavailability, broad spectrum of activity, and safety profile. This drug targets lanosterol 14-α-demethylase, Erg11, an enzyme that catalyzes the conversion of lanosterol to ergosterol, a key component of the fungal cell membrane. Recently, we found that iron loading conditions reduce fluconazole activity against Candida glabrata, the second most prevalent cause of nosocomial IFIs. In this work, we began to explore the molecular mechanisms underlying this phenotype.

Methods: Checkerboard assays were used to investigate the antagonism between iron and fluconazole. The expression of genes related to fluconazole resistance was evaluated by qRT-PCR and Western blotting in C. glabrata cultures exposed to mono- (iron or fluconazole) or co-treatment (iron and fluconazole). Ergosterol and fluconazole levels were quantified using HPLC.

Results: The antagonistic effect between iron and fluconazole is evident across a wide range of the compounds concentrations. Co-treatment with iron and fluconazole prevents ergosterol depletion induced by fluconazole and upregulates genes essential for its detoxification, specifically CDR1 and PDR1. However, increased intracellular levels of fluconazole were detected, ruling out enhanced drug extrusion as the primary cause of the antagonism. Remarkably, we found that although ERG11 mRNA levels were not as upregulated after co-treatment as they were under fluconazole alone, Erg11 protein levels were increased. This finding suggests that an as yet unidentified posttranslational mechanism, dependent on iron, may stabilize Erg11, contributing to higher tolerance to fluconazole under iron overload.

Conclusions: This work highlights the idea that iron may reduce fluconazole efficacy by strengthening pathways critical to its antifungal activity. Because iron excess is associated with many medical conditions or treatments, this finding can have important clinical implications for the management of IFIs caused by Candida glabrata in such contexts.

ID534 | Prebiotic potential of cellobiose for human gut modulation

Author: Joana Sousa

1 CEB - Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

E-mail: joana.sousa@ceb.uminho.pt

Other authors: Ana Cordeiro 1,2,3, Sónia Barbosa 1,4, Lígia Rodrigues 1,4, Sara Silvério 1,4

2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; 3 Laboratório Associado para a Sustentabilidade e Tecnologia em Região de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; 4 LABBELS - Associate Laboratory, Braga/Guimarães, Portugal

Prebiotics are defined as “substrates that are selectively utilized by host microorganisms conferring a health benefit”. Their intake has been associated with health maintenance and disease prevention, with effects on overall well-being. Several compounds have been identified as prebiotics, and recent research has highlighted the disaccharide cellobiose as a promising candidate. Unlike simple sugars readily absorbed in the small intestine, the β-(1→4) glycosidic bonds of cellobiose prevent its degradation by digestive enzymes, allowing it to reach the colon intact, where it becomes available to specific microbial groups. By acting as a selective substrate, cellobiose has been shown to modulate the composition and metabolic activity of the gut microbiota, stimulating beneficial species, reducing undesired bacteria, and enhancing the production of short-chain fatty acids (SCFAs), key metabolites involved in intestinal barrier integrity, immune regulation, and host metabolic health.

In this study, the prebiotic effect of cellobiose was investigated through batch culture fermentations using an in vitro static model inoculated with fecal microbiota from four healthy young donors. This system provided a simple and practical approach to evaluate preliminary functional properties of candidate prebiotic compounds. Experimental tests included pH monitoring, quantification of fermentation metabolites (SCFAs, lactate, and gases), and microbial community analysis by high-throughput sequencing (Illumina MiSeq).

The results showed that supplementation with cellobiose induced significant changes in both fermentation profiles and microbial composition. A marked decrease in pH was observed, consistent with enhanced microbial activity. Cellobiose fermentation yielded high levels of lactate, followed by acetate, propionate, and butyrate, although ~30% of the disaccharide remained unconsumed. Gas analysis revealed increased hydrogen and carbon dioxide production. Sequencing data confirmed a positive modulation of the gut microbiota, with increased abundance of Lactobacillus species, particularly Lactobacillus gasseri, in line with the elevated lactate levels. In contrast, the relative abundance of potentially harmful species, including Bacteroides sp. and Escherichia coli, was reduced.

In conclusion, these findings suggest that cellobiose holds potential functional properties for human health promotion, especially towards the modulation of lactate-producing strains.

ID551 | Repeated exposure to Hydrogen Peroxide does not induce bacterial resistance

Author: Ângela Rita Fernandes

Division of Microbiology, Department of Pathology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

E-mail: angelaritafernandes98@gmail.com

Other authors: Acácio Gonçalves Rodrigues (1,2), Luís Cobrado (1,2,3)

(1) Division of Microbiology, Department of Pathology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal; (2) RISE-Health, Department of Pathology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal; (3) Burn Unit, Department of Plastic and Reconstructive Surgery, University Hospital Centre of São João, 4200-319, Porto, Portugal

Background: Disinfection using Hydrogen Peroxide (H2O2) is common in healthcare settings as present a broad-spectrum activity and high effectiveness, even in shorter cycles and against biofilms. However, concerns over the potential induction of resistance in bacterial cells after repeated use of H2O2 persist and remain controversial. The aim of the present study was to evaluate the hypothetical development of bacterial resistance following 30 days exposure to different concentrations of H2O2.

Methods: Bacterial strains of Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterococcus faecium were selected based on their antimicrobial profile and subjected to H2O2 pre-defined concentrations corresponding to 1/2xMIC, 1xMIC and 2xMIC during 30 days of consecutive exposure.

Results: Exposure to sub-lethal (1/2xMIC), inhibitory (1xMIC) and lethal (2xMIC) concentrations of H2O2 over 30 days do not promote alteration in susceptibility against the disinfectant. Even, for the E. faecium strains that only sustained sub-lethal concentrations (1/16xMIC, 1/32xMIC and 1/64xMIC). During all period of time, H2O2 MIC maintained at 0.055%, with exception of a non-significant difference of 1 single dilution on after 30 days for 3 gram-negative strains.

Conclusions: Exposure to H2O2 for 30 days do not promote resistance development on commonly healthcare-associated bacterial species. In fact, H2O2 impact on bacterial strains was independent of species tested and do not relate to their antimicrobial profile. Furthermore, the adoption of H2O2 disinfection protocols should be considered for healthcare settings as infection prevention and control (IPC) measure.

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ID552 | Silencing PDR1 gene with antisense oligonucleotides reduces virulence of Candida glabrata infections in the Galleria mellonella model

Author: Daniela Araújo

1 National Institute for Agrarian and Veterinariay Research (INIAV), Rua dos Lagidos, 4485-655 Vila do Conde, Portugal;

2 Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

E-mail: daniela.araujo@iniav.pt

Other authors: Patrícia Serra1,2, Joana Castro 1,2, Mariana Henriques2, Sónia Silva 1,2,3
3 LABBELS–Associate Laboratory, Braga/Guimarães, 4710-057 Braga, Portugal

Background: Candida glabrata (formerly known as Nakaseomyces glabratus) is the second leading cause of invasive candidiasis, largely due to its intrinsic and acquired azole resistance. This resistance is primarily mediated by the transcription factor PDR1, which controls the activity of efflux pumps, membrane permeability and stress responses. The rise of multidrug-resistant C. glabrata highlights the urgent need for new therapeutic strategies. Antisense therapy (AST), which modulates gene expression at the mRNA level, has been little explored in pathogenic fungi. This study evaluates the in vivo effects of an antisense oligonucleotide (ASO) designed to target PDR1 in C. glabrata.

Methods: The efficacy of ASOs was tested in the Galleria mellonella infection model. The larvae were infected with a lethal dose of C. glabrata (1×10^7 cells per larva) and then treated with either free or liposome-encapsulated anti-PDR1 ASO. Larvae were monitored for survival and health indices over 72 h.

Results: This study investigated whether silencing PDR1 gene using an ASO could reduce C. glabrata viability by disturbing membrane integrity and other adaptive processes regulated by this factor. The results revealed that anti-PDR1 ASO had no therapeutic potential on its own. To address this, the ASO was encapsulated in cationic liposomes to enhance protection and facilitate its intracellular delivery. Two formulations (DOTAP:DOPC:PEG and DOTAP:DOPE:PEG) were tested, both of which improved larval survival after infection. Increases of 14 and 17% at 24 h, 21 and 23% at 48 h, and up to 30 and 32% at 72 h were observed respectively. The DOTAP:DOPC:PEG formulation yielded slightly better outcomes.

Conclusions: Liposomal delivery enhances ASO uptake and PDR1 silencing, supporting AST as a potential treatment for C. glabrata infections.

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ID553 | Combating Multidrug-resistance: novel metal-based complexes active against Klebsiella pneumoniae

Author: Raquel Almeida

Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB – NOVA), Oeiras, Portugal

E-mail: raquell.almeidaa@hotmail.com

Other authors: Nuno Alexandre Faria (1), Beatriz Royo (2), Oscar Lenis-Rojas (2), Maria Miragaia (1)

1 Laboratory of Bacterial Evolution and Molecular Epidemiology, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB – NOVA), Oeiras, Portugal; 2 Laboratory of Organometallic Chemistry and Catalysis, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB – NOVA), Oeiras, Portugal

Antibiotic resistance is a growing global threat, causing millions of deaths yearly. The lack of new antibiotics worsens this crisis, limiting treatment options. Multidrug-resistant Klebsiella pneumonia (MDR-Kp), a WHO priority pathogen, requires novel therapeutic strategies, however its

impermeable outer membrane and efflux pumps make antimicrobial development challenging. To answer this need, we synthesized and tested a new family of metal complexes (M) against MDR-Kp and perceived its mode-of-action.

Eleven M complexes (M1-M11) were evaluated for antimicrobial activity against clinical MDR-Kp by determining the minimum inhibitory concentrations (MIC) using broth microdilution assays. Among those, M3 and M4, showed the highest antimicrobial activities (MICs: 6.25 and 3.25µg/mL, respectively). M3 and M4 activity was characterized by cell viability and antibiofilm studies, and spectrum of activity assessment. The complexes' permeability was evaluated by checkerboard assays with polymyxin B (PMB) and efflux pump inhibitor (PAβN). M3 mode-of-action was explored by selecting M3-resistant mutants and identifying mutations by whole genome sequencing. Cross-resistance was checked by disk diffusion.

M3 and M4 were bactericidal, with a minimal bactericidal concentration (MBC) of 12.5 and 6.25ug/mL (MBC/MIC200µg/mL). Importantly, they showed activity against other clinical MDR Gram-negative pathogens (MIC<25µg/mL). Synergistic interactions with PMB (FICI≤0.5) suggest a membrane-targeting mechanism. In the presence of PAβN, M4 activity increased while in M3 it was unaffected, suggesting M3 is less prone to be extruded by efflux pumps. M3-resistant mutants developed after 6 passages (4xMIC), had one missense mutation in an outer membrane stress-sensor protein and lost a large plasmid (130kbp) containing multiple virulence genes, including iron acquisition and complement resistance. Moreover, M3 resistance did not cause cross-resistance to other antibiotics.

The novel complexes here tested exhibited high bactericidal activity, prevented biofilm formation and had broad-spectrum potential. Results suggest that M3 may decrease virulence potential, can overcome extrusion by efflux pumps and likely causes membrane permeability. Moreover, M3 mode-of-action appears to be new, emerging as a promising candidate for novel antibiotics against MDR-Kp.

ID561 | Ten years (2015–2024) of Lyme Borreliosis diagnosis in Portugal: serological and molecular insights from IHMT-NOVA laboratory

Author: Ana Armada

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Rua da Junqueira 100, 1349-008, Lisbon, Portugal

E-mail: aarmada@ihmt.unl.pt

Other authors: Inês Sena Lopes, Maria Luísa Vieira

Background: Lyme borreliosis (LB) is a multisystemic inflammatory zoonosis caused by infection with Borrelia burgdorferi sensu lato and transmitted by ticks of the genus Ixodes. As a vector-borne disease with broad clinical expression, LB presents diagnostic and public health challenges. Although early suspicion relies on clinical presentation and exposure history, laboratory confirmation remains crucial due to the non-specific nature of symptoms. In Portugal, LB has been notifiable since 1999; however, underreporting suggests that the recommended diagnostic approach, integrating clinical, epidemiological, and laboratory data, is not uniformly implemented. This highlights the need to strengthen clinical awareness, laboratory capacity, and surveillance.

Methods: This retrospective study analyses LB cases and diagnostic trends from 2015 to 2024 based on data from the Leptospirosis and Lyme Borreliosis Laboratory (LBL) at IHMT-NOVA. A total of 616 patients (295 males, 321 females), referred by Portuguese hospitals from the South, Lisbon metropolitan, and Central region, were included. A total of 1,303 serological tests (Indirect Immunofluorescence Assay and immunoblotting) and molecular analyses (nested PCR targeting the 5S-23S intergenic spacer) were performed on 651 biological samples, following ECDC guidelines.

Results: LB was laboratory-confirmed in 186 patients (30.2%), with similar positivity in males (29%) and females (30%), yielding 19 confirmed cases/year. Excluding the COVID-19 pandemic period, the positivity showed a rising trend, ranging from 32%-49%, with no significant differences between genders. The tests performed (IFA, immunoblotting, and PCR) contributed 23%, 67% and 10% of positive cases, respectively. Conclusions: The results highlight the fundamental role of reference laboratories in confirming LB, enabling timely diagnosis, clinical treatment, and strengthening surveillance systems. The observed upward trend in positivity may reflect both increased awareness among physicians and greater human exposure to infected ticks, possibly due to outdoor activities. The data also suggest that in several hospitals, laboratory diagnosis is performed, with reference laboratories confirming preliminary results. However, it is essential to maintain diagnostic surveillance and reporting to reduce the impact of LB and improve the population's literacy about vector-borne diseases.

ID575 | Role of 3'-nucleotidase/nuclease in Candida spp. – neutrophil interaction

Author: Lisa Rodrigues

1 Medical Microbiology Research Group, iCBR - Coimbra Institute for Clinical and Biomedical Research & CiBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; 2 FMUC- Faculty of Medicine, University of Coimbra, Coimbra, Portugal

E-mail: lisa1cor@gmail.com

Other authors: Mariana Afonso 1,2; Rita Domingues 1,2; Bernardo Bandeira 1,2; Rodrigo A. Cunha 2,3; Teresa Gonçalves 1,2

1 Medical Microbiology Research Group, iCBR - Coimbra Institute for Clinical and Biomedical Research & CiBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; 2 FMUC- Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 3 Neuromodulation Research Group, CNC-UC – Center for Neuroscience and Cell Biology- University of Coimbra & CiBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Background: Fungi constitute a significant, yet understudied, component of the human microbiota, capable of existing as commensals or opportunistic pathogens. Fungal infections are a major concern worldwide, with Candida albicans as the leading cause of mucocutaneous and systemic candidiasis, employing several virulence mechanisms to avoid immune defences and thrive across multiple host niches. In this context, ectonucleotidases have been described as key regulators of the immune responses, contributing to microbial persistence.

Methods: Ecto-5'&3' nucleotidase/nuclease (ecto-5'&3' NT/NU) activity was evaluated in C. albicans clinical isolates derived from oral, bloodstream, vaginal, medical device, and gut-associated infections. Enzymatic activities were quantified at different pH values (4-8), using Fiske Subbarow spectrophotometric method. Neutrophil-Candida interactions were assessed during 1 and 3h, quantifying neutrophil extracellular trap (NET) induction and nucleic acid degradation. 3'NT/NU inhibition with TTM was also determined, evaluating its immune modulation role.

Results: All isolates exhibited ecto-5'&3' NT/NU activity and induced NET release. Regarding 5'-NT activity, vaginal and oral isolates exhibited, respectively, the higher and lower values (198.1 ± 18.0 vs 174.3 ± 27.1 nmol Pi $h^{-1}10^{-9}$ cells). An elevated 3'-NT/NU activity was found in strains from medical devices and vaginal niches (286.9 ± 32.2 & 293.9 ± 24.9 nmol Pi $h^{-1}10^{-9}$ cells), when compared to oral and gut-associated ones (236.7 ± 28.8 & 228.8 ± 24.1 nmol Pi $h^{-1}10^{-9}$ cells). At 1 and 3 h of interaction, oral (5654 ± 449; 8187 ± 752 RFU) and medical device-associated isolates (6769 ± 593; 8847 ± 731 RFU) induced the higher NET formation, in opposition to vaginal strains (3473.3 ± 313.7; 4476.6 ± 790.3 RFU). However, vaginal isolates presented higher NET degradation (381.5 ± 11.4 ADU), suggesting a more effective immune escape. Also, 3'NT/NU inhibition reduced NET degradation, confirming this enzyme contribution to neutrophil evasion.

Conclusions: This work highlight the value of analyzing diverse clinical isolates to better understand host-pathogen interactions. Evidence also indicates that ecto-3'NT/NU contributes to NET degradation and immune evasion, though its role in C. albicans infection requires further confirmation. To address this, target genes linked to this activity, are being deleted to assess its impact on this interaction.

ID582 | Bioprospecting of antibiofilm and antimicrobial activity of Bacillus sp. strains from the Azores against Cutibacterium

Author: Ana Carolina Mendonça

Centre of Biotecnology of Azores (CBA), Faculty of Science and Technology, University of Azores, University of Aveiro- Biology Departement

E-mail: anamacanita99@gmail.com

Other authors: 1- José A. B. Soares; Carla Cabral; Nelson Simões; Duarte Toubarro; 2- Artur J.C.P. Alves

1-Centre of Biotecnology of Azores (CBA), Faculty of Science and technology, University of Azores-Portugal (azores);

2-University of Aveiro, Biology Department- Portugal

Cutibacterium acnes is a Gram-positive bacterium that exists on human skin microbiome but is strongly implicated in the pathogenesis of Acne vulgaris skin condition. A key feature underlying its pathogenicity is the ability to form biofilms, which confer protection against host defences and markedly reduce susceptibility to conventional antibiotic therapies. Consequently, there is an urgent need to identify novel antimicrobials capable of inhibiting both planktonic growth and biofilm formation.

In this study, we evaluated the antibacterial and anti-biofilm properties of cell-free supernatants (CFS) derived from 35 Bacillus isolates obtained from Azorean soils. The antimicrobial activity of the CFS was initially screened using agar diffusion assays, followed by broth microdilution assays in 96-well microplates to determine inhibitory activity against C. acnes. Biofilm inhibition and degradation were further assessed through resazurin staining and adhesion assays. The most active supernatant was subjected to semi-purification steps, with subsequent determination of its minimum inhibitory concentration (MIC), evaluation of its proteinaceous nature, and assessment of activity against established biofilms.

Among all isolates tested, one Bacillus strain exhibited strong and reproducible antimicrobial activity against C. acnes, with inhibition values in microdilution assays falling within ranges considered effective for therapeutic potential. The semi-purified protein fraction of high molecular weight displayed marked antibacterial activity, significantly reducing both bacterial growth and biofilm formation. These results suggest that the bioactive compounds secreted by this isolate are likely of protein nature and may act through mechanisms targeting adhesion and biofilm integrity.

Overall, this work highlights the Azorean soil microbiota as a valuable reservoir of Bacillus species capable of producing bioactive molecules with antimicrobial and anti-biofilm activities. The findings underscore the potential of these microorganisms as promising sources for the development of alternative therapeutic agents against C. acnes, while also emphasising the importance of exploring unique ecosystems for the discovery of novel compounds with clinical applications.

ID586 | Matricaria chamomilla L. for Oral Care: Dual Action in Pathogen Control and Microbiota Homeostasis

Author: Eva Olo-Fontinha

AquaValor - Centro de Valorização e Transferência de Tecnologia da Água-Associação,Rua Dr. Júlio Martins n.º 1, 5400-342 Chaves, Portugal; 2Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327,4169-005 Porto, Portugal; 4Research Centre for Active Living & Wellbeing (LiveWell), Instituto Politécnico de Bragança, Bragança

E-mail: evafontinha@hotmail.com

Other authors: Juliana Garcia 1,3,4, Jani Silva 1,4, Manuela E. Pintado2, Maria José Alves1,4,5,6

1AquaValor - Centro de Valorização e Transferência de Tecnologia da Água-Associação,Rua Dr. Júlio Martins n.º 1, 5400-342 Chaves, Portugal; 2Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327,4169-005 Porto, Portugal; 3CITAB —Centre for the Research and Technology of Agro-Environmental and Biological Sciences and Inov4Agro, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal; 4Research Centre for Active Living & Wellbeing (LiveWell), Instituto Politécnico de Bragança, Bragança; 5 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; 6 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Background: The oral cavity is a complex and dynamic ecosystem, where pathogenic species such as Streptococcus mutans and Actinomyces naeslundii are key contributors to the development of oral diseases. The growing resistance of these microorganisms to conventional antimicrobials highlights the need for natural alternatives capable of controlling pathogens while preserving beneficial bacteria. Chamomile (Matricaria chamomilla L.) and thermal water have emerged as promising candidates due to their antimicrobial and anti-inflammatory properties.

Methods: In this study, the antimicrobial activity of chamomile extract was assessed by Minimum Inhibitory Concentration (MIC) assays and bacterial viability analysis using flow cytometry.

Results: The MIC values were 31.25 mg/mL for S. mutans and 3.90 mg/mL for A. naeslundii. Flow cytometry confirmed pronounced alterations in membrane integrity, with 82% of A. naeslundii and 56% of S. mutans cells showing double labeling (SYTO9+/PI+), indicating susceptibility to chamomile extract.

Selectivity was further evaluated using the probiotic Lactobacillus reuteri as a reference commensal species. Although its MIC was also 31.25 mg/mL, bacterial viability assays demonstrated ~80% viable cells (SYTO9+/PI−), indicating that chamomile extract inhibits growth but does not exert lethal effects on this beneficial bacterium. This differential response highlights a favorable selective action: strong antimicrobial activity against pathogenic species while preserving probiotic viability, thereby contributing to the maintenance of oral microbiota balance.

Conclusions: Overall, chamomile extract demonstrates significant antimicrobial potential, particularly against A. naeslundii, while safeguarding commensal bacteria. These findings reinforce its promise as a natural therapeutic strategy for oral care, combining pathogen control with the preservation of microbial homeostasis.

ID594 | Fast and reliable identification of hypervirulent Klebsiella pneumoniae sublineages using FT-IR spectroscopy

Author: Ângela Novais

UCIBIO, i4HB, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

E-mail: aamorim@ff.up.pt

Other authors: Rita Martins 1, Sylvain Brisse 2, Kathryn E. Holt 3, Senjuti Saha 4, Luísa Peixe 1

1 UCIBIO, i4HB, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; 2 Institut Pasteur, Université Paris Cité, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France; 3 Department of Infection Biology, London School of Hygiene & Tropical Medicine, London, United Kingdom; 4 Child Health Research Foundation, Dhaka, Bangladesh

Background: Hypervirulent Klebsiella pneumoniae (Hv-Kp) can lead to severe and disseminated infections and is caused by sublineages carrying specific genomic capsule loci (KL) and hypermucoid (HM)/ hypermucoviscous (HMV) phenotypes. They are increasingly reported in Europe and early detection is essential for both clinical and public health but remains challenging due to limitations in available detection methods. We aimed to assess FT-IR spectroscopy as a rapid, affordable, and simple diagnostic tool for distinguishing and identifying global Hv-Kp.

Methods: We gathered a collection of 220 well-characterized and whole-genome sequenced strains of K. pneumoniae representing the main Hv-Kp (n=45, 23 ST, 7 KL), MDR-Kp (n=166, 18 ST, 19 KL) and convergent strains (both phenotypes) (n=9, 7 ST, 7 KL). Bacteria were cultured on Blood Agar and Mueller-Hinton Agar, and spectra were directly obtained using a Spectrum Two FT-IR ATR instrument (PerkinElmer). The spectra were then processed and compared with Clover MS Data Analysis software, considering different spectral regions and machine-learning (ML) models.

Results: As expected, FT-IR perfectly identified the 3 main Hv-Kp KL types (100%, KL1, KL2, KL5), using the carbohydrates region. Strains sharing the same KL-type (KL2) within both MDR and HV-Kp were additionally discriminated (94% correct predictions) using proteins+lipids regions. We used the same regions to build a model that was able to distinguish Hv-Kp from MDR-Kp populations (96.4%) with high KL diversity, independently of the culture media used (Fig.1). This model was further validated with a subset of external isolates (MDR=20, HV=7, convergent=9), yielding 100% sensitivity and 100% specificity. Interestingly, all but one convergent strain were classified as Hv-Kp, but this identification was not correlated with HM or HMV phenotypes (as determined by capsular quantification and sedimentation assays, respectively), suggesting that molecular features beyond capsule quantity or hypermucoviscosity are being recognized.

Conclusions: FT-IR spectroscopy captured differences in capsular structures, lipids and proteins, enabling accurate identification of HV-Kp and convergent strains. This study provides the first rapid and low-cost tool for reliable identification of global Hv-Kp sublineages of high value to support diagnostics and, consequently, patient management and surveillance of high-risk strains.

ID601 | Risk factors and bacterial etiology of neonatal sepsis at Maputo Central Hospital: preliminary data

Author: Lena Manhique-Coutinho

1 Instituto Nacional de Saúde, Maputo, Mozambique; 2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal;

E-mail: lena.coutinho@ins.gov.mz

Other authors: Sofia G. Seabra2, Isabel Couto2, Joyce Ventura3, Inacio Mandomando1,2,4,5, Ermelinda Covane1, Suzana Paque1, Sofia Santos Costa2, Jeronimo S. Langa1

1 Instituto Nacional de Saúde, Maputo, Mozambique; 2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal; 3 Hospital Central de Maputo, Maputo, Mozambique; 4 Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique. 5 Instituto de Salud Global (ISGlobal), Barcelona, Spain

Background: Neonatal sepsis is a dysregulated host response to a systemic infection occurring in infants at

≤ 28 days of life and is an important cause of morbidity and mortality of newborns in low- and middle-income countries. Early-onset neonatal sepsis is most consistently defined as occurring in the first 3 days of life and is mainly caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery. This study aimed to identify risk factors and the bacterial agents associated with neonatal sepsis in Maputo.

Methods: We conducted a cross-sectional study involving neonates admitted at quaternary neonatal unit at Maputo Central Hospital, Mozambique (June 2024 - April 2025), with clinical suspect of sepsis. All demographic, clinical, therapeutic and microbiological data was collected using a standard data collection form. Neonatal sepsis was defined as presence of clinical symptoms (e.g., fever) and a positive blood culture. Positive samples were subcultured in blood, chocolate, MacConkey, and/or Muller–Hinton agar. Isolated organisms were identified by Gram staining, biochemical reactions, and Vitek 2 when necessary for confirmation. Antibiotic susceptibility was tested by Kirby–Bauer disc diffusion and E-test when necessary. All data were recorded in Excel. Descriptive statistics and association analyses are currently being conducted using R Statistics platform.

Results: The study comprised 390 participants with suspected neonatal sepsis [males: 204/390 (52%), females 186/390 (48%)]. Of these, neonatal sepsis was confirmed for 193/390 (50%), of which 153 (79%) were considered early onset neonatal sepsis (onset at 50%).

Conclusions: The high frequency of neonatal sepsis associated with antimicrobial-resistant bacteria, in Maputo, Mozambique, highlights the need for novel preventive strategies and warrants formulation of a hospital-specific guideline for antibiotic use periodically reviewed.

ID602 | Emergence of linezolid-resistant Enterococcus faecalis in Portugal: Alert for a changing resistance landscape in hospitals

Author: Ana Raquel Freitas

3- UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 4-Laboratório Associado i4HB, Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 7-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Instituto Universitário de Ciências da Saúde (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal

E-mail: anarpf@gmail.com

Other authors: 1,2,3,4-Juan C. Vázquez-Ucha; 3,4-Ana C. Almeida-Santos; 1-Andrea García-Pose; 5-Guido Werner; 5-Jennifer K. Bender, 6-Antónia Read, 6-Mariana Romanholo, 3,4-Carla Novais; 3,4-Luisa Peixe

1- Servicio de Microbiología and Instituto de Investigación Biomédica A Coruña (INIBIC), Complexo Hospitalario Universitario A Coruña (CHUAC), A Coruña, Spain; 2-Ciber de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain; 3-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 4-Laboratório Associado i4HB, Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; 5-National Reference Centre for Enterococci, Nosocomial Pathogens and Antimicrobial Resistances Unit, Department of Infectious Diseases, Robert Koch Institute, Wernigerode, Germany; 6-Serviço de Patologia Clínica, Hospital Pedro Hispano, Matosinhos, Portugal; 7-UCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Instituto Universitário de Ciências da Saúde (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal

Background: Linezolid is an oxazolidinone critically important for treating Gram-positive infections. Although resistance rates remain low, acquired genes (optrA/poxtA), often horizontally transferred, are increasingly reported in Enterococcus spp., especially in livestock. In Portugal, clinical infections have so far been rare and linked exclusively to the chromosomal G2576T mutation. Here, we report the first cases of linezolid-resistant Enterococcus faecalis (LinR-Efs) in a large hospital in central Porto.

Methods: Between July-2024 and February-2025, LinR-Efs was detected in three male patients. Two isolates were preserved for phenotypic/molecular tests and whole-genome-sequencing. Clonality (MLST/cgMLST), antimicrobial susceptibility (9 antibiotics), and screening for antimicrobial resistance (AMR) genes, bacteriocins (76 genes), virulence markers and plasmid replicases was performed. Phylogenetic analysis compared these isolates with all GenBank genomes from the same ST/sequence-types. Results were contextualized within prior hospital studies.

Results: Two patients had bloodstream infections (urinary and foot-ulcer focus), and one had a UTI. Both isolates were multidrug-resistant, carrying 9–14 AMR genes, including optrA, which confers resistance to linezolid (MIC=4-8 mg/L). Virulence gene profiles were similar (n=18), while bacteriocin (Enterocin_MC4_1/enlA) and plasmid (rep9a/rep9b/repUS43/rep7a) content varied. They were unrelated to each other (ST16-CT4478-ST585-CT4477) and each clustered with strains from different sources (including pigs) and countries (2015–2025) (Figure1). Notably, a prior survey (October/2023-February/2024) of 714 patients at the same hospital found 2% colonized (rectal swab) with LinR enterococci (mostly optrA-carrying Efs), which at the time prompted staff alerts that infections by LinR enterococci were likely to emerge.

Conclusions: Our findings show that LinR-Efs colonization often precedes clinical infection, acting as a silent reservoir in hospitalized patients. Infections by different clones suggests independent acquisitions, while transferable resistance genes in gut carriage point to the food chain as a likely source. Remarkably, some LinR enterococci may carry optrA without phenotypic expression, as in one isolate here, enabling undetected spread. These findings indicate that clinical cases are not a

future risk but an ongoing reality, underscoring the need for proactive surveillance and infection control.

ID607 | Bioprospecting the Antimicrobial Properties of Essential Oils from Alentejo Lamiaceae Plants against Drug Resistant Pathogens

Author: Maria Rosário Martins

1 HERCULES Lab. and IN2PAST, University of Évora, 7000-809 Évora, Portugal. 2 Department of Medical Sciences and Health, School of Health and Human Development, University of Évora, 7000-671 Évora, Portugal.

E-mail: mrm@uevora.pt

Other authors: Sílvia Macedo Arantes 1,3; A. Teresa Caldeira 1,4,5

HERCULES Lab. and IN2PAST, University of Évora, 7000-809 Évora, Portugal. 3 Institute for Advanced Studies and Research, IIFA, University of Évora, 7000-809 Évora, Portugal.4 Department of Chemistry and Biochemistry, School of Sciences and Technology, University of Évora, 7000-671 Évora, Portugal 5 City U Macau Chair in Sustainable Heritage & Sino-Portugal Joint Laboratory of Cultural Heritage Conservation Science, Institute for Advanced Studies and Research, University of Évora, 7000-809 Évora, Portugal.

Background: Antimicrobial resistance (AMR) is a major global health concern. The emergence of resistance mechanisms, e.g. in nosocomial infections, compromises antimicrobial therapies and increases morbidity, mortality, and healthcare costs. The rise of multidrug-resistant (MDR) strains, driven by excessive antimicrobial use in medicine and agriculture, highlights the urgent need for alternative strategies. Essential oils (EOs), complex bioactive mixtures with antibacterial and antifungal activities, represent promising natural agents. In Portugal's Alentejo region, aromatic plants have long been used in food preservation and traditional medicine, suggesting their potential for EO-based preparations.

Methods: The antibacterial potential of essential oils (EOs) from wild-growing Calamintha, Lavandula, Mentha, Thymus, and Origanum species, in the Alentejo region was investigated. EOs were obtained by hydrodistillation and chemically characterized using GC-FID. Their antimicrobial activity was evaluated against clinically relevant, drug-resistant bacterial strains using disk diffusion and broth microdilution assays to determine inhibition zones, MICs, and MBCs. Additionally, synergistic interactions between EO combinations were assessed with the checkerboard method.

Results: Chemical analysis revealed that the EOs were rich in oxygenated monoterpenes, and Origanum species also showed a high content of hydrocarbon monoterpenes. Most EOs exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including E. coli and Klebsiella strains. Furthermore, several binary EO combinations demonstrated synergistic and/or additive effects, with significantly lower MIC and MBC values.

Conclusions: The results highlight the broad-spectrum antimicrobial potential of these essential oils and support their potential use as natural antibacterial agents in pharmaceutical formulations or as bio-based preservatives.

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ID610 | Characterization and immunomodulatory effect of Alternaria alternata extracellular vesicles in host-pathogen interactions

Author: Daniela Calheiros

iCBR - Coimbra Institute for Clinical and Biomedical Research & CiBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal and FMUC - Faculty of Medicine, University of Coimbra, Portugal

E-mail: danielamcalheiros@gmail.com

Other authors: Rita Domingues 1,2; Lisa Rodrigues 1,2; Célia Nogueira 1,2; Mariana Afonso 1,2; Maria da Cunha 3; Ana Todo-Bom 2,4; António Miguéis 2,4; Arturo Casadeval 5; Marcio Rodrigues 6,7; Kavita Sharma 8; Aishwarya Saha 8; Michael Strader 8; Jay Slater 8; Chantal Fernandes 1,2 and Teresa Gonçalves 1,2

1 iCBR - Coimbra Institute for Clinical and Biomedical Research & CiBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; 2 FMUC - Faculty of Medicine, University of Coimbra, Portugal; 3 Hospital de Vila Franca de Xira, Vila Franca de Xira, Portugal; 4 CNC-UC - Center for Neuroscience and Cell Biology- University of Coimbra & CiBB – Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; 5 Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 6 Instituto Carlos Chagas, Fundação Oswaldo Cruz (Fiocruz), Curitiba, Brasil; 7 Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Brasil; 8 Laboratory of Immunobiochemistry, Division of Bacterial, Parasitic and Allergenic Products, Office of Vaccines Research and Review, Center for Biologics

Background: Alternaria are filamentous fungi associated with human opportunistic infections and respiratory allergies, including severe asthma. Daily exposure to these fungi raises concerns about airway colonization as a potential continuous source of allergens. Here, we isolated the EVs produced by Alternaria alternata (AaEVs), the most common Alternaria species involved in human disease(s) to characterize their cargo and their impact on host cells.

Methods: Transmission electron microscopy (TEM) was used to characterize the morphology of AaEVs. Neutrophil activation was evaluated through extracellular trap formation and macrophage uptake was confirmed by immunocytochemistry, followed by quantification of TNF-α gene expression. Sera from 30 patients diagnosed with respiratory allergies were screened for IgE reactivity against AaEVs. Proteomic characterization of AaEVs was performed using RP LC-MS/MS and data-independent acquisition.

Results: TEM revealed that AaEVs are double-membrane vesicles with a mean diameter of 115 nm. Neutrophil interaction assays demonstrated extracellular trap release upon exposure to AaEVs. Macrophages internalized AaEVs, leading to a significant increase in TNF-α gene expression compared with controls. IgE from patient sera recognized allergens contained within AaEVs. Proteomic profiling identified over 1,400 proteins, with functional distribution including metabolism (27%), cell wall synthesis/vesicle formation and trafficking (14%), translation/transcription/replication (8%), stress/homeostasis (7%), mitochondrial/ATP synthesis (5%), and cytoskeletal proteins (2%), 37% remained uncharacterized. A putative sialidase was the most abundant protein and its enzymatic activity was confirmed. Virulence-associated phospholipases B and C and 7 of the 12 known A. alternata allergens were also identified in AaEVs cargo.

Conclusions: These results indicate that AaEVs are immunomodulatory and might be important vehicles of allergens. Proteome characterization provides insights into the physiopathology of respiratory diseases, particularly in allergy. Moreover, AaEVs are likely key players in the early interaction with human host cells (e.g. sialidase) and in the progression to allergic reactions (e.g. allergens). Further studies will focus on sialidase and other key proteins involved in the early interaction with human host cells and their role in the progression to a severe allergic reaction.

ID617 | Natural Mineral Waters in Wound Healing: Addressing Pathogenic Multidrug Resistance in Diabetic Foot Ulcers

Author: Ana Rita Pinto

AquaValor – Centre for the Valorization and Transfer of Water Technology, Association, 5400-342 Chaves, Portugal; CITAB/ Inov4Agro/UTAD - Centre for the Research and Technology of Agroenvironmental and Biological Sciences, University Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

E-mail: al60149@alunos.utad.pt

Other authors: Juliana Garcia[1,2,4]; Rafaela Guimarães [1,4]; Maria José Alves[1,3,4]; Maria José Saavedra[2]

[1] AquaValor – Centre for the Valorization and Transfer of Water Technology, Association, 5400-342 Chaves, Portugal [2] CITAB/ Inov4Agro/UTAD - Centre for the Research and Technology of Agroenvironmental and Biological Sciences, University Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal [3] CIMO/SusTEC – Mountain Research Centre, Associated Laboratory for Sustainability and Technology in Mountain Regions, Polytechnic Institute of Bragança, 5300-253 Bragança, Portugal [4] LiveWell – Research Centre for Active Living & Wellbeing, Polytechnic Institute of Bragança, 5300-253 Bragança, Portugal

Background: Diabetic foot ulcers (DFUs) are chronic wounds often colonized by multidrug-resistant (MDR) bacteria, including Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Acinetobacter baumannii. These microorganisms establish biofilms that protect them from antibiotics and host immunity, aggravating inflammation and delaying healing. MDR infections in DFUs are a major public health problem, contributing to treatment failures, prolonged hospitalizations, and increased mortality. Alongside microbial burden, chronic wounds also display enzymatic dysregulation, with matrix metalloproteinases (MMP-2, MMP-9) excessively degrading extracellular matrix (ECM). New therapeutic approaches are urgently required to counter both MDR pathogens and wound microenvironment imbalances.

Methods: Six natural mineral waters (NMWs) from Northern Portugal were analyzed for chemical composition and evaluated for antimicrobial, anti-inflammatory, and enzymatic modulatory activities. In vitro assays tested growth inhibition of MDR wound-associated bacteria and macrophage inflammatory response (RAW 264.7). Enzymatic activity assays focused on the regulation of MMP-2 and MMP-9 expression in fibroblasts and keratinocytes. Mineral content (bicarbonates, magnesium, silica, zinc) was correlated with bioactivity.

Results: All NMWs displayed distinct bioactive profiles. NMW-C and NMW-D achieved higher inhibition against S. aureus (53%) and K. pneumoniae (35%), respectively, while NMW-F was the only one active against P. aeruginosa (9%). Anti-inflammatory effects were most pronounced in NMW-B, NMW-E, and NMW-F (EC-- 24–25 µg/mL). Enzymatic assays revealed that NMW-C enhanced controlled MMP-2 expression, favoring keratinocyte migration, whereas NMW-F reduced MMP-9 activity, limiting excessive ECM degradation and inflammation. These effects were linked to the mineral composition, with zinc and copper supporting collagen cross-linking and antioxidant defense, while bicarbonates and magnesium promoted angiogenesis.

Conclusions: Our findings demonstrate that specific NMWs combine antimicrobial effects against MDR pathogens with enzymatic modulation, providing dual action in DFU management. These results reinforce the potential of NMWs as adjunctive, mineral-based therapeutic resources for tackling the microbiological and enzymatic barriers that hinder wound healing, bridging environmental microbiology and health biotechnology.

ID622 | Microbial Diversity in Chaves Thermal Natural Mineral Water: A Reservoir of Biotechnological and Therapeutic Potential

Author: Sara Araújo

AquaValor – Centro de Valorização e Transferência de Tecnologia da Água, Chaves, Portugal

E-mail: saravanessaaraujo07@gmail.com

Other authors: Cátia Fidalgo 1,2, Ana Cristina Gonçalves 2,3, Daniela Correia 1,2, Luís Pinto 1,2, Maria José Alves 1,2,4,5

1 AquaValor – Centro de Valorização e Transferência de Tecnologia da Água, Chaves, Portugal 2 Research Centre for Active Living and Wellbeing (LiveWell), IPB, Bragança, Portugal 3 Vigo University, Faculty of Law, Ourense Campus, Espanha 4 Centro de Investigação de Montanha (CIMO), IPB, Bragança, Portugal 5 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), IPB, Bragança, Portugal

Background: In recent years, thermal natural mineral waters (TNMW) have received increasing attention due to their therapeutic properties and the unique microbial communities they sustain. Traditionally used for health benefits in rheumatology, gastroenterology, pulmonology, and dermatology, TNMW s are also increasingly explored for ecological and biotechnological applications. TNMW of Chaves, located in the northern of Portugal, is distinguished by a unique geochemical profile, elevated emerging temperature (76ºC) and therapeutic indications, including rheumatology, gastroenterology, and pulmonology. Microbial communities thriving in such environments, adapted to extreme physicochemical conditions, might play important roles in human health, environmental and industrial processes. This study aimed to characterise the physicochemical composition and microbial taxonomic communities of Chaves TNMW.

Methods: Water samples were collected from the harvesting site (HS) and two reservoirs (R30 and R55), during three consecutive spring campaigns (2023-2025). Physicochemical parameters were measured, and microbial DNA was extracted and analysed using next-generation sequencing coupled with 16S rRNA gene profiling.

Results: Results revealed strong links between environmental gradients and microbial diversity, with distinct community profiles across 3 sites. HS, characterised by the highest temperature, neutral pH, and elevated sodium and bicarbonate levels, was dominated by the sulphur-reducing thermophile Caldimicrobium. R30, with lower temperatures, acidic pH, and the highest iron concentration, was enriched in Sideroxydans, Comamonas, and Curvibacter, consistent with iron-oxidising and heterotrophic activity. In contrast, R55, with intermediate temperature, neutral-to-slightly acidic pH, and higher silica content, was dominated by thermophiles such as Thermus and Hydrogenobacter.

Conclusions: These findings suggest that temperature, pH, and geochemical composition may act as key drivers of microbial variability in hydrothermal systems. The microbial traits observed suggest the potential of Chaves TNMWs as source of bioactive compounds with therapeutic, cosmetic, and environmental applications. Overall, this study highlights Chaves TNMWs as a natural laboratory of microbial diversity and a promising reservoir of metabolites with translational potential.

ID628 | Fungal microbiome changes and associated serum NMR metabolic signatures in stroke: A 3-month pilot study

Author: Isabel Miranda

1RISE-Health, Department of Surgery and Physiology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

E-mail: imiranda@med.up.pt

Other authors: Diana Martins1, Ana Beatriz Batista1, Micael Gonçalves2, António Carlos Ferreira1, Cláudia Silva1, Juliana Ferreira6, Joana Pinto3,4, Paula Guedes de Pinho3,4, Paulo Castro Chaves1,5, Sandra Marisa Oliveira1, Adelino Leite-Moreira1, Pedro Castro6,7, Sílvia Diaz1, António Barros1*

1RISE-Health, Department of Surgery and Physiology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal; 2Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, 3810 -193 Aveiro, Portugal; 3Associate Laboratory i4HB-Institute for Health and Bioeconomy, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 4UCIBIO/REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 5Department of Internal Medicine, Unidade Local de Saúde São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal; 6RISE-Health, Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal; 7Department of Neurology, Unidade Local de Saúde São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

Background: The gut-brain axis is essential in the pathophysiology of stroke, yet the role of the mycobiome in stroke recovery remains largely unexplored. This pilot study, conducted over time, examined the changes in the fungal microbiome and its relationship with serum metabolites in stroke patients.

Methods: We analysed fungal communities and serum NMR metabolite profiles in 32 stroke patients at emergency department admission (T0) and 3 months after stroke (T2). Alpha diversity metrics (Shannon, Simpson, Observed species), compositional analysis, and fungal-metabolite integration were assessed using ALDEx2 differential abundance analysis and multivariate approaches.

Results: Fungal alpha diversity did not differ significantly between T0 and T2. However, compositional analysis revealed substantial shifts in fungal communities. Candida dominated both time points but decreased from ~60% to ~45% relative abundance. Sclerotiniaceae increased markedly at T2 (~20%), whereas Wallemia emerged as a significant component (5%) only at T2. Integration analysis of fungal serum metabolite data showed clear temporal clustering with overlapping but distinct distributions between time points. The top variable loadings identified Byssomerulius, Ceratobasidiaceae, Phaeosphaeria, Pterula, and Aphanobasidium as key fungal discriminators, whereas serum NMR resonances at 2.24, 4.14, 2.28, 2.32, and 2.40 ppm showed the strongest associations. Correlation analysis revealed positive associations between all selected serum metabolites and fungi (r=0.3-0.8), suggesting systemic metabolic shifts. ALDEx2 analysis identified Fungi_gen_Incertae_sedis as significantly enriched at T2 (effect=-0.094, overlap=0.456), indicating potential recovery-associated fungal signatures.

Conclusions: While overall fungal diversity remained stable during stroke recovery, compositional and functional alterations occur within the mycobiome. The correlation between fungal taxa and serum NMR metabolites suggests systemic metabolic changes linked to mycobiome reorganisation during recovery. These findings highlight the potential role of the mycobiome in post-stroke recovery and identify fungal metabolite interactions that may represent potential therapeutic or prognostic targets.

*These authors contributed equally to this work

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ID643 | Ceftaroline resistant clinical MRSA strains accumulate a new cis-peptidated muropeptide in peptidoglycan

Author: Carolina Máximo

UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;

E-mail: c.maximo@campus.fct.unl.pt

Other authors: Jorge Silva Dias, Michele Karolak, Rita Gonçalves Sobral, Adriana Rosato

UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal; Center for Molecular Medicine, MaineHealth Institute for Research, Scarborough, 04074 ME, USA; Tufts University School of Medicine, Boston, MA, USA.

Methicillin-resistant Staphylococcus aureus (MRSA) poses a significant health problem worldwide and a therapeutic challenge. Resistance to β-lactams is due to the acquisition of the mecA gene, which encodes the extra penicillin-binding protein 2a (PBP2A), a β-lactam-insensitive protein that takes over cell wall synthesis in the presence of the antibiotic. In the effort to develop antimicrobial alternatives, ceftaroline (CPT), a "fifth-generation" cephalosporin, was introduced in clinical practice. In contrast to other β-lactams, CPT is active against MRSA strains because it binds to the allosteric site of PBP2A, inducing a conformational change that makes the active site accessible. A second molecule of CPT then binds irreversibly and acylates the active site residue serine (S403), inactivating the transpeptidase function of PBP2A.

Recently, resistance to CPT emerged in MRSA isolates from cystic fibrosis patients. The highly CPT-resistant 5007 clinical strain, that evolved from the CPT-susceptible strain 3957, gained three mutations in mecA gene, one in the allosteric site (E239K) and two in the penicillin-binding pocket of the transpeptidase region (Y446N and E447K).

Analysis of mutanolysin digested-peptidoglycan by RP-HPLC identified a new muropeptide structure that was absent from the peptidoglycan of 3957. NMR and mass spectroscopy analysis revealed that in this new structure the pentaglycine bridge assembles into a cyclic branch, originating cis-peptidation; therefore, averting the process of transpeptidation from occurring. The new muropeptide was not found in the cell wall of the 5007DmecA mutant, showing that the cyclization occurred due to the activity of the modified PBP2A. Furthermore, the intermediate clinical isolates 4519 and 5006, that harbor one mutation (Y446N) and two mutations (Y446N and E447K), respectively, both showed the presence of the cis-peptidated muropeptide, suggesting that the mutation in the allosteric site is not involved in the cell wall phenotype.

As a result of our study, we aim to improve understanding of the molecular basis of CPT resistance to advance its clinical application.

ID655 | Are Domestic Snakes Silent Carriers of Zoonotic Bacteria? A Public Health Perspective

Author: Ana Esteves

1 Unidade de Genómica e Bioinformática, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Doutor Ricardo Jorge, Portugal. 2 Centro de Ciência Animal

E-mail: ana.esteves@insa.min-saude.pt

Other authors: Andreia Rodrigues1, João C. Rodrigues3, Rui Matias3, Eduardo Marcelino4, Andreia Valença4, Rui Patrício4, Adriana Belas2,4,5*, Alexandra Nunes1,2,4*

1 Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal.
2 CECAV - Animal and Veterinary Research Center, Faculty of Veterinary Medicine, Lusófona University - Lisbon University Centre, Lisbon, Portugal. 3 Microbiology Laboratory, Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal. 4 I-MVET- Research in Veterinary Medicine, Faculty of Veterinary Medicine, Lusófona University- Lisbon University Centre, Lisbon, Portugal. 5 Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Lisbon, Portugal.

Background: Reptiles are rising as the 'new pet animals', yet their role as asymptomatic carriers of zoonotic bacteria remains unknow to the common folk. To everyone's surprise, snakes are the main reptiles that people choose to have in their homes, but this animal is recognized as a reservoir of Gram-negative bacteria, such as, Salmonella spp., Escherichia coli, among others, both highly associated with antimicrobial resistance (AMR). This study aims to investigate the diversity, and antimicrobial resistance of Gram-negative bacteria in domestic snakes in Portugal, from a public health perspective.

Methods:A total of 154 oral and cloacal swabs were collected from the cavities of 77 snakes (Python regius). The samples were thus processed by selective enrichment and culture. Antimicrobial susceptibility testing was performed to identify ESBL/CRE phenotypes, followed by bacterial identification using MALDI-TOF MS. Salmonella-positive isolates were subsequently analyzed by whole-genome sequencing (WGS) for genomic characterization.

Results: A total of 386 isolates were obtained, with 75% (n=288/386) originating from cloacal swabs and 25% (n=98/386) from oral samples. It was possible to identify twenty-five (25) different bacterial species, with the most frequent being E. coli (40%; n=154/386), Salmonella spp. (17%; n=67/386), and Klebsiella pneumoniae (10%; n=39/386). S. enterica was found in 49 snakes (63 cloacal, 4 oral), with co-infections in 83.7% (n=41) of the isolates. ESBL phenotypes were detected in some isolates, one of them being Salmonella enterica, confirming the circulation of β-lactamase-producing bacteria. In silico analysis emphasized the existence of S. enterica subsp. enterica serovar Paratyphi B (ST88), amongst other serotypes identified, such as S. enterica subsp. diarizonae ser. 47:z10:z35 (ST4578), being the most common in 19 serotypes identified.

Conclusions: Domestic snakes harbor a wide diversity of zoonotic bacteria, including multidrug-resistant strains, such as ESBL-producing Enterobacterales, and Salmonella enterica serovar Paratyphi B. Thus these findings underscore the public health risks posed by exotic pets and emphasize the urgency of implementing preventive measures within a One Health framework.

ID687 | Bioprospecting of antibiofilm and antimicrobial activity of Bacillus sp. strains from the Azores against Cutibacterium acnes

Author: Ana Carolina Mendonça

Centre of Biotecnology of Azores (CBA), Faculty of Science and Technology, University of Azores, University of Aveiro- Biology Departemen- Portugal

E-mail: anamacanita99@gmail.com

Other authors: Ana Mendonça1,3; 1- Carla Cabral1, Duarte Toubarro; 2- António Machado; 3- Artur J.C.P. Alves

1-Centre of Biotecnology of Azores (CBA), Faculty of Science and technology, University of Azores 2- Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales COCIBA, Instituto de Microbiología, Ecuador 3- University of Aveiro, Biology Department

Cutibacterium acnes is a Gram-positive bacterium that exists on human skin microbiome but is strongly implicated in the pathogenesis of Acne vulgaris skin condition. A key feature underlying its pathogenicity is the ability to form biofilms, which confer protection against host defences and markedly reduce susceptibility to conventional antibiotic therapies. Consequently, there is an urgent need to identify novel antimicrobials that can inhibit both planktonic growth and biofilm formation.

In this study, we evaluated the antibacterial and anti-biofilm properties of cell-free supernatants (CFS) derived from 35 Bacillus isolates obtained from Azorean soils. The antimicrobial activity of the CFS was initially screened using agar diffusion assays, followed by broth microdilution assays in 96-well microplates to determine inhibitory activity against C. acnes. Biofilm inhibition and degradation were further assessed through resazurin staining and adhesion assays. The most active supernatant was subjected to semi-purification steps, followed by determination of its minimum inhibitory concentration (MIC), evaluation of its proteinaceous nature, and assessment of its activity against established biofilms.

Among all isolates tested, one Bacillus strain exhibited strong and reproducible antimicrobial activity against

C. acnes, with inhibition values in microdilution assays falling within ranges considered effective for therapeutic potential. The semi-purified protein fraction of high molecular weight displayed marked antibacterial activity, significantly reducing both bacterial growth and biofilm formation. These results suggest that the bioactive compounds secreted by this isolate are likely of protein nature and may act through mechanisms targeting adhesion and biofilm integrity.

Overall, this work highlights the Azorean soil microbiota as a valuable reservoir of Bacillus species capable of producing bioactive molecules with antimicrobial and anti-biofilm activities. The findings underscore the potential of these microorganisms as promising sources for the development of alternative therapeutic agents against C. acnes, while also emphasising the importance of exploring unique ecosystems for the discovery of novel compounds with clinical applications

ID688 | Azorean Bacillus as a Source of Antimicrobial Peptides Targeting Staphylococcus aureus

Author: Carla Cabral

Centro de Biotecnologia dos Açores/Faculdade de Ciências e Tecnologia da Universidade dos Açores

E-mail: carla.mm.cabral@uac.pt

Other authors: Sabrina Pacheco1, Duarte Toubarro2, Jorge Frias1

1Centro de Biotecnologia dos Açores, Universidade dos Açores; 2 Centro de Biotecnologia dos Açores/Faculdade de Ciências e Tecnologia da Universidade dos Açores

Bacteria produce antimicrobial peptides (AMP) active against various microorganisms, that show potential for the development of new drugs. It is known that some Bacillus-derived AMPs act against multidrug-resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). This work aimed to identify antimicrobial compounds against Staphylococcus aureus, produced by Bacillus isolated from the soil of the Azores.

From 20 initial isolates tested by co-inoculation with Micrococcus luteus, S. aureus, and Escherichia coli, two were selected (S117B and S118E), with S118E demonstrating the greatest activity, particularly against

S. aureus. The activity once lost in the transition from solid to liquid medium was recovered by immobilizing Bacillus cells in agar beads, in this way optimizing antimicrobial peptide (AMP) production.

Proteinase K degradation (5mg/mL) and thermal inactivation of the supernatant confirmed the proteinaceous nature of the active compounds. The culture supernatant was collected, filtered, and tested at all stages, initially by the agar diffusion assay and, later, replaced by the liquid medium dilution method.

Culture supernatant from strain S118E was concentrated and fractionated by optimized acetone precipitation, followed by fractionation through Superdex peptide 10/300 GL column, C18 resin reversed phase column and RPC reversed phase column by Fast protein liquid chromatography (FPLC). During the process it was verified that the active compound was not detectable at absorbance of 280nm, that the molecules are smaller than 5kDa and were eluted mainly with 30% (30% C18) and 60% acetone (60% C18). The highest purification reached a 220-fold increase with 20% recovery. Both fractions were analyzed by MALDI-TOF MS, showing enrichment in peptides with molecular weight between 700 and 1024 Da. Analysis of the 16S gene and the genome sequencing (3.6 Mbp) identified isolate S118E as Bacillus pumilus. Genome mining resulted in the identification of 4 gene clusters with 78%, 80%, 85% and 91% similarity with gene clusters coding for lichenisin, bacilibactin, bacillysin and plantazolicin, respectively.

In this work it was possible to identify AMPs produced by Bacillus pumilus S118E against Staphylococcus aureus, requiring further tests to confirm their anti-MRSA activity.

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ID699 | Serological and Molecular Diversity of Streptococcus pneumoniae Isolated in Cabo Verde

Author: Sandrine de Pina

1Faculty of Science and Technology, University of Azores, Ponta Delgada, Portugal

E-mail: sandrinecruz16@gmail.com

Other authors: Inês Santos 1, Riselly Bettencourt 3, Delcy Santos 3, Carla Cabral 1,2, Isabel Araújo 3, Nelson Simões 1,2, Duarte Toubarro 1,2*

1Faculty of Science and Technology, University of Azores, Ponta Delgada, Portugal

2Center of Biotechnology in Azores, Ponta Delgada, Portugal

3Faculty of Science and Technology, University of Cabo Verde, Praia, Cabo Verde

Background: Streptococcus pneumoniae is a major human pathogen associated with high morbidity and mortality, particularly in children, the elderly, and immunocompromised individuals. It can also colonize the nasopharynx asymptomatically, especially in children, often preceding invasive pneumococcal disease. The polysaccharide capsule is the main virulence factor and the basis of current pneumococcal vaccines. Since 2017, S. pneumoniae has been included in the WHO Bacterial Priority Pathogens List (medium priority), emphasizing the need to address its

public health impact, especially in resource-limited settings such as Cabo Verde. Despite the global burden of pneumococcal disease and increasing antimicrobial resistance, no data are available on its epidemiology, serotype distribution, or resistance patterns in Cabo Verde.

Methods: A total of 120 suspected samples were collected between April 2024 and April 2025 from the Agostinho Neto University Hospital (HUAN), the Clinical Analysis Laboratory (LAC), and the Santa Maria Laboratory. PCR detection of the autolysin gene (lytA) confirmed 69 isolates as S. pneumoniae, which were stored in a DNA bank for further analyses. The serotypes were determined using multiplex PCR.

Results: Serotyping identified 21 distinct serotypes: 18C, 9V, 3, 8, 12F, 20, 5, 33F, 35B, 7F, 15B/C, 16F, 19F, 22F, 1, 4, 9N, 11A, 23F, 31, 35F, and 38. The six most frequent serotypes (18C, 9V, 3, 8, 12F, 20) accounted for 55.2% of isolates. Serotype 18C (18.9%) predominated in non-invasive disease among children, suggesting a role in asymptomatic colonization. Serotype 9V (10.2%) was mainly found in adults and the elderly, while serotypes 3 (8.7%) and 8 (5.8%) were strongly associated with IPD in older adults, consistent with their recognized virulence and association with international pneumococcal clones. Vaccine coverage analysis indicated that PCV13 would cover an estimated 62.5% of the identified serotypes. However, PCV13 is not part of Cabo Verde's National Immunization Program, leaving high-risk groups unprotected.

Conclusions: Despite limitations in sample size, this study provides the first serotype distribution data of S. pneumoniae in Cabo Verde, revealing substantial diversity and preliminary antimicrobial resistance insights. These findings are critical for surveillance, vaccine policy, and the prevention of pneumococcal disease in the archipelago.

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ABSTRACTS

TOPIC #5: Molecular Microbiology and Microbial Physiology

ORAL COMMUNICATIONS

ID220 | The great escape: loss of WTA glycosylation or L-form conversion protects against bacteriophage infection

Author: Martin J. Loessner

Department of Health Science and Technology, ETH Zurich, Switzerland

E-mail: martin.loessner@ethz.ch

Background: The first step in bacteriophage infection is recognition of, and binding to, cell-wall associated phage receptors. In Listeria monocytogenes, these have been identified as glycosylated wall teichoic acids (WTA). Phage predation rapidly selects for mutations leading to loss of glycosylation, providing stable immunity to phage infection.

Bacteriophages eventually kill their host bacteria through enzymatic lysis at the end of the lytic cycle. However, in Gram-positive bacteria, the release of such peptidoglycan-degrading endolysins not only results in explosive lysis of the infected host, but may also affect neighboring cells as a result of collateral damage.

Results: Loss of WTA glycosylation not only changes the surface antigens and serovar, but also causes strong attenuation of bacterial virulence through loss of essential factors such as InlB, which itself requires glycosylated WTA for presentation at the cell surface.

Furthermore, we show that Listeria monocytogenes can evade phage predation by undergoing transient conversion to a cell wall-deficient L-form state. The trigger for the escape is not phage infection itself, but the concommitant release and subsequent accumulation of endolysins in the environment during phage infection cycles. The enzymes can hydrolyze and induce lesions into the cell walls of non-infected bystander cells, resulting in the turgor-driven extrusion of wall-deficient, but viable L-form bacteria. Hence, phage infection and subsequent cell lysis can indirectly induce L-form conversion among phage-sensitive bacterial populations, providing resistance to further infection. Remarkably, in the absence of phage predation and endolysin-based selective pressure, L-forms may revert to the walled state.

Conclusions: Collectively, our results indicate that Listeria and other Gram-positive pathogens such as Enteroccus can effectively evade phage predation not only by loss of wall teichoic acid glycosylation, but also by transient switching of subpopulations to the L-form state. Consequently, L-form conversion serves as a population-level persistence mechanism to evade complete eradication by phage attack. This escape mechanism appears widespread amongst Gram-positive bacteria, and has important implications for the development of phage- and endolysin-based therapeutic interventions.

ID232 | Structural and mechanistic insights into the central respiratory complex in DSR metabolism

Author: Raquel M. Bernardino

Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier (ITQB NOVA), Universidade NOVA de Lisboa; Oeiras, Portugal

E-mail: rm.bernardino@itqb.unl.pt

Other authors: Max Dongsheng Yin2 , Ana C. C. Barbosa1, Jos A. Brito1, Andreia I. Pimenta1, In s A. C. Pereira1, Bonnie J. Murphy2

1 Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier (ITQB NOVA), Universidade NOVA de Lisboa; Oeiras, Portugal. 2 Redox and Metalloproteins Research Group, Max Planck Institute of Biophysics; Frankfurt am Main, Germany.

Dissimilatory sulfite reduction (DSR) is a central pathway in microbial sulfur metabolism and is believed to be an ancient respiratory process sustaining life in the early Earth. Microbial reduction of sulfur compounds plays a key role in driving the biogeochemical sulfur and carbon cycles [1]. Furthermore, its relevance for human health has become increasingly recognized, as gut microbial sulfide production has been associated with chronic inflammatory disorders and cancer [2].

Over the last years, our group has made significant advances in elucidating the biochemical mechanisms involved in the reduction of sulfite to sulfide and how this process is coupled to energy conservation. The central proteins of the DSR pathway are DsrAB, DsrC, and the membrane-bound DsrMKJOP complex. DsrAB, together with its cosubstrate DsrC, reduces sulfite to a zero-valent sulfur (S0), forming a trisulfide in DsrC [3]. The DsrC-trisulfide is subsequently reduced to sulfide by the transmembrane DsrMKJOP complex, which is believed to couple sulfite reduction to chemiosmotic energy conservation [4]. Here, I will present the first Cryo-EM structure of the DsrMKJOP complex, which reveals key insights into the mechanism of DsrC-trisulfide reduction and its coupling to energy conservation.

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ID234 | Inhibition of toxic sulfide production by sulfidogenic members of the human gut microbiota

Author: Madalena Duarte

Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
E-mail: madalena.duarte@itqb.unl.pt

Other authors: In s A.C. Pereira, Andreia I. Pimenta

Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Excessive proliferation of sulfide-producing bacteria (SB) in the human gut, often driven by Western diets, rich in animal protein and fat, leads to the accumulation of hydrogen sulfide (H S). This increase has been associated with taurine respiration via the dissimilatory sulfite reductase (Dsr) pathway, a metabolic pathway also used by many SB for sulfate and sulfite respiration, which is among the major microbial sources of intestinal H S. The accumulation of H S can mediate epithelial cell injury, mucous layer disruption and inflammation. Such effects contribute to intestinal barrier dysfunction and have been strongly implicated in the onset and progression of inflammatory bowel disease (IBD) and colorectal cancer, among other diseases. This project aims to identify novel inhibitors of the DsrMKJOP membrane complex, a crucial component of the Dsr pathway and responsible H2S production. Based on recently resolved Cryo-EM structural data, two quinone-binding sites within DsrMKJOP were identified as potential drug targets due to their likely role in the complex s energy-conserving function. The DsrMKJOP native complex was purified from Bilophila wadsworthia (Bw), while the other Dsr components were heterolougously expressed in Escherichia coli using genes cloned under an inducible promoter. In vitro enzymatic assays were performed to test the inhibitory efficacy of several quinone-analogs and to characterize the inhibition profile of the DsrMKJOP. The expression and purification of the DsrMKJOP complex from Bw yielded functionally intact protein suitable for biochemical characterization. In vitro inhibition assays have been established, allowing for the evaluation of candidate inhibitors targeting the quinone-binding site within the DsrMKJOP complex. By integrating microbial genetics and structural biology, this study proposes a targeted strategy to suppress harmful bacterial metabolism. Targeting the DsrMKJOP complex may provide a specific means to reduce H S production without disrupting overall gut microbial balance, presenting a promising therapeutic avenue for the restoration of intestinal homeostasis in IBD and potentially other H S-associated disorders.

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ID248 | To be or not to bi(ofilm): the influence of mediator-complex subunits in Candida biofilm formation

Author: Inês Vieira Costa

1 iBB - Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior T cnico, Universidade de Lisboa Lisboa, Portugal; 2 I4HB Institute for Health and Bioeconomy - Laborat rio Associado, Portugal.

E-mail: inesvcosta@tecnico.ulisboa.pt

Other authors: Maria Zolotareva 1,2; Andr Horta Maior 1,2; Miguel Cacho Teixeira 1,2

1 iBB - Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior T cnico, Universidade de Lisboa Lisboa Portugal; 2 I4HB Institute for Health and Bioeconomy - Laborat rio Associado, Portugal.

Background: Millions of people worldwide are affected by fungal infections every year. Some major fungal pathogens are from the genus Candida, like Nakaseomyces glabratus (former Candida glabrata) whose incidence is rising. This fungal pathogen is inherently less susceptible to azoles, and its ability to form biofilms and adhere to different substrates, further exacerbate its virulence. Even though biofilms are valuable tools to pathogenic yeasts, the molecular basis of biofilm formation evolution and transcriptional control in clinical isolates is understudied .

Methods: The ability to form biofilms was characterized in a collection of N. glabratus clinical isolates. Using an experimental microevolution assay, we evolved low biofilm producers into high biofilm-producing isolates. Through comparative genomics of isolates before and after evolution, several key contributors to biofilm formation were found with mutations, including in the CgPGD1A gene, encoding a tail-subunit of the Mediator Complex (MC). The role of CgPGD1A in biofilm formation was assessed using biofilm quantification assays and RNA-sequencing.

Results: After evolution from low- to high-biofilm producing N. glabratus isolates, consistent mutations in CgPGD1A were found. Biofilm quantification showed that in the absence of either CgPGD1A or CgPGD1B biofilm production is nearly halved. Other tail subunits were tested (GAL11A and GAL11B) but their deletions increased biofilm formation, highlighting distinct functional roles among MC components. Transcriptomic analysis showed the influence of CgPGD1A in the gene expression profile of N. glabratus under biofilm growth conditions. Specific contributors and critical pathways that govern biofilm formation and potential drug resistance were identified, such as those involved in adhesion, cell wall organization, carbon and nitrogen metabolism, and drug resistance.

Conclusions: These findings underscore the MC's influence on fungal pathogenesis, supporting continued exploration of MC subunits and their role in N. glabratus infections.

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ID322 | Peroxisomal engineering for improved lactones sustainable biosynthesis within a circular economy

Author: Miguel Francisco

CEB Centre of Biological Engineering, University of Minho, Braga, Portugal

E-mail: miguelfrancisco8507@gmail.com

Other authors: Eduardo Coelho (1,2), Luc lia Domingues (1,2)

1 CEB Centre of Biological Engineering, University of Minho, Braga, Portugal; 2 LABBELS Associate Laboratory, Braga, Portugal

Lactones, like γ-decalactone, are highly valuable cyclic esters used as fragrances and flavor enhancers. These are produced mainly by chemical synthesis or bioconversion of hydroxy fatty acids (HFAs), which possess high industrial value, making HFAs requirement a major drawback of these strategies. Therefore, alternatives are required for the development of sustainable production processes. Ashbya gossypii, a filamentous fungus industrially used for riboflavin production, is capable of de novo lactone production and low-cost carbon source utilization, indicating a potential candidate for sustainable processes. Peroxisomes are organelles that provide acetyl-CoA from fatty acid (FA) degradation through the β-oxidation pathway, from which intermediate compounds can derive and be converted to lactones. Organelle compartmentalization of metabolic pathways can improve target compound production, as shown in yeasts using enzyme surface display at the peroxisome together with enlarged membrane surface area to successfully increase acetyl-CoA levels and derived products. Therefore, translation of peroxisome engineering in A. gossypii poses as a potential strategy to enhance de novo γ-decalactone production.; Metabolic engineering of A. gossypii was performed to regulate expression of genes involved in peroxisome formation, along with expression of endogenous genes involved in lipogenesis. Developed strains were characterized, γ-decalactone production was analyzed by GC-FID, lipid quantification and profiling were achieved by GC-MS and peroxisome associated morphological changes were observed by microscopy and fluorescence analysis.; An A. gossypii peroxin for peroxisome formation and a fusion of a transmembrane peroxin for peroxisome surface display with reporter gene were successfully integrated in the genome. These constructions supported the study of differences in peroxisome number and/or size by assessing gene reporter signal. Microscopy showed the potential of peroxin for peroxisome surface display of enzymes in A. gossypii. Developed strains showed similar biomass production to the wild-type, indicating no additional metabolic burden despite no increment in γ-decalactone production.; Altogether, these results showed the feasibility of peroxins for peroxisome modifications and organelle surface display in A. gossypii, while showcasing new metabolic engineering strategies to enhance γ-decalactone production towards a more sustainable bioprocess.

ID337 | Multi-omic Data Integration and Modeling for Yeast Physiology: Case Studies in Regulation, Metabolism, and Biomass Dynamics

Author: David Henriques

Acuabiotec Lab. Institute of Marine Research, Spanish National Research Council (IIM-CSIC), 36208 Vigo, Spain

E-mail: davidh@iim.csic.es

Background: Integrating multi-omic datasets is still a major challenge in systems biology, as the information often remains fragmented across different cellular layers. Saccharomyces cerevisiae is arguably the best model to tackle this challenge, given the wealth of data on its metabolism, signaling, and gene expression. Bringing these aspects together is a promising route toward a more holistic description of yeast physiology, although many gaps remain.

Methods: We combined genome-scale metabolic models (GSMMs), 13C/15N flux analysis, proteomics, and physiological assays in three case studies: (i) modeling the dynamics of HAP-regulated genes to link regulation with metabolism and growth; (ii) metabolic modeling of wine yeasts using isotope-labeled flux data; and (iii) empirical analysis of biomass composition and growth regulation. In parallel, we curated the

S. cerevisiae GSMM to identify and correct structural inconsistencies.

Results: Crossing models with data generated insights at several levels. Curation of the GSMM revealed problems with transporter annotation. In particular, ambiguous kinetics from reconstituted liposome studies left one mitochondrial carrier unresolved, which prevented correct operation of anaplerotic fluxes. Re-examining the evidence suggested malate and oxaloacetate as likely exchange substrates, improving model performance. Revisiting proteomic datasets enabled dynamic modeling of HAP gene expression, providing a better explanation of regulatory effects on metabolism and growth transitions. Time-resolved isotope labeling showed that temporal resolution matters: without it, incorporation dynamics are misrepresented. Finally, the combined use of empirical data and models allowed us to estimate biomass fractions and flux distributions in non-conventional yeasts, extending the analysis beyond S. cerevisiae.

Conclusions: These case studies show how data model integration can refine GSMMs, resolve mechanistic gaps, and link regulation with metabolism. The approach is not free of limitations, but it illustrates how iterative use of published and new data can move us closer to a systems-level understanding of yeast physiology and, by extension, eukaryotic cells.

ID420 | Intraspecific Variation in Nitrogen Utilisation of Torulaspora delbrueckii: Growth and Genomic Perspectives

Author: Maria João Sousa

CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal

E-mail: mjsousa@bio.uminho.pt

Other authors: Flávia Silva-Sousa 1,2, Ricardo Franco-Duarte1, Carole Camarasa2

1 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal 2 UMR SPO (Sciences pour l'Œnologie) - INRAE, Montpellier, France

Background: Nitrogen metabolism critically influences yeast growth, fermentation performance, and aroma formation. While this relationship has been extensively characterized in Saccharomyces cerevisiae (Sc), it remains less understood in non-Saccharomyces yeasts. These yeasts are gaining popularity in winemaking for their ability to create more complex and desirable aroma profiles. Among them, Torulaspora delbrueckii (Td) is increasingly used for its ability to enhance aroma complexity, improve mouthfeel, and reduce volatile acidity.

Methods: To address the limited understanding of amino acid utilization variability linked to genomic diversity within Td, we investigated the growth patterns of fifteen Td strains compared with a commercial Sc strain under diverse nitrogen conditions. In addition, the genomic repertoire of nitrogen transporter genes in Td was explored and compared to Sc genetic background.

Results: Substantial intraspecific diversity was observed among Td strains regarding nitrogen source preference and growth efficiency. Cysteine utilization emerged as a particularly distinctive trait relative to Sc. Td generally exhibited shorter lag phases than Sc across all nitrogen sources. Genomic analysis revealed the absence of several nitrogen transporters genes (ATF1-2, HIP1, TAT1-2) and duplications of others (YCT1, SAM3), suggesting potential differences in Td's nitrogen uptake mechanisms. For instance, although Td strains lack specific branched-chain amino acid transporters, they still utilized these substrates, showing variable preferences, particularly between leucine and valine.

Conclusions: Our findings highlight important genotypic and phenotypic differences between Sc and Td, along with high intraspecific diversity within Td. This deeper understanding of Td-specific traits, emphasizing the importance of nitrogen nutrition for growth, has potential applications in winemaking by guiding strain selection and managing co-cultures of Td and Sc.

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ID559 | Construction and analysis of a CRISPRi library to identify essential genes required for cell cycle progression in S. aureus

Author: Dominik Alwardt

Bacterial Cell Biology, Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: dominik.alwardt@itqb.unl.pt

Other authors: Ant nio D. Brito, Mariana G. Pinho

Bacterial Cell Biology, Instituto de Tecnologia Qu mica e Biol gica Ant nio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Background: Over 300 million people are expected to die from antibiotic-resistant bacteria in the next 35 years, underscoring the need for alternative antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) strains are currently the second leading cause of death from antibiotic-resistant infections. An understudied target for antimicrobial agents is the bacterial cell cycle. In this project, we aim to find new cell cycle regulators of S. aureus, as impairment of cell cycle progression will lead to bacterial growth arrest.

Methods: We optimized a CRISPR interference system to facilitate gene knockdown in the gram-positive pathogen S. aureus. Our approach uses a CRISPRi system from S. pyogenes, co-expressing dcas9, encoding an inactive Cas9, and a customizable single guide RNA. In our system, dcas9 is expressed from a single chromosomal copy in the MRSA strains COL or JE2, controlled by a tightly regulated promoter inducible by anhydrotetracycline. The sgRNAs are expressed from a plasmid under a constitutive promoter. This enables efficient, inducible knockdown of both essential and non-essential genes. With this approach, we constructed the Lisbon CRISPRi Mutant Library (LCML) of 261 strains in the JE2 background, with sgRNAs targeting 200 essential genes/operons. Using Super-resolution microscopy we screened the mutant library, aiming to identify phenotypes that provide insights into the regulation of the cell cycle. The deep-learning software eHooke was utilized to analyse each tested strain for alteration of the cell cycle phases, cell area and cell eccentricity.

Results: The resulting comprehensive dataset allowed us to identify numerous mutants with altered cell morphology and/or that are impaired in the cell cycle progression due to the repression of an essential gene, indicating the importance of the corresponding protein.

We identified mutants where depletion of well-known cell shape and division proteins, like FtsW and FtsZ, showed the expected morphology phenotypes and impaired cell cycle progression, validating the screen. Furthermore, our analysis revealed multiple mutants with strong impairment in the cell progression and morphological changes that had not been linked to the cell cycle progression yet.

Conclusion: Our work led to a comprehensive mutant library and corresponding dataset, allowing the study of S. aureus cell biology. We have identified promising candidates whose further study might reveal novel cell cycle regulators.

ID570 | Engineering of Dct-02 transporter to improve the biomanufacturing of succinate

Author: Francisca Ferreira

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

E-mail: pg52236@alunos.uminho.pt

Other authors: João Alves, Toni Rendulić, Pedro Soares, Margarida Casal, Isabel Soares-Silva

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

The dicarboxylate exporter Dct-02 of Aspergillus niger, a member of the Tellurite-resistance/Dicarboxylate Transporter (TDT) family, has been used in the bioproduction of succinate by increasing production yields in microbial cell factories. This study aimed to engineer Dct-02 with the ultimate goal of improving the bioproduction efficiency through increasing succinate export.

Approximately 10,000 proteomes were retrieved from NCBI Assembly, and a BLAST search was executed using the Dct-02 sequence from A. niger, with a threshold e-value of 10-6 and a query-cover value exceeding 65 %. For engineering the activity and specificity of Dct-02, amino acid residues were selected for site-directed mutagenesis based on their conservation across the TDT family, 3D structural modelling, and molecular docking analyses. The phenotypic impact of mutations was assessed by expressing the Dct-02 alleles in the Saccharomyces cerevisiae CEN.PK 113-5D strain, by evaluating cell growth on carboxylic acids, the ability to secrete succinate, and the subcellular localisation of GFP-tagged protein.

The phylogenetic relationships of Dct-02 homologues within the TDT family revealed a close association with known dicarboxylate transporters found in eukaryotic organisms, such as SpMae1 from Schizosaccharomyces pombe and AoMae from Aspergillus oryzae. Molecular docking of the 3D model of the Dct-02 protein, obtained by AlphaFold, identified seven binding positions for dicarboxylates. The site-directed mutagenesis strategy involved the selection of 20 amino acid residues, focusing on highly conserved residues within the TDT family and those predicted to interact with malate and succinate, resulting in the design of 31 mutations. Nine mutations affected the proper localisation of Dct-02 at the plasma membrane. Among the properly localised transporters, 11 mutations enabled an improved cell growth on carboxylic acids, suggesting a decreased succinate export activity of Dct-02. Three mutants presented a growth phenotype corresponding to a potential increase in succinate export capacity of Dct-02. In liquid cultures, one of these mutants presented improved succinate secretion.

With this approach we were able to develop Dct-02 variants with improved capacity to export succinate.

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POSTERS

ID214 | Exploring the fluoride resistome of two defluorinating bacteria

Author: Diogo A. M. Alexandrino

1 CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal 2 Department of Environmental Health, School of Health, P. Porto, Portugal

E-mail: dalexandrino@ciimar.up.pt

Other authors: Maria de Fátima Carvalho 1,3

3 ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Portugal

With the increasing interest in bacterial defluorination as a bioremediation strategy to combat organofluorine pollution (including PFAS), fluoride toxicity arises as a bottleneck. Fluoride, an inevitable end product of these biocatalytic processes, is highly bacteriostatic even at micro- and millimolar levels, limiting the efficiency of enzymatic defluorination. Intracellular fluoride leads to rapid metabolic arrest by inhibiting essential enzymes in central metabolism and may also cause cytoplasmic acidification in low pH environments. While bacteria have evolved specialized membrane shuttles regulated by fluoride-specific riboswitches to export intracellular fluoride from the cell, their deployment during bacterial defluorination has shown to be insufficient in preventing fluoride-induced loss of metabolic viability. And yet, some defluorinating bacteria can accommodate the stoichiometric defluorination of fluorinated compounds that lead to the release of fluoride significantly beyond the known toxic thresholds of this halide for bacteria. In this light, this work aimed to study the transcriptome of two model defluorinating bacteria under fluoride stress, to characterize their homeostatic response and potentially highlight new functional traits linked to their tolerance to intracellular fluoride.

Delftia acidovorans MFA5 and Labrys portucalensis F11 were used as model strains for this study, given their capacity to defluorinate aliphatic and aromatic organofluorines. We conducted Minimum Inhibitory Concentration (MIC) assays with these strains to establish their susceptibility threshold towards fluoride. Then, we conducted an expression study by exposing these strains to fluoride at sub-MIC levels for 72 hours, taking samples for RNA-seq every 24 hours. Once we acquire the RNA-seq transcriptomic datasets, we plan to map them to each strain's genome and perform quantitative expression analyses to reveal differentially-regulated transcripts under fluoride exposure.

Results obtained so far showed high fluoride MICs for both MFA5 (500 mM) and F11 (250 mM), confirming their unusual tolerance to this halide. Resolving their transcriptomes under fluoride exposure at sub-MIC levels will allow us to establish a functional map of the homeostatic response of F11 and MFA5 towards fluoride, focused on looking for potentially novel traits of resistance that may explain their unusual tolerance to the halide.

ID256 | Optimization of Antifungal Agents for Molecular Genetics Studies in Starmerella bombicola

Author: Jirameth Angchuan

UCIBIO-i4HB, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

E-mail: j.angchuan@fct.unl.pt

Other authors: Carla Gonçalves, Paula Gonçalves

Background: Yeast species belonging to the Wickerhamiella/Starmerella genera (W/S clade) appear to have acquired many metabolic genes from bacteria and filamentous fungi through horizontal gene transfer. Over the last decade, our laboratory started dissecting this phenomenon at the molecular genetics level in Starmerella bombicola, a genetically tractable species. This, like many other species in the W/S clade, is highly resistant to various stresses. Probably due to this characteristic, the types and standard concentrations of selective agents commonly used for genetic selection in yeasts tend not to be applicable. So far, only Hygromycin B at very high concentrations is effective as selective agent for generating single mutants in this yeast. However, an additional selective agent is required to enable the straightforward construction of double mutants. This study aimed to investigate the adequacy of a set of anti-fungal agents, including Zeocin for positive selection, and 5-Fluoroorotic acid (5-FOA) and 5-Fluorocytosine (5-FC) as counter-selection agents, for the construction of double mutants in S. bombicola.

Methods: S. bombicola PYCC 5882 and the isogenic ΔbetAΔbetB-HYGR mutant (carrying a deletion of an horizontally acquired betaine synthesis gene cluster and exhibiting ample transcriptional deregulation) were inoculated in selective medium, including YPD supplemented with 1500 and 1800 µg/mL of Zeocin, 50 - 800 µg/mL of 5-FC, and YNB (+Uracil) supplemented with 2 mg/mL 5-FOA.

Results: At high cell density, both WT S. bombicola PYCC 5882 and the ΔbetAΔbetB-HYGR mutant exhibited robust growth on YPD containing 1500 and 1800 µg/mL of Zeocin, whereas only a slight background growth was observed at low cell density. For 5-FOA, no growth and no resistant colonies were observed for both strains, suggesting the concentration employed is too high and that lower concentrations should be studied. For 5-FC, no growth of S. bombicola strains other than a few isolated colonies were observed across a concentration range of 50 µg/mL to 800 µg/mL, even at high cell densities.

Conclusions: Zeocin effectiveness is strongly affected by inoculum density, so it is not suitable as selective agent for the construction of mutants, even at very high concentrations. Of the counter-selection agents, 5-FC represents so far the most promising alternative for the construction of double mutants in S. bombicola, since it is effective even at high inoculum density.

ID268 | Development of synthetic devices to modulate intracellular O2 levels in Synechocystis strains

Author: Bárbara Pires

i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;
IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;
ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

E-mail: bpires@i3s.up.pt

Other authors: Filipe Pinto1, Catarina Coutinho Pacheco1,2,4, Peter Lindblad5, Paula Tamagnini1,24

1 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;
2 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; FCUP – Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
3 Microbial Chemistry, Department of Chemistry-Ångström, Uppsala University, Uppsala, Sweden;
4 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;
5 FCUP – Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

Background: Cyanobacteria are photosynthetic prokaryotes, being increasingly used as green cell factories. However, the oxygen generated in photosynthesis impairs the activity of several enzymes (e.g. hydrogenases). This can be overcome by developing Oxygen Consuming Devices (OCDs) based, for e.g., on proteins that catalyze the oxidation of a substrate or proteins that bind O2. Here, to create an environment suitable for O2-sensitive processes in the model cyanobacterium Synechocystis sp. PCC 6803, we designed OCDs based on hemoglobins, namely on (i) cyanoglobin (GlbN) from Nostoc commune UTEX 584, which was successfully used to decrease O2 levels in the heterocysts of Nostoc sp. PCC 7120, increasing H2 production1; (ii) leghemoglobin a (Gmlb3) from Glycine max2; and (iii) the hemoglobin (Vhb) from Vitreoscilla sp. HG1, shown to increase respiration levels in Escherichia coli mutants lacking native terminal oxidases3.

Methods: Design and synthesis of synthetic devices for constitutive and regulated expression of different hemoglobins, including combinations of promoters and ribosome binding sites (RBS) of varying strengths/affinities; generation of Synechocystis mutants by natural transformation; analysis of protein expression by Western Blot; assessment of O2 evolution using a Clark-type electrode.

Results: Results obtained so far show that hemoglobin expression levels are higher with regulated devices than with constitutive devices. Expression also increased when cells were grown in BG11 medium with 2x ferric ammonium citrate, most likely due to the presence of a heme group in the hemoglobin. Analysis of O2 evolution showed a small difference between non-induced and induced cells.

Conclusions: Expression of a heterologous hemoglobin in Synechocystis shows potential to modulate intracellular O2 levels, e.g. for H2 production.

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ID277 | Dissecting multiple hybridization events in a complex yeast lineage using genomics

Author: Margarida R. Silva

1 UCIBIO, Department of Life Sciences, Nova School of Science and Technology, Caparica 2829-516, Portugal.

2Associate Laboratory i4HB, Nova School of Science and Technology, Caparica 2829-516, Portugal

E-mail: mri.silva@fct.unl.pt

Other authors: Patrícia H. Brito 1,2, José Paulo Sampaio 1,2

1 UCIBIO, Department of Life Sciences, Nova School of Science and Technology, Caparica 2829-516, Portugal.

2Associate Laboratory i4HB, Nova School of Science and Technology, Caparica 2829-516, Portugal

Background: Understanding the evolutionary processes underlying the domestication of non-conventional yeasts is key to unlocking their potential for novel biotechnological applications. The sister genera Torulaspora and Zygosaccharomyces serve as prime examples of yeast lineages with both ecological and industrial relevance. For example, T. delbrueckii can enhance wine and bread fermentations and Z. bailli, despite being a spoilage agent, is seen as a promising cell-factory for high-valued organic acids. We are carrying out a comprehensive phylogenomic analysis of these genera to refine species boundaries, explore ecological patterns, uncover signs of domestication, and detect and characterize hybridization events. Additionally, we aim to dissect the genomic architecture and ancestry of inter-species hybrids, with particular attention to potential links between hybridization and transition from wild to domesticate.

Methods: We performed a large-scale phylogenomic study across the two genera, encompassing nearly 300 genomes and including representatives from all currently described species. Using single-copy orthogroups (SCOs) derived from predicted proteomes, a Maximum Likelihood phylogeny was inferred. Next, potential hybrids were identified using SppIDer and analyzed with complementary methods to explore genome structure and ancestry.

Results: Our analyses uncovered multiple inter-species hybridization events, particularly in Zygosaccharomyces. Several previously unreported hybrids were identified, some of which involve a newly recognized parental species, Z. progenitor, that contributed to the formation of at least three hybrid lineages, one of them with triparental ancestry. While fewer hybrids were found in Torulaspora, the events we observed differ significantly from each other in terms of parental divergence and genomic composition.

Conclusions: Our study uncovered several hybridization events with remarkably distinct levels of genetic divergence between parent species. In some cases, an association between hybridization and transition to anthropic settings and domestication could be made, thus providing valuable insights into the evolutionary changes and emergence of novel adaptations that occurred in this complex yeast lineage.

ID285 | Improving the heterologous production of glycine betaine in engineered Synechocystis strains

Author: Manuel Correia

i3S – Instituto de Investigação e Inovação em Saúde IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;

ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

E-mail: manuel.correia@i3s.up.pt

Other authors: David Rodrigues1, Pedro Lamosa2, Catarina Coutinho Pacheco1,3, Paula Tamagnini1,3

1 i3S – Instituto de Investigação e Inovação em Saúde, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;

2 ITQB NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Lisboa, Portugal;

3 i3S – Instituto de Investigação e Inovação em Saúde, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal;

4 i3S – Instituto de Investigação e Inovação em Saúde, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

Background: Compatible solutes (CS) are low molecular weight molecules that stabilize cell components under stress. Among these, glycine betaine (GB) has high commercial value, with applications in feed and pharmaceutical/cosmetic formulations. GB is currently obtained from sugar beets or chemical synthesis, but more sustainable production methods are required. For this purpose, a synthetic device – Ahbet – was introduced into a cyanobacterial strain deficient in the production of the native CS glucosylglycerol (Synechocystis ΔggpS), enhancing its growth in 3% NaCl and achieving a production of 1.9 µmol GB/mg protein [1]. Furthermore, extracellular polymeric substances (EPS) and glycogen were identified as competing pathways [1], and medium supplementation with glycine (GB precursor) was also shown to enhance GB production [2]. Thus, strategies such as deleting competing pathways and/or increasing glycine availability could be used to further improve GB production.

Methods: Generation of Synechocystis mutants by natural transformation, growth experiments in 3% (w/v) NaCl without or with glycine, CS extraction with ethanol/chloroform, CS quantification by H-NMR, RNA extraction with TRIzol, RNA-Seq.

Results: The Ahbet device was introduced into a Synechocystis strain impaired in EPS export (ΔkpsM), available in the host group [3]. The ΔkpsM_Ahbet strain achieved a production of 3.4 µmol GB/mg protein, but its growth was not enhanced. Supplementing the medium with 6 mM glycine further increased ΔkpsM_Ahbet's production (12.4 µmol GB/mg protein), while in ΔggpS_Ahbet the production was not increased by any of the glycine concentrations tested. Transcriptomic studies were performed to identify additional targets.

Conclusions: The highest GB production obtained so far was achieved on a Synechocystis strain deficient in EPS production. This was further improved by supplementing the medium with the GB precursor glycine, providing new insights for metabolic engineering.

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ID361 | The roles of CadF and CiaB in Arcobacter butzleri–host interactions

Author: Susana Ferreira

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal

E-mail: susana.ferreira@fcsaude.ubi.pt

Other authors: Cristiana Mateus 1, Fernanda Domingues 1, Roland Bücker 2, Mónica Oleastro 3

1 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, 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.

Background: Arcobacter butzleri has emerged as a relevant enteropathogen, yet the molecular basis underlying its interaction with human host remains poorly understood. Among the putative virulence determinants, the fibronectin-binding protein CadF and the invasion-associated protein CiaB are thought to mediate early events of infection. Thus, this study aimed to explore the contribution of cadF and ciaB genes in A. butzleri-host interactions.

Methods: A. butzleri knockout mutants for cadF and ciaB genes, along with the parental strain, were subjected to a panel of assays addressing bacterial-host interactions. These included fibronectin-binding, adhesion to and invasion of a human intestinal epithelial cell line, cytotoxicity assessment, interleukin expression, transepithelial electrical resistance (TEER), bacterial transmigration in mono- and co-culture systems, and analysis of the tight junction protein expression (barrier-maintaining claudins).

Results: Both cadF and ciaB mutants displayed significantly impaired fibronectin binding compared to parental strain. Using HT-29 cells as intestinal epithelial model, both mutants showed reduced adhesion, invasion, and cytotoxic effects compared to the parental strain. Conversely, IL-8 expression remained unaffected. Both parental and mutant strains altered barrier integrity in HT-29/B6 monocultures and HT

-29/B6+THP-1 co-cultures (epithelial and immune cells), as reflected by changes in TEER and bacterial transmigration. Specifically, the cadF mutant showed a reduced effect on TEER, whereas the ciaB mutant presented a more pronounced effect in transmigration in the co-culture, when compared with the parental strain in the same conditions. Nonetheless, no significant differences in the expression of claudin-1, -3, -5 and E-cadherin were observed when compared to the parental-infected cells, but cytotoxicity was present in all strains tested.

Conclusions: CadF and CiaB enhanced A. butzleri adhesion, invasion, and cytotoxicity, while barrier integrity and IL-8 responses remained mostly unaffected. These findings provide novel mechanistic insight into A. butzleri pathogenesis. Altogether, these findings support a model in which CadF and CiaB may have a potential contribution to Arcobacter butzleri virulence, by enhancing epithelial colonization and host cell damage.

ID386 | Development of a Gene-PNA-FISH approach to analyze Listeria monocytogenes persistence in biofilms

Author: Rui Macedo

LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;
ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;
INIAV – National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Vairão, 4485-655 Vila do Conde, Portugal

E-mail: up201603392@edu.fe.up.pt

Other authors: Nuno F. Azevedo 1,2; Joana Castro 3,5; Ricardo Oliveira 3; Carina Almeida 1,2,3,5; Darla Goeres 1,2,4; Laura Cerqueira 1,2

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 in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal;
3 INIAV – National Institute for Agrarian and Veterinary Research, Rua dos Lagidos, Vairão, 4485-655 Vila do Conde, Portugal;
4 The Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;
5 CEB – Centre of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

Listeria monocytogenes is a foodborne pathogen that persists in food-processing environments through biofilm formation. Biofilms are 3D structures of spatially arranged cells that interact with their environment. Their cellular arrangement influences L. monocytogenes biofilm behavior. This diversity within the biofilm drives differences in gene expression, metabolism, and physiology, increasing disinfectant tolerance and hindering eradication. Preserving biofilm structure is essential to study gene expression at the cellular level. This study intends to apply Gene-PNA-FISH as a spatial transcriptomic approach to investigate genes involved in biofilm formation and benzalkonium chloride (BAC) tolerance. Gene-PNA-FISH preserves biofilm structure, allowing in situ gene expression analysis via confocal laser scanning microscopy (CLSM), revealing spatial heterogeneity.

Peptide Nucleic Acid (PNA) probes were designed to target the L. monocytogenes genes actA (biofilm formation), sigB (biofilm formation and BAC resistance), and mdrL and sugE2 (BAC tolerance). Probe hybridization conditions are being optimized for multiplex mapping of gene expression within biofilms. MIC assays determined subinhibitory BAC concentrations to mimic stress. Gene expression quantification was compared by RT-qPCR at 24 h and 48 h post-BAC exposure.

RT-qPCR results showed upregulation under BAC stress (4 µg/mL), with sigB increasing ~11-fold at 24 h and ~4.5-fold at 48 h. Although actA is not directly related to disinfectant tolerance, it was also upregulated (~1.8-fold at 24 h; ~2.3-fold at 48 h), supporting its involvement in tolerance.

The upregulation of key genes like sigB under stress highlights their role in survival. Future spatial transcriptomics studies will offer critical insights for improved disinfection strategies and food safety.

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ID387 | Co2 reduction and O2-tolerance by a highly active w/sec-dependent enzyme

Author: Inês A. C. Pereira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: ipereira@itqb.unl.pt

Other authors: Ana Rita Oliveira1, Cristiano Mota2,3, Rita R. Manuel1, Guilherme Vilela-Alves2,3, Maria João Romão2,3

2 Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal 3UCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

Background: Metal-dependent formate dehydrogenases (Fdh) catalyze under mild conditions the reversible reduction of CO2 to formate, a versatile C1 feedstock that can contribute to a carbon-neutral economy. One of the most active CO2 reductases reported is the W/Sec-FdhAB isolated from the bacterium Nitratidesulfovibrio vulgaris Hildenborough. This enzyme has a simple composition and the presence of both W and selenocysteine at the active site contribute to its high CO2 reduction activity. Enzymes containing W and Sec are usually extremely oxygen sensitive, whereas the W/Sec-FdhAB displays a considerable O2 tolerance and is surprisingly robust, and can be purified in aerobic conditions.

Methods: To investigate the mechanism behind this O2 tolerance we produced variants of W/Sec-FdhAB that were studied by solution kinetic assays, electrochemistry, EPR spectroscopy, by X-ray crystallography, coupled to in vivo experiments with whole cells.

Results: We showed that the activity of W/Sec-FdhAB is controlled by a redox switch based on an allosteric disulfide bond at the surface of the protein [1]. Here, we will present the molecular basis for this mechanism and the first structure of W/Sec-FdhAB in the active state.

Conclusions: The redox switch mechanism operates in vivo and prevents enzyme reduction by physiological formate levels, conferring a fitness advantage during transient cell exposure to O2. Due to its robustness, the W/Sec-FdhAB is an excellent model for biocatalytic applications for CO2 reduction.

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ID406 | Pear scab disease suppression by Pseudomonas capeferrum NFX1: direct antagonistic activities and pear defense priming

Author: Sara Tedesco

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal 2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

E-mail: sara.tedesco@ibet.pt

Other authors: Margarida Pimenta1,2, Filipa Silva1,2, João Baixinho1, Frédéric Bustos Gaspar 1,2, Maria Teresa Barreto Crespo1,2, Francisco Nascimento1,2

1 iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal 2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

Background: Pear scab, caused by Venturia pyrina, is a major disease of pear trees, severely affecting Portugal's high-value Rocha cultivar. Biocontrol options are limited, emphasizing the need for alternatives to chemical fungicides. Here, we investigated the potential of Pseudomonas capeferrum NFX1 against V. pyrina through integrated genomic, phenotypic, and transcriptomic analyses.

Methods: The genome of strain NFX1 was sequenced and mined for secondary metabolite clusters using antiSMASH. Antifungal activity was evaluated in vitro via confrontation assays against V. pyrina mycelial growth and spore germination. Detached leaf assays were developed to evaluate NFX1 activity, with lesion severity quantified by a novel image-based method using lesion darkness as a proxy, and fungal DNA measured by a newly developed qPCR assay targeting the V. pyrina EF-1α gene. Transcriptomic responses of NFX1-treated and untreated pear scab-infected leaves were assessed by differential expression in OmicsBox.

Results: NFX1 genome analysis revealed biosynthetic clusters for putisolvin, siderophores, homoserine lactones, and a type IVB secretion system, involved in pathogen antagonism and plant colonization. In vitro, NFX1 significantly inhibited V. pyrina mycelial growth and spore germination, with efficacy comparable to the commercial fungicide Cerimonia®. In detached leaf assays, NFX1 reduced lesion severity by >50% and fungal DNA accumulation by ~4-fold ($p < 0.05$). Transcriptomic analysis showed that NFX1 primed host defenses by upregulating hormonal pathways (ethylene, abscisic acid, and auxin), defense-related transcription factors, and signaling kinases, enhancing immune perception and transcriptional control. At the same time, canonical resistance (R) proteins and pathogenesis-related proteins were predominantly downregulated, consistent with reduced pathogen pressure.

Conclusions: This study presents the first experimental framework for biocontrol discovery against V. pyrina and identifies P. capeferrum NFX1 as a promising bacterial candidate for sustainable control of pear scab in Rocha pear cultivation.

ID433 | Occurrence of norA and other multidrug efflux pump determinants across the Staphylococcus genus

Author: Diana Martinho

Instituto de Higiene e Medicina Tropical, 1349-008 Lisboa, Portugal
E-mail: a21002174@ihmt.unl.pt

Background: Multidrug efflux pumps (MDR EPs) play a major role in the emergence of antimicrobial resistance. NorA is a main native MDR EP in Staphylococcus aureus, contributing to reduced susceptibility towards fluoroquinolones and biocides. Its encoding gene is part of the S. aureus core genome, but less is known about the occurrence of norA and other MDR EPs across the Staphylococcus genus. This study aims to analyze the presence of norA across staphylococci, to identify other putative MDR EP determinants in five pathogenic staphylococcal species and to assess the genetic variability of main MDR EP genes in these bacteria.

Methods: The norA nucleotide sequences from 61 Staphylococcus species were retrieved from public databases and aligned. Other MDR EP genes were searched in public databases for S. aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis and Staphylococcus hominis. Putative homologues of S. aureus main MDR EP genes were BLAST searched against complete genomes of each species. The nucleotide sequences of norA, norB, norC (or norB/C) and mepA of the five staphylococcal species were retrieved from public databases and aligned to assess gene variability.

Results: The norA phylogenetic tree follows the phylogenetic relations within the Staphylococcus genus highlighting the presence of norA in the early branching of the genus. Putative MDR transporter genes were identified for S. aureus (n=46), S. lugdunensis (n=50), S. haemolyticus (n=45), S. epidermidis (n=43) and S. hominis (n=35). Potential alleles were found for S. aureus norB: norBI, present in 98% of the genomes, and norBII associated with CC45 strains.

Conclusions: Our data highlights norA as part of the staphylococcal core genome and a possible conservation of NorA function across species. The identification of main MDR EP determinants in other staphylococcal species of clinical relevance opens new avenues for the study of their impact on antimicrobial resistance.

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ID434 | Walking towards the characterization of Staphylococcus haemolyticus and Staphylococcus hominis effluxome

Author: Diana Martinho

Instituto de Higiene e Medicina Tropical, 1349-008 Lisboa, Portugal

E-mail: a21002174@ihmt.unl.pt

Background: Multidrug resistance phenotypes are often reported for Staphylococcus haemolyticus and Staphylococcus hominis. In S. aureus, the multidrug efflux pump (MDR EP). NorA is linked to the emergence of multidrug resistance phenotypes, by extruding fluoroquinolones and biocides. However, efflux as a resistance mechanism remains unexplored in these two coagulase-negative species. The present study aimed to investigate the presence and variability of norA and other EP genes potentially associated with efflux-mediated resistance in S. haemolyticus and S. hominis.

Methods: The study collection comprised 24 S. haemolyticus and 24 S. hominis clinical isolates. Species confirmation was performed by nuc-specific PCR amplification. The presence of norA and of the plasmid-encoded MDR EP genes smr, qacA/B, qacG and qacJ genes (reduced susceptibility to biocides) was assessed by PCR amplification. The norA promotor and coding regions were amplified by PCR, sequenced and the resulting nucleotide/polypeptide sequences aligned and compared with representative norA/NorA sequences for each species. The detection of efflux activity was evaluated using two approaches based on assessment of the intracellular accumulation of ethidium bromide (EtBr), a common substrate for MDR EPs: the EtBr-Cartwheel method and real-time fluorometry.

Results: Several amino acid alterations were detected in NorA, particularly for S. hominis. Over 70% of the isolates displayed EtBr accumulation profiles consistent with increased efflux activity, which could be mediated by the EPs encoded by norA and/or other MDR EP genes detected by PCR, namely smr (S. haemolyticus: 4/24; S. hominis: 5/24), qacA/B (S. haemolyticus: 10/24; S. hominis: 16/24) or other EPs yet to be identified.

Conclusions: These results highlight the importance of studying efflux in clinically-relevant pathogens, such as S. haemolyticus and S. hominis, reinforcing the need to integrate the assessment of EP determinants into antimicrobial resistance surveillance and control protocols.

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ID438 | Plasmodium falciparum Ubiquitin-proteasome system (UPS) modulating antimalarial resistance

Author: Adriana F. Gonçalves

1Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho. Gualtar, 4710-057, Braga, Portugal 2Life and Health Sciences Research Institute (ICVS)/ Biomaterials, Biodegradables and Biomimetics Research Group (3B's)-PT Government Associate Laboratory, 4710-057 Braga, Portugal

E-mail: id10896@alunos.uminho.pt

Other authors: Ana Lima-Pinheiro 1,2, Gustavo Capatti Cassiano 3, Pedro Cravo 3, and Pedro E. Ferreira 1,2

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho. Gualtar, 4710-057, Braga, Portugal 2 Life and Health Sciences Research Institute (ICVS)/ Biomaterials, Biodegradables and Biomimetics Research Group (3B's)-PT Government Associate Laboratory, 4710-057 Braga, Portugal 3 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

Background: The emergence of drug resistance in malaria parasites remains a major obstacle to malaria control. While artemisinin resistance has been mainly linked to mutations in the k13 gene in clinical settings, other components of the ubiquitin-proteasome system (UPS) have also been implicated in modulating parasite susceptibility. Artemisinin acts by inducing protein damage, which the UPS manages through ubiquitination and degradation via the 26S proteasome. The UPS has become a promising drug target given its essential role in protein homeostasis. Notably, proteasome inhibitors show synergy with artemisinin, supporting their potential use as partner drugs. Ubiquitin receptors, such as the RPN2 subunit of the 19S regulatory particle, play a crucial role in recognizing damaged proteins.

Methods: Previously, we investigated the functional relevance of an E738K variant in

the rpn2 gene, previously identified in an artemisinin-resistant Plasmodium chabaudi line. Using Plasmodium falciparum as a model, we demonstrate that the 738K mutation confers resistance to dihydroartemisinin (DHA) by stabilizing proteasome activity during drug exposure.

Now, we aim to understand how the rpn2 mutation modulates artemisinin response in P. falciparum parasites in the vicinity of a K13-resistant genetic background and its implications for other antimalarial susceptibility. We introduced both E738K variants into a k13-mutant background (C580Y) using the Selection-Linked Integration technique and performed drug susceptibility and proteasome function assays.

Results: Interestingly, we found that the resistance phenotype of rpn2738K is partially reduced in DHA susceptibility assays. Despite the increased survival of k13-mutant parasites, functional tests revealed no direct alterations of P. falciparum proteasome function, confirming that k13 acts upstream of the proteasome. Additionally, we observed a pleiotropic effect of rpn2 mutation towards resistance to other antimalarial drugs, highlighting its broader implications in parasite drug resistance.

Conclusions: Altogether, our findings offer new insights into the role of the UPS in antimalarial action and resistance in Plasmodium spp. This work underscores the therapeutic potential of targeting the UPS in malaria treatment strategies.

ID460 | Staphylococcus aureus RIC interacts with the SUF machinery for the assembly of iron-sulfur clusters

Author: Frederico M. Lourenço

Instituto de Tecnologia Química e Biológica- Universidade Nova de Lisboa, Avenida da República- EAN 2780-157 Oeiras, Portugal

E-mail: frederico.lourenco@itqb.unl.pt

Background: When a pathogen invades the host, the cells of the innate immune system produce Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which damage several pathogens components including the Fe-S cluster containing proteins. To survive in this harsh condition, pathogens have developed detoxifying and repair proteins including the repair of iron centre proteins (RIC). These proteins are widely spread in the bacteria and in some eukaryotic pathogens and are involved in the repair of essential Fe-S cluster proteins, such as aconitase and fumarase that are crucial for the tricarboxilic acid (TCA) cycle [1][2]. Staphylococcus aureus is a human pathogen, which is the causative of many nosocomial life-threatening infections [3][4]. S. aureus RIC has been shown to contribute to the virulence. For instance, the wax moth larvae Galleria mellonella infected with a S. aureus strain lacking the ric gene had a higher survival rate compared to the wild-type strain [5].

Methods: The goal in this work is a comprehensive study of S. aureus RIC of iron donating properties, at biochemical and structural levels. We also aim the establishment of the network of interactions of RIC with proteins forming the S. aureus Sulfur mobilization (SUF) system, which is the only Fe-S assembling machinery active in this bacterium, using three protein-protein interaction methodologies: Bimolecular Fluorescence Complementation (BiFC), Pull-downs assays and Fluorescence anisotropy [6].

Results: For the first time, it was demonstrated that proteins of the SUF system interact with RIC. Furthermore, we could assemble Fe-S clusters in vitro using S. aureus RIC as the only source of iron.

Conclusions: The ability of RIC to provide iron to the scaffold as well the interaction with the early-acting components of the Fe-S biogenesis is consistent with the role of iron donor properties.

ID467 | Mobilome analysis of Staphylococcus pseudintermedius strains associated with antimicrobial resistance

Author: Catarina Morais

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal

E-mail: a21000758@ihmt.unl.pt

Other authors: Sofia Santos Costa 1, Dennis Hanke 2,3, Ana Santos 1, Henrike Krüger-Haker 2,3, Constança Pomba 4, Andrea T. Feßler 2,3, Stefan Schwarz 2,3, Isabel Couto 1

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisbon, Portugal; 2 Institute of Microbiology and Epizootics, Center for Infection Medicine, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 3 Veterinary Center for Resistance Research (TZR), School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 4 Laboratory of Antimicrobial Resistance, CIISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

Background: Previous work from our group revealed high rates of antimicrobial resistance (AMR) in a collection of 155 Staphylococcus pseudintermedius causing skin and soft-tissue infections (SSTIs) in companion animals in Lisbon, Portugal. We now aim to analyze the carriage of mobile genetic elements (MGEs) and the associated AMR properties in S. pseudintermedius.

Methods: We studied 56 representative S. pseudintermedius from predominant and emerging clonal lineages previously characterized regarding AMR and clonality. Plasmid DNA was extracted and digested with EcoRI and XbaI. Each unique restriction pattern was assigned to a plasmid profile. A subset of 17 strains was selected for hybrid whole genome sequencing (WGS) (MinION and Illumina MiSeq).

Results: Thirty-one of the 56 S. pseudintermedius strains carried one or more plasmid(s), mostly of small (≤ 5 kb) or medium (> 5 kb, ≤ 23 kb) size, corresponding to eight plasmid profiles. Only two of these plasmids carried AMR determinants; plasmid pSP-G3C4 (tet(K), tetracycline resistance), from ST71 strains, and plasmid pSP5912 (qacG, biocide resistance), from an ST2061 strain. Other AMR determinants were detected as part of MGEs integrated into the chromosomal DNA, namely Tn552, Tn552-like, Tn553, Tn916, Tn5405-like, Tn5801, Tn5801-like GI6287 and pRE25-like element. A new chromosomal cassette, carrying fusC, was identified in a ST1183 strain. The 12 methicillin-resistant S. pseudintermedius studied carried SCCmec type III (n=5), SCCmec type IVg (n=3), SCCmecNA45 (n=1), ΨSCCmec57395 (n=1), SCCmec7017-61515 (n=1), or SCCmec type V(T)SL/154 (n=1). Ten strains carried intact prophages without AMR determinants. Intact restriction–modification systems were detected in 15/17 strains and CRISPR/Cas in 5/17 strains.

Conclusions: This study suggests that AMR content in S. pseudintermedius is mainly associated with MGEs integrated into the chromosomal DNA rather than in plasmids. These results may lead to a better understanding of multidrug resistance in S. pseudintermedius towards improved SSTIs treatment in companion animals.

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ID483 | Coded to Kill: Uncovering New Families of Rearrangement Hotspot Toxins from the Bee Gut Microbiome

Author: Margarida Moreira

Gulbenkian Institute for Molecular Medicine, 2780-156, Portugal

E-mail: margarida.moreira@gimm.pt

Other authors: Samuel Acheampong

1 Gulbenkian Institute for Molecular Medicine, 2780-156, Portugal

Background: The bee gut microbiota, though composed of only 8–10 core bacterial clusters, is highly specialized, with each species contributing to host health. Among bacterial competitive mechanisms, Type VI Secretion Systems (T6SS) stand out for their versatility. This molecular apparatus mediates interbacterial antagonism by delivering toxic effectors, allowing certain species to dominate the community. Among T6SS effectors, Rearrangement Hotspot (Rhs) proteins are modular toxins with a conserved N-terminal and highly variable C-terminal region.

This study investigates the role of Rhs in mediating competition, integrating computational and experimental approaches to identify novel toxin clusters, predict their structure and function, and validate selected candidates.

Methods: Profile Hidden Markov Models were built from representative Rhs C-terminal sequences of bee gut bacteria and applied to metagenomic datasets to detect homologous domains. Sequences were clustered with CD-HIT to define distinct toxin families. Functional annotation of representative sequences was performed with InterProScan, BLASTP, and AlphaFold, enabling inference of domain architectures and possible toxicity mechanisms.

Primers were designed for seven candidate Rhs toxins, which were cloned and expressed in E. coli Mach1 and BL21 to test their activity.

Results: While previous studies identified close to 208 Rhs C-terminal families, this analysis uncovered over 900, highlighting extensive sequence divergence and structural diversity. Several domains show homology to known enzymatic effectors, including nucleases and ADP-ribosyltransferases. Some families appear conserved across metagenomes, suggesting functional relevance or horizontal transfer. Experimental validation confirmed that six of seven candidates exhibited toxic activity in E. coli, supporting computational predictions.

Conclusions: This study expands knowledge of Rhs toxins in the bee gut microbiome by classifying over 900 distinct C-terminal families. Experimental assays confirmed toxic activity in most tested candidates, validating computational methods. The results emphasize the modularity and domain shuffling of Rhs proteins and provide a comprehensive catalog of toxin diversity, establishing a foundation for future functional studies of these effectors.

ID495 | FunMETAromaDB: A curated database of proteins involved in fungal aromatic compound metabolism for comparative genomics

Author: Tiago Miguel Martins

Cristina Silva Pereira Lab, ITQB-NOVA, Av. da República 2780-157 Oeiras, Portugal

E-mail: tiagomartins@itqb.unl.pt

Other authors: Rita Caturrinho Carmo, Cristina Silva Pereira

Cristina Silva Pereira Lab, ITQB-NOVA, Av. da República 2780-157 Oeiras, Portugal

Background: Aromatic compounds represent the second most abundant organic class after carbohydrates, and the study of their microbial metabolism is relevant across several research fields. Numerous peripheral pathways converge on a small number of central intermediates that undergo aromatic ring-opening in the central pathways. Over the past decade, alongside numerous peripheral pathway genes, most of the catabolic genes constituting the central metabolic pathways have finally been characterized in fungi [1–4].

Methods: Here we present FunMETAromaDB, a manually curated database of proteins involved in fungal aromatic compound metabolism, along with associated bioinformatics tools.

Results: The database currently includes 296 proteins, primarily enzymes, but also includes transcription factors and transporters. Entries span 53 species and 36 genera of fungi. Most entries are from Ascomycota (88%), with a significant number from Aspergilli (54%). Application of FunMETAromaDB and its tools enables the quick and accurate representation of aromatic metabolism across various fungal proteomes and genomes.

Conclusions: This resource is designed to provide a useful and accessible platform for researchers worldwide, even those without specialized expertise in fungal aromatic catabolism, facilitating omics analysis and genomic comparisons.

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ID498 | Glucose Transport in *Torulaspora delbrueckii* as a Key to Enhancing Its Potential in Winemaking

Author: Bruna Oliveira

CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal

E-mail: bruna.oliveira9973@gmail.com

Other authors: Flávia Silva-Sousa1, Susana Chaves1, Ricardo Franco-Duarte1, Maria João Sousa1

1 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal

Background: To enhance wine aroma and flavor complexity, non-Saccharomyces species are being increasingly used in grape must fermentation, usually caried out by Saccharomyces cerevisiae. This is especially important in the context of climate change, as higher grape sugar levels can result in wines with excessive alcohol content and low aromatic diversity. However, the low fermentative capacity of many non-Saccharomyces yeasts limits sugar consumption and fermentation reliability. Despite this, Torulaspora delbrueckii has attracted interest for its ability to improve the sensory profile and contribute to a more balanced composition of wines. Since sugar transport strongly influences fermentative capacity, but it remains poorly studied in T. delbrueckii, unraveling transport mechanisms and their regulation in this yeast is essential to improve sugar consumption efficiency.

Methods: In this context, we are characterizing T. delbrueckii glucose transporters, their regulation and kinetics. Comparative genome analysis of 41 strains was performed to identify candidate glucose transporter genes, and their expression is being studied throughout fermentation. Also, to address the lower fermentative performance of T. delbrueckii, possibly associated to its ethanol-sensitive transporters, we are testing if introducing the most ethanol-tolerant transporters from S. cerevisiae can enhance its fermentative capacity.

Results: Genome analysis revealed, besides the previously described LGT1 and IGT1, at least 4 additional transporter genes across all strains. Expression analysis of the 4 most conserved genes, present in a cluster, revealed that LGT1 is expressed at relatively constant levels throughout fermentation, while IGT1 expression is very low or undetectable. The 2 remaining genes displayed substantial expression variation. Preliminary tests with T. delbrueckii strains expressing S. cerevisiae's HXT1 indicate no improvement in growth rate or fermentative performance compared with the wild type.

Conclusions: This work provides new insights into glucose transport regulation in T. delbrueckii, identifying distinct transporter expression patterns during fermentation. The lack of improvement with S. cerevisiae HXT1 expression suggests that the higher ethanol tolerance observed in S. cerevisiae cannot be replicated in T. delbrueckii.

Acknowledgments: This work was supported by FCT – UID/04050 (CBMA) and COMPETE2023-FEDER-00733000 Oper. Nº16175

ID511 | Profiling the metabolic responses of *Candida albicans* in the gastrointestinal tumor microenvironment: moving beyond correlative microbiome studies

Author: Rosana Alves

Universidade do Minho, 4710-057 Braga, Portugal
E-mail: rosana@bio.uminho.pt

Other authors: Wouter Van Genechten, Rudy Vergauwen, Patrick Van Dijck

2, KU Leuven, 3000 Leuven, Belgium

C. albicans infections are highly prevalent in cancer patients, largely due to gastrointestinal (GI) dysbiosis triggered either by broad-spectrum antibiotics or chemotherapeutic agents. Current epidemiological and experimental evidence supports C. albicans involvement in GI cancer, including an association of these pathogens with poor prognosis. While the ability of C. albicans to colonize tumors is established, it remains unclear whether these fungi act as oncomicrobes promoting cancer progression or instead exploit the tumor microenvironment to thrive and evade immune surveillance.

To address this question, we are employing in vivo models of gastrointestinal cancer to profile the metabolic responses of *C. albicans* to the tumor environment. Using high-throughput screenings, we have characterized the responses of multiple clinical isolates and targeted null mutants to tumor-derived metabolites. Our findings suggest that tumor cells may fuel C. albicans growth through circulating lactate. Ultimately, our goal is to elucidate the molecular mechanisms underlying the involvement of *C. albicans* in GI carcinogenesis and determine whether these pathogens play a causal or consequential role in the development of GI cancer.

This work was supported by Fundação para a Ciência e Tecnologia through the GastroFungi Project (grant no. 2023.12236.PEX) and the FCT Mobility Program (grant no. FCT/Mobility/1332981647/2024-25.

ID512 | Breaking the mold: Alternative mechanisms of yeast arsenate detoxification

Author: Teresa Pissarro

Instituto de Tecnologia Química e Biológica António Xavier - ITQB NOVA, Oeiras, Portugal

E-mail: t.pissarro@itqb.unl.pt

Other authors: Sofia M. da Silva 1, Catarina Amaral 1, Andreia Pedras 1, Inês A. Pereira 1, Américo G. Duarte 1, Catarina Pimentel 1

Introduction: Arsenic contamination poses a serious threat to public health worldwide, with chronic exposure linked to a spectrum of life-threatening disorders. The development of sustainable and environmentally friendly processes able to mitigate arsenic contamination relies on the comprehensive knowledge of arsenic detoxification pathways. In Saccharomyces cerevisiae, a powerful eukaryotic model organism, the arsenate (AsV) reductase Acr2 and the arsenite (AsIII) permease Acr3 are key players in arsenic detoxification, and their mode of action is well documented. However, our recent data indicate that arsenic detoxification is more complex than previously assumed.

Methods: Intracellular As was quantified by ICP-AES and AsV/AsIII speciation was determined by HPLC-MS. To investigate additional pathways of arsenate detoxification, RNA sequencing was used to identify differential expressed genes in Δacr2 cells exposed to AsV. To determine whether AsV is a substrate of the Acr3 permease, an AsIII/H+ antiporter, a recombinant form of the protein was expressed in the Δacr2Δacr3 double mutant and everted plasma membrane vesicles were prepared by two-phase partitioning. The ability of these vesicles to transport AsV was then evaluated by monitoring the pH gradient generated inside the vesicles.

Results: Deletion of both ACR2 and ACR3 genes increases S. cerevisiae sensitivity to AsV and leads to a massive cellular accumulation of arsenic, which is not observed in the Δacr2 single mutant. These findings suggest a direct role for the Acr3 transporter in AsV detoxification or the presence of an additional arsenate reductase in yeast. Our data indicates that both alternative pathways coexist. We provide genetic and functional evidence for the existence of alternative AsV reductases that operate independently of Acr2 and demonstrate that Acr3 is not solely an AsIII transporter but also contributes directly to AsV efflux.

Conclusions: This work challenges the prevailing model of AsV detoxification in yeast.

ID520 | Bacterial diversity in industrial microalgal cultures and screening for *Vibrionaceae*-inhibiting strains

Author: Veronica Rossetto

Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal.

E-mail: vrossetto@ualg.pt

Other authors: Manuel Murtinho1, Tamára Santos1, Mariana Carneiro2, Luísa Barreira1,3, João Varela1,3.

1 Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal. 2 Necton S.A., Portuguese Company of Marine Cultures, 8700-152 Olhão, Portugal. 3 GreenColab – Associação Oceano Verde, Universidade do Algarve, Campus de Gambelas, 8005-139, Portugal.

The holobiont concept, once considered mostly ecological, is now gaining importance in industrial systems. In microalgae production, associated microbial communities can provide essential metabolites and vitamins, while also protecting cultures from pathogenic bacteria such as those in the Vibrionaceae family. This work aims to isolate and identify bacteria from microalgal cultures and evaluate their potential antagonistic activity against Vibrio spp., with the main goal of supporting more stable and resilient large-scale microalgal production. Fresh microalgal culture samples were collected at different culture stages at Necton facilities, ranging from 5L laboratory-scale flasks to 19m³ industrial photobioreactors. Aliquots (50mL) were collected in sterile tubes, serially diluted, and plated on marine agar to recover heterotrophic bacteria. Plates were incubated at 30°C for up to 14 days, and morphologically distinct colonies were purified by repeated streaking. Bacterial DNA was extracted, and the 16S rRNA region was amplified with primers F27 and R1492, sequenced, and compared with BLASTn (NCBI). Identification thresholds were ≥94.5% similarity for genus and ≥97% for species. Selected isolates were co-cultivated with Vibrio spp. strains in marine broth media (30°C, shaking, 24 h), then plated on TCBS agar and compared with Vibrio-only controls. From 60 samples, more than 350 plates were screened, yielding over 70 isolates subjected to molecular identification. Most belonged to the phylum Pseudomonadota (>50%), with Alphaproteobacteria and Gammaproteobacteria as the dominant classes. Orders like Hyphomicrobiales and Rhodobacterales were particularly frequent, including genera such as Marinobacter and Roseovarius spp., the latter genera commonly associated with antimicrobial activity. Twenty-five isolates were tested in inhibition assays, and about half showed qualitative antagonistic effects against Vibrio spp. Although these assays were qualitative, quantitative CFU reduction experiments are planned. This work shows that microalgal cultures contain bacterial strains with antagonistic activity against Vibrio spp. These interactions may contribute to reducing culture collapse and improving stability in large-scale production. Further work aims to validate these findings, extend the tests to additional Vibrio strains with the aim of supporting more sustainable and resilient microalgae production processes.

ID574 | Elucidation of the Structure-Function Relationship of the Dicarboxylate Transporter Mae1 in Saccharomyces cerevisiae

Author: Isabel Soares-Silva

1Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal;

2Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

E-mail: ijoao@bio.uminho.pt

Other authors: Filipe Kuhlmann1 2, Tayane Fontes1 2, João Alves1 2, Francisca Ferreira1 2, Sandra Paiva1 2, Margarida Casal1 2

1Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal;

2Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Carboxylic acids are important compounds with various industrial applications. To find environmentally sustainable production processes, microbial cell factories have been exploited to produce these compounds from renewable resources. Their productivity can be improved by genetically manipulating transporter proteins to optimize substrate uptake and/or product export. However, challenges persist due to a limited understanding of transport mechanisms and the structure-function relationships of these proteins. The transporter Mae1 from Schizosaccharomyces pombe is known for its ability to transport malate, succinate, and fumarate bidirectionally across the plasma membrane. However, its underlying mechanism and structure-function relationship are not fully understood.

In silico tools were used to predict the 3D structure of Mae1 and identify key residues potentially involved in substrate translocation. Seventeen amino acid residues within the central cavity of Mae1 were selected for mutagenesis. These mutants were expressed in Saccharomyces cerevisiae to analyzed their impact on cell physiology. AlphaFold3 and CaverDock were applied to analyze structural alterations of Mae1 mutants.

Among six Mae1 mutants the proper subcellular localization at the plasma membrane was compromised, and one the expression was fully inhibited. For the remaining Mae1 mutants, growth assays revealed a broad spectrum of phenotypes, seemingly indicating its capacity for malate export, succinic acid import, or both was affected. The exception was one mutant allele promoting an enhanced malate export of the cells. In silico modeling suggests that a novel hydrogen bond between malate and this residue may underlie this increased export activity.

Our findings identify several residues crucial for Mae1 activity and subcellular localization, enhancing our understanding of its structure-function relationship. We also identified a particular amino acid residue whose mutation has potential biotechnological applications for increasing malic acid export in engineered microbial cell factories.

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ID592 | Capsular Rheological Properties of Klebsiella pneumoniae: a double-edged sword mechanism of bacterial pathogenesis

Author: Liliane Pinto Ferrador

UCIBIO - Applied Molecular Biosciences Unit, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

E-mail: liliane.lmdpf@gmail.com

Other authors: Michele Loiodice, Paulo Costa 2,3, Helena Amaral 2,3, Ângela Novas 1,3, Luísa Peixe 1,3

1 UCIBIO - Applied Molecular Biosciences Unit, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 2 UCIBIO - Applied Molecular Biosciences Unit, MEDTECH, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; 3 Associate Laboratory i4HB, Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

Background: Capsular overproduction and hypermucoviscosity (HMV) in Klebsiella pneumoniae (Kp) are traits associated with increased virulence and invasiveness. Yet, the impact of capsular composition/amount on bacterial fluid dynamics remains unclear. Our study explores how variation in capsule expression shapes Kp fluid dynamics.

Methods: 21 Kp strains representing multidrug-resistant (MDR-Kp) and hypervirulent (hvKp) pathotypes with variation in HMV or capsular amount [hypermucoid (HM), regular-mucoid (RM) and non-mucoid (NM)] were evaluated for mechanical and fluid dynamics properties. NM variants were generated in vitro by serial passages in LB broth and carried cps-locus mutations. HMV and capsule abundance were assessed by sedimentation assay and uronic acid quantification, respectively. Rheological and textural properties were assessed after growth (CLED agar, 37ºC/24h) in a rotational rheometer (Malvern Kinexus Lab+) and TA-X2i texture analyser (Stable Micro Systems), with 1% xanthan gum as reference. Shear viscosity (SV, resistance to flow) was measured at shear rates of 0.1–100 s^{-1} at 25°C and 37°C. Work of shear (WS, resistance to shear/firmness) and adhesiveness (energy required for detachment) were measured at 25ºC. Genomes were sequenced (Illumina) and compared with Geneious Prime (v.2025.2).

Results: Strain properties varied by capsule amount rather than composition. HM strains displayed higher sedimentation and capsule content than RM or NM strains, and lower SV (Fig.1. A) and WS (Fig.1. B), which favours flow dynamics. In contrast, NM-Kp exhibited higher WS and adhesiveness (Fig.1. B) than RM-Kp or HM-Kp, indicating increased stickiness. At 37ºC, these differences were even more pronounced, with values being roughly twofold lower or higher, respectively (Fig.1. A). Of note, one ST147-KL64 HMV strain displayed exceptional capsule stretchability (168 mm), while its corresponding mutant displayed markedly higher SV (3x), WS (12x) and adhesiveness (7x) than other mutants (Fig.1).

Conclusions: Capsule abundance rather than composition modulates bacterial rheological and textural properties, influencing strain behaviour in different environments. Shifts in capsule amount alter bacterial mechanical behaviour, providing a double-edged sword mechanism for bacterial virulence and invasion.

ID603 | Exploring CAZyme-Driven Functional Potential in GIT Microbiomes of Catrina and Holstein-Friesian Cattle for Sustainable Agriculture

Author: Maria Susana Lopes

University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal

E-mail: maria.sb.lopes@uac.pt

Other authors: Ana Rita Azevedo1, Artur da Câmara Machado1, Ricardo Franco Duarte2, Duarte Mendonça1, Maria Susana Lopes1

1 University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal; 2 Centre of Molecular and Environmental Biology (CBMA), Biology Department, Minho University, Campus de Gualtar, 4710-057 Braga, Portugal

Background: The bovine gastrointestinal tract (GIT) harbours a complex microbiome that drives fibre degradation, carbohydrate fermentation, and hydrogen turnover. In this study, we integrate metagenome-assembled genomes (MAGs), functional annotation of carbohydrate-active enzymes (CAZymes), and correlation-based network analysis to uncover breed-specific microbial and metabolic architectures in the bovine GIT of two breeds: the Catrina, a native Azorean breed and the Holstein-Friesian.

Methods: Ruminal fluid and faecal samples were collected from the two breeds under mixed-ration and pasture-based diets. Shotgun metagenomic MAGs reconstructed were functionally annotated for CAZy categories. Weighted scores were calculated by MAG relative abundance. Microbial co-occurrence and functional networks were inferred using Spearman correlations with false-discovery-rate correction within and between breeds.

Results: In Catrinas, glycoside hydrolases (GHs) and glycosyltransferases (GTs) were predominantly encoded by Ruminococcaceae. Bacteroidales, Lachnospiraceae, Rikenellaceae, and Prevotella reflecting a strong fibrolytic capacity. Carbohydrate-binding modules (CBMs), although less abundant, were notable in Ruminococcaceae, indicating roles in polysaccharide binding and degradation. Holsteins exhibited broader CAZyme diversity, with GHs and GTs distributed across Ruminococcaceae, Lachnospiraceae, Bifidobacterium spp., Prevotella spp., and Akkermansiaceae, reflecting more versatile carbohydrate utilisation.

Conclusions: By integrating genome-resolved metagenomics, functional annotation, and network analysis, we reveal high-resolution, breed-specific GIT microbiome architectures. Catrina cattle maintain a GH-rich, fibrolytic, methanogen-centred microbiome optimised for complex polysaccharide degradation and efficient hydrogen disposal. Holstein-Friesians possess a diverse, saccharolytic–lactate microbiome supporting rapid carbohydrate fermentation and metabolic flexibility. These contrasting microbial networks have practical implications: methanogenic hubs in Catrinas present potential targets for methane mitigation, whereas the distributed Holstein network supports resilience to dietary change. Collectively, our findings demonstrate that host genetics, diet, and microbial interactions jointly shape the rumen ecosystem, offering insights for precision feeding and sustainable cattle production.

ID606 | COG Functional Signatures of the GIT Microbiome in Two Cattle Breeds: Towards Sustainable Ruminant Production

Author: Ana Rita Azevedo

University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal

E-mail: ana.rf.azevedo@uac.pt

Other authors: Artur da Câmara Machado1, Ricardo Franco Duarte2, Duarte Mendonça1, Maria Susana Lopes 1

1 University of the Azores, Biotechnology Centre of the Azores (CBA), School of Agricultural and Environmental Sciences, 9700-042 Angra do Heroísmo, Azores, Portugal; 2 Centre of Molecular and Environmental Biology (CBMA), Biology Department, Minho University, Campus de Gualtar, 4710-057 Braga, Portugal

Background: The bovine GIT microbiome supports fibre degradation, fermentation, and hydrogen metabolism, with breed-specific differences influencing energy yield and methane emissions. To uncover breed-specific microbial and metabolic architectures in the GIT of two breeds: the Catrina, a rustic native Azorean and the Holstein-Friesian, a high-productivity dairy breed, functional annotation of Clusters of Orthologous Groups (COGs) and correlation-based network by metagenome-assembled genomes (MAGs). Methods: Ruminal fluid and faecal samples were collected from animals fed under two diets. Metagenomic data were processed, resulting in high-quality MAGs. These genomes were annotated based on COG functional categories, and their contributions were weighted by relative abundance. Microbial and functional interaction patterns were analysed through correlation-based network inference with false discovery rate adjustment.

Results: COG-weighted profiles identified 12.874 significant associations. In Catrinas, Ruminococcaceae bacterium prevailed across major functional categories, including carbohydrate transport and metabolism (G), transcription (K), translation, ribosomal structure, and biogenesis (J), amino acid transport and metabolism (E), replication, recombination, and repair (L), cell wall/membrane/envelope biogenesis (M), and energy production and conversion (C). Bacteroidales bacterium and Lachnospiraceae bacterium contributed strongly to categories G, M, and K, while methanogenics were central hubs in hydrogen disposal pathways. Fibrolytic bacteria showed tight co-occurrence, mainly in rumen samples, reflecting coordinated fibre degradation. Holstein-Friesians displayed reduced Ruminococcaceae dominance, with Lachnospiraceae, Succiniclasticum ruminis, Bifidobacterium pseudolongum, and Bacteroidales driving categories G, E, K, and J, indicating a community specialised in soluble carbohydrate fermentation and metabolic regulation.

Conclusions: Functional annotation revealed high-resolution, breed-specific GIT microbiome architectures. These microbial networks have practical implications: archaeal–fibrolytic hubs in Catrinas present potential targets for CH4 mitigation, whereas the distributed Holstein saccharolytic–acidogenic network supports resilience to dietary change. This study demonstrates that host genetics, diet, and microbial interactions jointly shape the rumen ecosystem, offering insights for precision feeding and sustainable cattle production.

ID644 | The Skf system of Staphylococcus aureus – role in inter-species and host interactions

Author: Rita Sobral

1Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 2UCIBIO- Applied Biomolecular Sciences Unit, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal;

E-mail: rgs@fct.unl.pt

Other authors: Ana Rita L. Sequeira1,2, Raquel Portela1,2,3, Jorge S. Dias1,2

1Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 2UCIBIO- Applied Biomolecular Sciences Unit, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; 3 current address: Molecular, Structural and Cellular Microbiology Unit (MOSTMICRO). Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Oeiras, Portugal.

Staphylococcus aureus is a major human pathogen responsible for a wide range of clinical infections. Due to the emergence of methicillin-resistant S. aureus (MRSA) and its increased resistance to antibiotics, it is extremely important to find new approaches to decrease this clinical threat.

Two small genes of unknown function, skfA and skfB, were identified as highly over-expressed in the transcriptome of a S. aureus cell wall mutant. These genes encode two highly positively charged peptides, only present in Staphylococcal species. NMR studies and in silico analysis showed that SkfA-B are unstructured in solution and can bind to membranes through a putative inducible and amphipathic alpha-helix.

We analysed the role of the Skf system in the interaction with other bacteria. The promoter of the skfAB operon was placed upstream of the gfp reporter gene in a replicative plasmid. The S. aureus promoter fusion constructs were grown in co-culture with E. coli, K. oxytoca, M. luteus and B. subtilis, at several cell proportions, and the fluorescence intensity was monitored along time. These assays showed that the presence of other bacteria, in particular of gram-negative species, activated the expression of the Skf system at the promoter level.

Furthermore, the susceptibility of Skf overexpression mutants to phagocytosis and killing by activated THP-1 macrophages was tested. Macrophages were infected at a MOI of 5 and bacteria internalization and survival was assessed at t=1h, t=6h and t=24h. The overexpression of SkfAB promoted an ~5-fold increase in internalization of S. aureus in macrophages and the survival of the bacteria was enhanced for the first 6h post-infection. These assays were also performed with the promoter fusion constructs and fluorescence microscopy showed that the expression of the skf genes is increased during the macrophage infection process.

Summarizing, our results suggest that the Skf system of S. aureus plays a relevant role in inter-species interaction, and also in the process of macrophage internalization.

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ID647 | Mechanical Behavior of Staphylococcus aureus in the presence of different antibiotics

Author: Carolina Máximo

UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal;

E-mail: c.maximo@campus.fct.unl.pt

Other authors: Raquel Portela, Pedro Lúcio Almeida, Rita Gonçalves Sobral, Catarina Rosa Leal

Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University of Lisbon, Campus Caparica, 2819-516 Caparica, Portugal;UCIBIO—Applied Molecular Biosciences Unit, Molecular Microbiology of Bacterial Pathogens Laboratory, NOVA School of Science and Technology, NOVA University of Lisbon, Campus Caparica, 2819-516 Caparica, Portugal;Molecular, Structural and Cellular Microbiology Unit (MOSTMICRO). Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Oeiras, Portugal; UnIRE e Departamento de Mecânica, ISEL - Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, 1959-007 Lisboa, Portugal; Departamento de Física, ISEL - Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, 1959-007 Lisboa, Portugal; CeFEMA - Centro de Física e Engenharia de Materiais Avançados, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; CENIMAT/I3N, Departamento de Ciência dos Materiais, Faculdade Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

In the laboratory, studies on antibiotic resistance of bacterial pathogens are normally performed in static conditions or under orbital shaking. However, in host conditions, bacteria are commonly exposed to external mechanical forces, such as the continuous shear stress generated by flowing blood.

To characterize the dynamics and mechanical properties of planktonic bacterial populations, when exposed to the presence of antibiotics, we monitored by rheology, the growth of a methicillin resistant Staphylococcus aureus strain (MRSA). The viscoelastic behavior of the culture was determined by measuring the viscosity as a function of time and shear rate.

We addressed the impact of oxacillin, a b-lactam, with a bacteriolytic mode of action and of chloramphenicol, a bacteriostatic antibiotic that inhibits protein synthesis. The antibiotics were added, at concentrations below the minimum inhibitory concentration (sub-MIC) and at a concentration that inhibited growth, to the culture of S. aureus. Steady-state shear flow curves were obtained for discrete time points, showing distinct viscosity progress over time. In a second approach, the viscosity growth curves were measured at a constant shear rate of 10 s-1, during time.

Distintive characteristics associated to each concentration were identified and related with the antibiotic mode of action and impact in culture growth. Also, the obtained rheological profiles, revealed specific signatures associated with the different tested antibiotics, which were screened through the viscosity growth curves.

These results bring new insights to the bacteria response to two different antibiotics, with different modes of action and cellular targets, one with a bacteriolytic and the other with a bacteriostatic mechanism.

ID664 | Predicting metabolic potential with PICRUSt2 in Bathymodiolus azoricus from a whale-fall: chemoautotrophic traits and symbionts

Author: João Moreira

Faculty of Science and Technology, University of the Azores, 9500-321, Portugal; OKEANOS Institute of Marine Sciences, Azores, 9901-862, Portugal

E-mail: joao.cs.moreira@uac.pt

Other authors: Juliette Debost-Mallevialle, Daphne Cuvelier, Ana Colaço, Raul Bettencourt

1- OKEANOS Institute of Marine Sciences, Azores, 9901-862, Portugal; 2- OKEANOS Institute of Marine Sciences, Azores, 9901-862, Portugal; 3- OKEANOS Institute of Marine Sciences, Azores, 9901-862, Portugal; 4- Faculty of Science and Technology, University of the Azores, 9500-321, Portugal; OKEANOS Institute of Marine Sciences, Azores, 9901-862, Portugal

Background: Chemosynthetic environments like hydrothermal vents, cold seeps and whale falls host holobiontes such as the hydrothermal-vent mussel Bathymodiolus azoricus that lives at the Azores triple Junction vents. These animals hosts sulfur- and methane-oxidizing symbionts. We recovered small size B. azoricus from a whale-fall vertebra and hypothesized that this vent mussel living in the vertebra hosts a microbiome consistent with chemosymbiosis typically from vents. and screened pathway/gene predictions linked to chemoautotrophy and host symbiosis.

Methods: Small size mussel from a whale fall underwent 16S V3–V4 sequencing (Illumina) and QIIME2 processing to ASVs and taxonomy. PICRUSt2 inferred KOs/ECs/MetaCyc; stratified outputs linked functions to contributing taxa. ASV contributions were aggregated to genus and normalized to compare taxon shares per function; pathway presence (e.g., CALVIN-PWY, DENITRIFICATION-PWY) was also assessed. We summarized canonical markers for sulfur oxidation (Sox K17222–K17225; SQR K17218), carbon fixation (Calvin K01601/K00855), methane monooxygenase (pMMO K10944–K10946; sMMO K16157), and denitrification (K00368/K15864/K04561/K00376). Analyses reflect one specimen and 16S-based predictions.

Results: Genus-level, stratified contributions were led by the SUP05 cluster (Thioglobaceae) and “Candidatus Campbellbacteria”. Sulfur oxidation markers were prominent: Sox and SQR were detected, with SUP05 carrying ~58% of Sox and ~81% of SQR normalized shares. Calvin-cycle KOs (K01601, K00855) were present and ~99% assigned to SUP05; CALVIN-PWY was detected. A denitrification KO set and DENITRIFICATION-PWY were present, contributed by “Candidatus Campbellbacteria” and SUP05 (SUP05 ~24%). pMMO-like KOs (K10944–K10946) were assigned mainly to Cycloclasticus; sMMO (K16157) was not detected, leaving methanotrophy unresolved. Values are normalized, stratified shares from one specimen.

Conclusions: PICRUSt2 predicts sulfur-based chemolithoautotrophic potential in this whale-fall B. azoricus, with SUP05 dominating Calvin-cycle and sulfur-oxidation markers, and denitrification shared with “Candidatus Campbellbacteria.” Methanotrophy remains inconclusive (pMMO-like without sMMO). These findings suggest vent-type, symbiont-like functions may persist outside vents and warrant targeted meta-omics and geochemical validation.

ABSTRACTS

TOPIC #6: Bioprocess Engineering

ORAL COMMUNICATIONS

ID240 | Inline Monitoring of Induced Pluripotent Stem Cell Growth Kinetics with Stirred-Tank Bioreactor Oxygen Uptake Modeling

Author: William de Oliveira e Sousa Salvador

Department of Bioengineering, iBB – Institute for Bioengineering and Biosciences and Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal

E-mail: william.salvador@tecnico.ulisboa.pt

Other authors: Diogo do Espírito Santo Nogueira; Joaquim Manuel Sampaio Cabral; Klaus Graumann; Carlos André Vitorino Rodrigues

ISEL – Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro 1, 1959-007 Lisbon, Portugal; Department of Bioengineering, iBB – Institute for Bioengineering and Biosciences and Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal; Phoenestra GmbH, Huemerstrasse 3-5, 4020 Linz, Austria; Department of Bioengineering, iBB – Institute for Bioengineering and Biosciences and Associate Laboratory i4HB – Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal

Background: Human induced pluripotent stem cells (hiPSCs) are at the forefront of drug discovery, disease modeling and regenerative medicine. However, their applications remain hampered by several shortcomings related to the large-scale manipulation of hiPSCs, which usually relies on stirred-tank bioreactors (STBRs). Notable among these shortcomings is the lack of inline monitoring of viable cell density (VCD), traditionally measured by periodic cell counting, impeding process automation and timely reaction to VCD changes. As such, this work sought to employ common bioreactor sensor data to accurately estimate hiPSC number in real time during STBR culture.

Methods: hiPSCs maintain glycolytic metabolism as long as they do not undergo differentiation, making the modeling of cellular oxygen uptake rate (OUR) a promising approach for tracking VCD over time. Since hiPSC-STBR culture relies on headspace aeration and perfusion is the most efficient bioreactor operation mode, OUR was modeled theoretically to account for O2 headspace transfer, O2 perfusion transfer and variations in O2 concentration of bioreactor medium. The model relies on theoretical constants and variables deducible from commonly employed bioreactor sensors. An automated protocol for determining the impact of agitation speed on O2 liquid-side mass transfer coefficient (kL) was implemented to precisely describe headspace O2 transfer, the most significant model term.

Results: A robust relationship between kL and agitation speed was obtained, with the resulting linear regression model having a coefficient of determination (R2) of 0.999 and a normalized root mean squared error (nRMSE) of 0.38%. Comparing the model to manual cell counts for three hiPSC-STBR runs, an average nRMSE of 11.24% was observed, indicating a good fit of the model to the cell counts despite some discrepancy between the two. The discrepancy is limited to the start of culture, indicating that the model's assumption of stable cellular metabolism may not be entirely correct, or that cell counts performed when VCD is relatively low are somewhat unreliable.

Conclusions: Overall, the model shows excellent potential for near real-time monitoring of hiPSC growth kinetics, contributing to process know-how by capturing trends in VCD that cannot be gleaned with traditional cell counting and making advanced automation and control strategies possible. By relying solely on readily available sensor data its usefulness is further enhanced.

ID324 | Integrated Bioconversion of Grape Pomace-Derived Sugars into 2,5 furandicarboxylic acid Using Engineered and Wild-Type Yeasts

Author: Cristiana Martins

1 Departamento de Enxeñaría Química, Universidade de Vigo, Facultade de Ciencias, Ourense 32004, Spain; 2 Instituto de Agroecoloxía e Alimentación, Universidade de Vigo – Campus Auga, Ourense 32004, Spain

E-mail: cristiana.fernandes@uvigo.gal

Other authors: Beatriz Gullón1,2; Aloia Romaní1,2; Lucília Domingues3,4

1 Departamento de Enxeñaría Química, Universidade de Vigo, Facultade de Ciencias, Ourense 32004, Spain; 2 Instituto de Agroecoloxía e Alimentación, Universidade de Vigo – Campus Auga, Ourense 32004, Spain; 3 CEB – Centre of Biological Engineering, University of Minho, Braga 4710-057, Portugal; 4 LABBELS – Associate Laboratory, Braga/Guimaraes, Portugal

The reliance on fossil-derived resources for chemical synthesis presents significant sustainability challenges. Agro-industrial residues, such as grape pomace—a by-product of the winemaking industry—offer a renewable and underutilized feedstock, particularly due to their high content of fermentable sugars. These sugars can be valorised into 5-hydroxymethylfurfural (HMF), a key platform molecule with broad industrial relevance. Through environmentally friendly processes, HMF can be converted into high-value compounds such as 2,5-furandicarboxylic acid (FDCA), a bio-based monomer used in the production of bioplastics, coatings, and resins. Microbial whole-cell biocatalysts, notably using Saccharomyces cerevisiae genetically engineered to express HMF oxidase (HMFO), enables the selective oxidation of HMF to FDCA [1], presenting an alternative to chemical catalysis.

Engineered S. cerevisiae strains expressing HMFO either intracellularly or on the cell surface were evaluated for their ability to convert HMF into its derivatives, specially FDCA. In parallel, wild-type non-conventional yeasts were screened for their natural tolerance to HMF and their bioconversion potential. To obtain a renewable source of hexoses, white grape pomace was subjected to aqueous extraction for sugar recovery, followed by acid-catalyzed microwave-assisted dehydration to produce HMF from the sugar-rich extract.

Results: Intracellular expression of HMFO in S. cerevisiae led to significantly higher FDCA production (3.9 mM) compared to surface expression (0.2 mM), demonstrating the advantage of intracellular enzyme localisation. Among non-conventional yeasts, Pichia fermentans stood out achieving FDCA concentrations of 7.2 mM, demonstrating both, high HMF tolerance and effective bioconversion. The aqueous extraction process confirmed that white grape pomace is rich in fermentable sugars, and microwave-assisted dehydration converted these sugars into HMF with 34% yield. Notably, the tested yeast strains were able to convert HMF derived from grape pomace into FDCA and other derivatives, validating the feasibility of an integrated valorisation route.

This study demonstrates the potential of combining green chemistry with microbial biotechnology to convert grape pomace into value-added chemicals. The efficient conversion of grape pomace into FDCA via engineered and naturally tolerant yeast strains supports the development of circular bioeconomy strategies for sustainable chemical production.

ID342 | Valorisation of Seafood Canning Industry Residues for Biosurfactants Production

Author: Joana Dias de Almeida

1: Department of Bioengineering, iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal; 2: Associate Laboratory, i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal; 3: Surfactinnov Lda, 4730-423 Ribeira do Neiva, Braga, Portugal

E-mail: joana.d.almeida@tecnico.ulisboa.pt

Other authors: Diogo Rodrigues (1,2), Frederico Castelo Ferreira (1,2,3), Nuno Torres Faria (1,2,3)

1: Department of Bioengineering, iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal; 2: Associate Laboratory, i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisbon, Portugal; 3: Surfactinnov Lda, 4730-423 Ribeira do Neiva, Braga, Portugal

Background: Mannosylerythritol lipids (MELs) are glycolipids produced by non-conventional yeasts with strong commercial potential. Beyond reducing surface tension like typical surfactants, they show antioxidant, antimicrobial, and ceramide-like properties, making them valuable in food, pharma, and personal care. MELs stand out for low ecotoxicity, high biodegradability, and biocompatibility, but remain costly versus synthetic surfactants. Cost-reduction strategies include fermentation optimization, strain engineering and medium engineering. This work explores the use of undervalued Portuguese seafood cannery residues as alternative carbon and water sources for MELs production.

Methods: Cannery water and oil effluents were collected and characterized. Moesziomyces antarcticus yeasts were cultivated in fermentation medium with water effluent to replace fresh water and pretreated oil effluent as hydrophobic carbon source. Cell growth and MELs production was monitored.

Results: In shake-flask, the use of oil effluent led to a MELs productivity of 1.5 g/L.day and 0.9 purity (gMELs/gMELs+residual lipids). When both oil and water effluents were used, a productivity of 1.2 g/L.day and a purity of 0.8 were achieved, values comparable to the control with refined vegetable oil and fresh water (1.8 g/L.day and 0.9 purity). In a fed-batch bioreactor fermentation, with both effluents in the medium, productivity increased to 4.1 g/L.day. The MELs produced under these conditions were highly like those obtained in standard media.

Conclusions: In this study a new valorisation pathway for aqueous and oily side-streams from the canned seafood industry was developed, through their incorporation in fermentation medium for biosurfactants production.

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ID487 | Microbial Community Selection Under Controlled Conditions for PHA (Polyhydroxyalkanoates) Production

Author: Marta Catalão

1 Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Caparica 2825-149, Portugal. 2 UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, Caparica 2825-149, Portugal

E-mail: m.catalao@campus.fct.unl.pt

Other authors: Cristiana A. V. Torres 1,2 , Juan F. Martínez-Blanch 3 , Rui Oliveira 1,2 , Filomena Freitas 1,2 , Maria A.M. Reis 1,2

1 Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA University Lisbon, Caparica 2825-149, Portugal. 2 UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology, NOVA University Lisbon, Caparica 2825-149, Portugal 3 ADM-Biopolis S.L., University of Valencia Science Park, Carrer del Catedràtic Agustín Escardino Benlloch 9, Edificio 2, 46980, Paterna, Valencia, Spain

Microorganisms naturally inhabit complex ecosystems, known as microbiomes, where interspecies interactions and division of labour lead to community-level functions beyond the capabilities of individual species (Su et al., 2023). These microbial consortia exhibit enhanced productivity, resilience, and adaptability that are highly valuable for biotechnological applications, including the synthesis of biopolymers. Polyhydroxyalkanoates (PHAs) are biodegradable polyesters produced by certain bacteria and are considered promising sustainable alternatives to conventional petroleum-based plastics. This study applied a feast/famine (F/F) strategy to enrich natural microbiomes from diverse environments, including soil, lake and saltwater samples, plant roots, and muds, for PHA production. Over a 33-day enrichment period in shake flask cultivation using acetate as carbon source, distinct mixed microbial consortia (MMCs) were cultivated. PHA accumulation was monitored throughout the process. Genetic characterization revealed that most microbiomes evolved toward a dominance of the phyla Bacteroidetes, Proteobacteria and Actinobacteria. Among the tested sources, the consortia derived from the Tagus River Marshland and Olive Tree Moss exhibited the highest PHA yields, reaching 116.47 ± 3.46 mgPHA/gVSS and 95.44 ± 0.16 mgPHA/gVSS, respectively. The Tagus River Marshland consortium was subsequently cultivated in 2 L bioreactors under controlled conditions, resulting in increased PHA accumulation over time and achieving a final volumetric productivity of 1.84 gPHA/(L d) after 3 hydraulic retention times (HRT) in an accumulation bioreactor. Overall, this work provides valuable insights into the evolution and functional potential of natural microbiomes under selective pressures for biopolymer production. These findings support the development of robust, predictive strategies for engineering high-performance MMCs and advancing the sustainable manufacture of PHAs.

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Su, Y., Xu, M., Cui, Y., Chen, R., Xie, L., Zhang, J., Chen, Y. and Ding, T. (2023) Bacterial quorum sensing orchestrates longitudinal interactions to shape microbiota assembly. Microbiome, 11, 241. https://doi. org/10.1186/s40168-023-01699-4

ID530 | Be@t project: Valorization of Waste Cooking Oil and Grapevine Pruning for Sustainable Rhamnolipid Production and Application in Textile Softening

Author: João Rainha

Centre of Biological Engineering, Campus de Gualtar, University of Minho, 4710-057, Braga, Portugal

E-mail: joao.rainha@ceb.uminho.pt

Other authors: Adelaide Braga 2, Beatriz Cardoso 1, Ana Margarida Fernandes 3, Catarina Rodrigues 3 , Maria Santos 3, Joana Santos 1, Armando Venâncio 1,2, Carla Joana Silva 3, Lígia Raquel Rodrigues 1,2

2 LABBELS –Associate Laboratory, Braga, Guimarães, Portugal. 3 CITEVE-Portuguese Technological Centre for the Textile and Clothing Industries, Rua Fernando Mesquita, 2785, 4760-034 Vila Nova de Famalicão, Portugal

The development of sustainable alternatives to synthetic surfactants is increasingly important for reducing environmental impact in industries such as textiles. Rhamnolipids, glycolipid biosurfactants produced by Pseudomonas aeruginosa, combine excellent surface activity, biodegradability, and versatility. However, the high production costs, largely due to substrate expenses, limit their commercial use. The Be@t project aims to address this challenge by investigating the use of low-cost, renewable residues—waste cooking oil (WCO) and hydrolysed grapevine pruning (VP)—as substrates for rhamnolipid biosynthesis by P. aeruginosa #112. WCO supplied hydrophobic fatty acids while VP hydrolysates provided fermentable sugars, enabling the design of a cost-effective fermentation medium. At shake-flask scale, WCO treatment and glucose supplementation resulted in biosurfactant yields reaching 2.8 g/L. VP hydrolysates obtained after alkaline pretreatment and enzymatic hydrolysis further supported production, with titers up to 2.6 g/L. Bioreactor experiments confirmed scalability, achieving comparable concentrations in reduced time, supported by improved oxygen transfer. The physicochemical characterization of the produced biosurfactant mixture (surface tension, emulsification, CMD, and oil displacement assays) confirmed strong surface-active properties. HPLC-MS analysis identified 16 congeners, dominated by Rha-C10-C10 and Rha-Rha-C10-C10, representing over 85% of the biosurfactant mixture. Application trials demonstrated the effectiveness of biosurfactant-rich culture media as textile softening agents. A sensory panel rated treated cotton fabrics significantly softer than untreated controls, with performance comparable to a commercial softener, highlighting the potential to reduce downstream purification steps. Overall, this study illustrates a key objective of the Be@t project, namely coupling food and agro-industrial waste valorisation with rhamnolipid biosurfactant production, contributing to circular economy approaches while delivering functional and sustainable alternatives for textile finishing.

ID543 | Biotechnological Production and Functional Characterisation of a Nematode-Derived Sterol-Binding Protein with Bioinsecticidal Potential

Author: Jorge Frias

Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Açores, Portugal;

E-mail: jorge.mv.frias@uac.pt

Other authors: Duarte Toubarro1, Tiago Paiva1 e Nelson Simões1

1 Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Açores, Portugal;

The urgent need for sustainable pest control alternatives has positioned bioinsecticidal molecules as promising tools in agricultural applications. The Steinernema carpocapsae nematode releases several excretory/secretory products (ESPs) upon contact and during the parasitic infection process of its host. Different virulence factors have been identified in nematode ESPs, some of which are immunomodulators, while others cause tissue damage and insect lethality. One family of proteins found in the ESPs of this nematode is the CAP (cysteine-rich secretory protein/antigen 5/pathogenesis related-1) protein; however, the functional role of these proteins in their respective hosts remains unknown.

Here, we report the cloning, heterologous expression, and characterisation of a CAP protein (Sc-CAP) secreted by the entomopathogenic nematode Steinernema carpocapsae. Using Escherichia coli as a production system, we successfully expressed and purified this protein, enabling structural and functional studies. The results showed that Sc-CAP protein has a strong binding affinity for cholesterol, and docking analyses of sterols on the CAP protein revealed potential molecular interactions. Immunoassays performed on Galleria mellonella larvae revealed that this venom protein has an inhibitory effect on phenoloxidase and the antimicrobial response of insects. Moreover, treatment with this virulence factor led to nonviable eggs and a marked decrease in offspring.

This study not only uncovers the potential of Sc-CAP as a bioinsecticidal candidate but also demonstrates the feasibility of microbial systems, such as E. coli, to produce nematode-derived proteins for sustainable pest management solutions.

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ID571 | Near-infrared spectroscopy for real-time monitoring and optimization of polyhydroxyalkanoates production

Author: Ana Marta Rodrigues

i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

E-mail: amsi.rodrigues@campus.fct.unl.pt

Other authors: Maria Reis; Nídia Dana Lourenço

i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal; UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal

Background: Bioprocess monitoring is oftentimes performed using time-consuming analytical techniques. Conversely, real-time monitoring enables early problem detection and solving, being advantageous for process efficiency enhancement. Near-infrared (NIR) spectroscopy is a promising tool for the real-time monitoring of bioprocesses, including the waste-based production of polyhydroxyalkanoates (PHA) with mixed microbial cultures (MMCs). This process consists of 3 stages: acidogenic fermentation for volatile fatty acid (VFA) production, culture selection, and PHA accumulation, the latter being a rate-limiting stage where VFA exhaustion triggers PHA consumption and VFA accumulation can lead to substrate inhibition.

Methods: The accumulation stage of a pilot-scale, 3-stage, MMC PHA production process was operated with a fed-batch supply of a fermented fruit waste stream rich in VFA. Bioreactor samples were collected throughout the process and analyzed for intracellular PHA content and VFA concentration. At-line NIR spectra of the same samples were acquired using a Matrix-F Fourier Transform-NIR spectrometer (Bruker Optik GmbH, Germany) coupled to a fiber optic transflection probe (mechanical pathway of 2 mm). Spectral data pre-processing and development of partial-least-squares (PLS) models through cross-validation were performed using the software OPUS Quant2, version 8.2.28 (Bruker Optik GmbH, Germany).

Results: PLS calibration models were developed for the prediction of PHA and VFA profiles throughout the PHA accumulation process. The models with the best performance were subjected to external validation, presenting a R2 of 0.98 and a RMSEP of 1.75% for intracellular PHA content, and a R2 of 0.97 and a RMSEP of 0.11 gCOD/L for VFA concentration.

Conclusions: The good prediction abilities of the developed models demonstrated the potential of NIR spectroscopy for the real-time monitoring of PHA production, to be used to optimize the fed-batch strategy, and to determine the end of the accumulation stage, maximizing productivity and reducing energy costs.

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ID649 | Integrative process for bacterial production and separation of tellurium particles

Author: Jorge F. B. Pereira

1 University of Coimbra, CERES, Department of Chemical Engineering, Coimbra, Portugal.

E-mail: jfbpereira@eq.uc.pt

Other authors: Pedro Farias2, Inês Costa3, Alexandre M. S. Jorge1, Rita Branco2, Paula V. Morais2, José António Paixão4

2 University of Coimbra, CEMMPRE, Department of Life Sciences, Coimbra, Portugal. 3 University of Coimbra, CEF, Department of Life Sciences, Coimbra, Portugal. 4 University of Coimbra, CFisUC, FCTUC, Department of Physics, Coimbra, Portugal

The recovery and upcycling of Tellurium to produce advanced sensors and particles for therapy and bioimaging, particularly through bio-based strategies, has garnered significant interest. Bioproduction of tellurium-particles (TePs) offers an efficient and eco-friendly alternative to conventional hydrothermal and chemical processes. However, downstream processing, specifically the recovery and separation of TePs, remains a major challenge for the practical implementation of these biogenic approaches. Therefore, innovative, eco-friendly, and low-energy extraction are urgently needed to replace existing techniques using energy-intensive processes or hazardous chemicals. We aim to enhance Te(IV) reduction using the Paenibacillus pabuli ALJ109b strain, as well as through careful process integration, improve the extraction and separation of TePs.

This study's novel approach for room-temperature biogenic TePs production, with strain 109b, only depends on nitrogen enriched growth medium, and the mid/late growth phase addition of Te(IV) to improve TePs yields. Notably, in our integrated process TePs separation is achieved with an eco-friendly solvent. Optimal bacterial growth and tellurite reduction, 24%, were achieved after 20 h, with Te(IV) added in the late log phase. Various cell disruption methods were evaluated for the TePs recovery, with 3 min of sonication in water providing the most effective for particle release. Additionally, a 1:1 mixture of water and ethanol emerged as the most efficient solvent for separating TePs from cell debris. TePs recovered after 8 h of bacterial growth exhibited smaller and more stable aggregates compared to those recovered after 24 h. These findings confirm the potential of P. pabulis ALJ109b for the bioproduction of TePs and the integration of low-cost and eco-friendly solvents in a solid-liquid extraction processes provides a sustainable and efficient solution for cell lysis and recovering of these microparticles from bacterial biomass.

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POSTERS

ID257 | Removal of additives from plastic to enhance enzymatic depolymerization

Author: Marco Prazeres

CICECO - Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Aveiro, Portugal

E-mail: marcoprazeres16@ua.pt

Other authors: Daniela Espínola 1, Mariana I. S. Aguiar 1, João A. P. Coutinho 1, Ana M. Ferreira1

1- CICECO - Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Aveiro, Portugal

Background: Plastics, while integral to both the global economy and modern daily life, present a pressing environmental challenge due to their persistence and resistance to degradation. Although recycling holds considerable promise as a mitigation strategy, its effectiveness is compromised by the presence of additives, particularly colorants, which lead to lower-quality recycled materials. To overcome this limitation, we propose the removal of these additives as a crucial step preceding enzymatic depolymerization. This initiative focuses on the extraction of both organic and inorganic colorants from widely used polymers, such as polyethylene terephthalate (PET) and polyethylene (PE), thereby enhancing the versatility and applicability of the proposed purification approach.

Methods: To extract the undesired colorants, we selected renewable solvents based on COSMO-RS predictions and applied solid liquid extraction under mild heating conditions (30 °C to 100 °C), using a solid liquid ratio of 0.02. From all the systems tested, we retained the most effective ones and evaluated their performance in enzymatic depolymerization using cutinase and laccase.

Results: Colorants present in some polymers, such as PET and low-density polyethylene (LDPE), were partially removed using solvent mixtures comprising N-methylpyrrolidone (NMP), gamma-valerolactone (GVL), and propylene carbonate, in combination with nitrogen-containing salts and alkanols. Initial enzymatic depolymerization assays are ongoing, with some solvent combinations showing promising effects, while others have shown limited improvement.

Conclusions: The results support the potential of tailored solvent systems for the selective removal of colorants and for improving substrate quality for enzymatic depolymerization. Further optimization and validation are ongoing. This integrated approach may contribute to the development of more efficient and sustainable plastic recycling processes.

ID260 | Influence of choline chloride–butyric acid natural deep eutectic solvent composition on astaxanthin extraction from Phaffia rhodozyma

Author: carmem gonçalves

1 CERES - Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790, Coimbra, Portugal

E-mail: carmem@eq.uc.pt

Other authors: nuno oliveira, cassamo mussagy, jorge pereira

1 CERES - Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790, Coimbra, Portugal; 2 Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Quillota 2260000, Chile

Background: Natural astaxanthin (AXT), a potent antioxidant with various biological functions, has gained considerable relevance in the cosmetic, nutraceutical, and food sectors. Its intracellular localization in Phaffia rhodozyma (P. rhodozyma) poses challenges for efficient extraction. Given recent advances in green technologies, Natural Deep Eutectic Solvents (NADES) have emerged as sustainable alternatives to conventional organic solvents. This study studied the potential of using choline chloride: butyric acid NADES (1:2 to 1:7 molar ratios) for AXT recovery from P. rhodozyma wet biomass. Methods: P. rhodozyma was cultivated under controlled conditions using YPM medium (comprising yeast extract, malt extract, glucose, peptone bacteriologic, and xylose) for the pre-inoculum stage at 25°C and 230 rpm. The fermentation process was carried out in YPDMGl medium (YPM supplemented with glycerol and mineral salts: MgSO-, ZnSOn, CaCl-, and KnHPOn) at 22 °C and 230 rpm. After harvesting, the cells were centrifuged and stored frozen for further processing. For the extraction process, 0.2 g of wet biomass was mixed with 1 mL of NADES. Extractions were performed at 65 °C, 300 rpm for 1 h in glass bottles sealed, followed by vortexing and centrifugation. Liquid–liquid separation was applied, and the AXT content was quantified by UV–Vis spectrophotometry. Results: All NADES formulations enhanced the AXT extraction. The 1:7 ratio led to the highest extraction efficiency, reaching ~143 µg/mL of AXT. Conclusions: P. rhodozyma is a promising source of natural AXT. NADES-based extraction proved effective and sustainable, with the molar ratio of 1:7 demonstrating optimal performance and highlighting the positive influence of increased butyric acid content on the recovery process, reinforcing its potential for biotechnological applications.

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ID261 | Modified Electrospun Polysulfone Membranes for Arginine Immobilization

Author: Diana Pereira

RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal. | CDRSP-PL-Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, Marinha Grande, 2430-028 Leiria

E-mail: diana.carvalho.pereira1@gmail.com

Other authors: Juliana Dias, Nuno Alves, Ângela Sousa and Joana F. A. Valente

1 CDRSP-PL-Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, Marinha Grande, 2430-028 Leiria, Portugal; 2 CDRSP-PL-Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, Marinha Grande, 2430-028 Leiria, Portugal; 3 RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal; 4CDRSP-PL-Centre for Rapid and Sustainable Product Development, Polytechnic of Leiria, Marinha Grande, 2430-028 Leiria, Portugal

Polysulfone (PSU) is a highly suitable polymer for applications requiring mechanical robustness, thermal stability, and chemical resistance and can be electrospun into membranes which can be functionalized with ligands of interest, like arginine, to attract certain molecules such as nucleic acids for purification purposes. However, first there's a need to produce uniform nanostructured membranes and optimize the immobilization process which is described in this work.

Membrane production started by dissolving PSU in dimethylformamide (DMF) testing 7-20% [w/v] for 16 h; and different electrospinning conditions of flow rates (0.1–3.5 mL/h), distances (10–25 cm), and applied voltages (10–25 kV). Then, heating membranes at 180 °C for 6h for mechanical integrity reinforcement and DMF removal. After, chemically activation involved a three-step process: alkaline treatment with NaOH; reaction with epichlorohydrin; and ligand coupling with arginine. Time in contact with NaOH (1/2/3h) and temperature (37–60°C) was optimized, as well as the solvent for epichlorohydrin/arginine. Chemical/morphological characterization with SEM, FTIR and contact angle (CA) measurements was applied. Arginine was partially quantified by UV absorbance at 235 nm.

From the above-described conditions, two yielded uniform fibres without bead formation: 12.5% and 20% [w/v] PSU/DMF. Thermal treatment did not alter fibre morphology but improved mechanical/structural resistance. Membranes treated 1h in NaOH at 37°C, with epichlorohydrin in ultrapure water and arginine saturated solution in sodium bicarbonate showed more wettability in CA analysis, however in FTIR the arginine functional groups were too faint or absent. After optimization to 3h NaOH at 60°C and epichlorohydrin and arginine saturated solution in borate buffer, the wettability remained but the arginine functional groups became more pronounced in FTIR.

PSU electrospun membranes can be reproducibly fabricated and modified to immobilize arginine, especially under optimized physiochemical conditions. Parameters like solvent concentration, flow-rate, and thermal treatment are critical for obtaining homogeneous, resistant membranes. Chemical immobilization improved by optimizing buffer composition and incubation conditions. These membranes offer a promising platform for further functionalization and application in bioseparation and filtration processes.

ID266 | Optimizing the co-fermentation of sewage sludge and agri-food residues for the production of volatile fatty acids (VFAs)

Author: Joana Batista

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, 2819-516 Caparica, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, 2819-516 Caparica, Portugal

E-mail: jba.batista@campus.fct.unl.pt

Other authors: Mónica Carvalheira, Mariana Matos, Maria Ascensão Miranda Reis, and Nídia Dana Lourenço

1. Associate Laboratory i4HB - Institute for Health and Bioeconomy, 2819-516 Caparica, Portugal; 2. UCIBIO – Applied Molecular Biosciences Unit, 2819-516 Caparica, Portugal

Background: The huge volume of residues from agri-food production and of sewage sludge (SS) from wastewater treatment has driven the development of sustainable technologies for their valorization. Both wastes are suitable feedstocks for the production of volatile fatty acids (VFA) by acidogenic fermentation (AF), and their co-fermentation may enhance VFA production yields. This work aimed to valorize nutrient-exhausted SS (treated SS) and agri-food residues through the production of VFA, which can be used by mixed microbial cultures to produce biodegradable polymers, namely polyhydroxyalkanoates (PHA).

Methods: Two sets of 10-day batch tests were carried out to: (1) assess the feasibility to convert fruit waste (orange, kiwi, tomato or their mixtures) and treated SS into VFA; (2) determine the optimal conditions for VFA production using response surface methodology (RSM) with two independent variables to assess their impact on the acidification degree (AD): X1-treated SS content as volatile solids (VS, 3.8-46.2%gVS); X2-substrate to inoculum (S/I) ratio (2.9-17.1 gVSresidue/gVSinoculum).

Results: In single fruit assays, an AD of 60.4-71.7% was attained, with all fruits showing a similar VFA profile, mostly composed of acetic acid (AA) and propionic acid (PA). Treated SS obtained an AD of 10.5

±0.1%. Co-fermentation assays using fruit mixtures and treated SS resulted in a maximum VFA production and AD of 2.98±0.02 gCOD/L and 42.0±0.3%, respectively, with similar VFA profiles across mixtures (31-42% AA and 30-41% PA). RSM showed that the S/I ratio had a greater influence on AF performance than the treated SS content, with the lowest S/I ratios (2.9 and 5 gVSresidue/ gVSinoculum) achieving the highest AD (53-58%). VFA composition profile varied depending on the conditions applied, but AA was the main acid produced in most of the assays (68-28%). Optimal conditions were found at an S/I ratio of 2.5 and 20% SS content.

Conclusions: This work demonstrated the feasibility of VFA production through co-fermentation of nutrient-exhausted SS and fruit waste, highlighting the S/I ratio as a key factor for VFA production.

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ID314 | Lactobacilli consortium and Corynebacterium glutamicum cultivation in sequential bioreactors to produce SCP from marine by-products

Author: Diogo Jacinto Marques

(1) - iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Portugal; (2) - Associate Laboratory i4HB — Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Portugal

E-mail: diogo.j.marques@tecnico.ulisboa.pt

Other authors: Diogo Miguel (1,2), Marília Mateus (1,2), Helena Pinheiro(1,2), Carla Motta (3) and Maria Teresa Cesário (1,2)

(1) - iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Portugal; (2) - Associate Laboratory i4HB — Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Portugal; (3) - Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA IP, Lisboa, Portugal

The demand for sustainable food production, e.g. protein sources, is steadily increasing. Single-cell protein (SCP), industrially produced mainly for feed, offers the amino acid profiles required for human nutrition. However, a lack of sustainable media limits its production. Marine by-products, rich in nutritional value, are underexplored as potential SCP growth medium components. Current research uses diverse approaches to enhance the cost-effectiveness of SCP production.

Our research aims to innovate in utilizing marine by-products and bacterial fermentation towards SCP production. We use Gelidium corneum industrial by-products (after agar extraction), as nutrient source for fermentation due to their favourable carbohydrate and protein contents (27.3 ± 3.0 % DW and 36.0 ± 1.2 % DW, respectively). G. corneum biomass was enzymatically hydrolysed in a bench-scale bioreactor to release fermentable sugars. The obtained hydrolysate was supplemented with minerals and corn steep liquor to create a rich medium. This was inoculated with a consortium of 4 Levilactobacillus (LAB) species, and a fed-batch fermentation was carried out. After cell harvesting, the lactic acid rich supernatant (LA, >80 gLA/L) was thereafter supplied to Corynebacterium glutamicum as the feed stream in a fed-batch bioreactor fermentation. C. glutamicum can assimilate LA as single carbon source in aerobic conditions, producing a final broth enriched in SCP in high yield.

Broth protein content increased during C. glutamicum fermentation, more than doubling the initial level. The lyophilized cell pellet from the LAB fermentation contained 55.8 ± 0.7% protein (DW), and the final C. glutamicum broth product reached 25.0 ± 0.3% protein (DW).

This sequential process yielded 0.30 and 0.41 g SCP protein per gram of initial marine by-product from LAB and C. glutamicum, respectively. Protein bioaccessibility levels were 62.9 ± 6.0% for the LAB product and 71.7 ± 8.0% for C. glutamicum.

This innovative process increased protein production from marine by-products by optimizing metabolite use. Ongoing work explores single-step C. glutamicum fermentation on marine hydrolysates and product characterization (e.g., amino acid profiles) for comparison purposes.

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ID332 | Valorization of Tomato Pomace and Brewer's Spent Grain for Polyhydroxyalkanoate (PHA)

Author: Cristiana A. V. Torres

1 UCIBIO, Chemistry Department, NOVA School of Science and Technology, NOVA University Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal 2 Associate Laboratory i4HB – Institute for Health and Bioeconomy, School of Science and Technology, NOVA University, Campus da Caparica, 2829-516 Caparica, Portugallp.chaves@campus. fct.unl.pt

E-mail: c.torres@fct.unl.pt

Other authors: Lara Chaves1,2, Francisco Santos1,2, Filomena Freitas1,2, Maria A.M. Reis1,2

1 UCIBIO, Chemistry Department, NOVA School of Science and Technology, NOVA University Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal 2 Associate Laboratory i4HB – Institute for Health and Bioeconomy, School of Science and Technology, NOVA University, Campus da Caparica, 2829-516 Caparica, Portugallp.chaves@campus. fct.unl.pt

Background: Transitioning to a circular bioeconomy is key to mitigate environmental impacts and reducing reliance on fossil resources. Polyhydroxyalkanoates (PHAs) are microbial polyesters with high application potential as sustainable bioplastics. The use of agroindustrial residues, such as tomato pomace and brewer's spent grain (BSG), as feedstocks offer a sustainable and cost-effective alternative, providing abundant carbon sources for microbial growth and polymer synthesis.

Methods: Oil extracted from tomato pomace (TP) and from Brewer Spent grains (BSG) with ethyl acetate was used as feedstock for the cultivation of several bacterial strains to evaluate their ability for PHA accumulation. The best-performing strain, Cupriavidus necator DSM 428 was selected for 2 L bioreactor-scale experiments.

Results: Fatty acid profiles revealed palmitic, linoleic, and oleic acids as predominant components of both oils. All strains produced PHA, with C. necator achieving high yields on biomass and high PHA content in the biomass, in the bioreactor experiments. A PHA content of 87.73 ± 2.04wt.% was reached from BSG oil, while using TP oil resulted in an accumulation of 79.31 ± 0.00wt.%. Moreover, the oil extraction leftovers were treated to obtain sugar-rich hydrolysates for further PHA production.

Conclusions: This study outlines a waste-based strategy for PHA production using both lipid and carbohydrate fractions of tomato pomace and BSG. The dual valorization approach enhances feedstock utilization and process sustainability, contributing to a circular model for biopolymer production.

ID336 | Nanobubble-Enhanced Oxygen Transfer in Bacterial NanoCellulose Production: Comparative Evaluation with Static and Airlift Systems

Author: Ana Cristina Rodrigues

1 CEB- Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

E-mail: anacris38599@gmail.com

Other authors: Daniela Martins1,2, Ricardo Silva-Carvalho1,2, Susana Marques3, Isabel Belo1 2, Begoña Espiña4, Fernando Dourado1, 2, Miguel Gama1,2

1 CEB- Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 LABBELS – Associate Laboratory, Braga, Guimarães, Portugal; 3 LNEG- Laboratório Nacional de Energia e Geologia, I.P. Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal; 4INL-International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal

Background: Bacterial nanocellulose (BC) is a multifunctional biomaterial with high mechanical strength, crystallinity, and a nanofibrillar 3D porous network. These features makes it suitable for diverse applications, including food, cosmetics, paper, textiles, and biomedical areas such as drug delivery, tissue engineering, and wound dressing. BC is mainly produced by strictly aerobic Gram-negative bacteria. In BC culture systems, oxygen transfer limitations frequently constrain productivity. This inefficiency, together with high production cost, reduces its industrial adoption. The production of BC in agitated systems offers enhanced oxygen transfer and improved process efficiency, allowing for greater control of culture conditions and higher productivity.

Methods: Recognizing the need for a highly efficient oxygen supply to improve the efficiency of BC production, this study applied for the first time an agitated bioreactor equipped with a nanobubbles (NB) generator, comparing its performance with static and airlift (AL) systems. BC productions were conducted using a newly in-house isolate from kombucha (Komagataeibacter sp.) and a culture medium containing Eucalyptus bark hydrolysate (EBH) and corn steep liquor. Parameters monitored included dissolved oxygen, cell density, pH, sugars, lactic acid, and BC concentration. The cellulose obtained was characterized for crystallinity, thermal stability, polymerization degree, morphology, and fiber bundle size.

Results: The NB generator produced bubbles of 95.8 ± 12.9 nm with a zeta potential of –14.17±8.58 mV. At 1 L.min-1 aeration, oxygen transfer was markedly superior in NB versus AL (kLa 35.9 ± 1.2 vs 6.0±0.0 hn^{1}; OTR 270.3±8.9 vs 45.2±0.07 mg.L-1.h-1). The NB bioreactor supported higher cell density, maintaining BC productivity comparable to AL (0.023 g.L-1.h-1) and significantly greater than static production (0.0082 g.L-1.h-1). However, increased oxygen favored biomass growth over cellulose production, highlighting the need for process optimization. Structural analysis showed denser networks and lower polymerization degrees in AL- and NB-derived BC compared to static cultures.

Conclusions: This work demonstrates that NB aeration effectively improved oxygen transfer in BC culture without causing cytotoxicity effects. Although optimization is needed to enhance cellulose yield, NB technology represents a promising strategy for BC production and other fermentative processes.

ID356 | Probing Carbonic Anhydrase Structural Modulation by Choline-Based Ionic Liquids through Molecular Dynamics

Author: Michele Fraga

CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal. University of Coimbra, CERES, Department of Chemical Engineering, Coimbra, Portugal.

E-mail: michelefraga@ua.pt

Other authors: Mara G Freire; Matheus M Pereira

1 CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal. 2 University of Coimbra, CERES, Department of Chemical Engineering, Coimbra, Portugal.

Background: Carbonic anhydrase (CA), a metalloenzyme of high biotechnological relevance, exhibits catalytic proficiency that sustains diverse applications, including COn sequestration, bioremediation, and biosensor engineering. Nevertheless, CA activity and stability are critically ruled by intermolecular interactions, emphasizing the relevance of assessing novel enzyme-friendly environments. Within this context, choline-based ionic liquids (Ch-ILs) can act as tunable and sustainable solvents, whose molecular composition enables precise regulation of enzyme performance.

Methods: The effect of five Ch-ILs on CA-II (Carbonic anhydrase II) from Bos taurus was investigated through molecular dynamics (MD) simulations. Simulations were conducted in YASARA Structure. Systems were optimized, solvated, adjusted to pH 7.4, and simulated for 250 ns with 1.25 fs timesteps (both in the presence of ILs and in a control system without ILs). Simulation results were subsequently validated through enzymatic activity assays using a colorimetric kit.

Results: Analysis of MD results revealed distinct effects of the ILs on CA-II structural dynamics. The studied Ch-ILs act as modulators that either stabilized or perturbed CA-II structural features. Corresponding enzymatic activity assays confirmed these structural alterations, with a significant increase in activity (>300%) observed for structures where active site exposure was enhanced, facilitating substrate and product mass transfer.

Conclusions: Choline-based ILs demonstrate the capacity to modulate CA-II structure and activity. Enhanced enzymatic performance observed in select systems highlights the potential of these ILs as stabilizing and performance-promoting agents. The combination of molecular dynamics simulations and enzymatic assays proved crucial for elucidating IL-enzyme interactions and identifying candidates with broad applicability in biocatalysis and industrial biotechnology.

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ID360 | Engineered Bacterial Laccase Enables One-Step Synthesis of Bioactive (Neo) Lignans from Lignin Phenolics

Author: Vânia Brissos

ITQB NOVA, 2780-157 Oeiras, Portugal

E-mail: vbrissos@itqb.unl.pt

Other authors: Márcia Reino1, Magdalena Lejmel1, Ricardo Estevinho1, Maria P. Robalo2,3, M. Rita Ventura1 and Lígia O. Martins1

1ITQB NOVA, 2780-157 Oeiras, Portugal; 2 Departamento de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, 1959-007 Lisboa, Portugal; 3 Centro de Química Estrutural, Institute of Molecular Sciences, Complexo I; Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

Background: Laccases are biocatalysts with immense potential in lignocellulose biorefineries to valorize lignin monomers for sustainable chemicals and materials [1]. Although enzyme costs have significantly decreased during the past two decades, they remain a major expense in biorefining. Protein engineering offers the ability to tailor enzyme properties.

Methods: We combined multiple rounds of directed evolution (error-prone PCR and high-throughput screening) with rational design to enhance activity for lignin-related phenolic compounds. Selected variants were biochemically characterized, and the best-performing enzyme was tested against a broad panel of lignin-phenolics. One-step oxidative conditions for synthesizing lignans and neo-lignans were optimized, with products analyzed by NMR.

Results: The catalytic efficiency (kcat/Km) of a hyperthermostable bacterial laccase [2] toward syringol improved >400-fold. The evolved variant also showed enhanced activity for hydroxycinnamyl alcohols, cinnamic acid, and vanillyl derivatives across neutral to alkaline pH, demonstrating broad applicability. Optimized reaction parameters (pH, catalyst load, time, temperature, oxygenation) enabled one-step synthesis of compounds like syringaresinol, pinoresinol, dehydrodiconiferyl alcohol, thomasidioic acid, biseugenol, dehydrodiisoeugenol, and diapocynin. Compared to previous methods, our system offers solvent-free reactions, faster rates (1–24 h), and excellent yields (up to 100%). These (neo)lignans hold potential applications in medicinal chemistry and polymer synthesis. These natural products derived from plants possess structural scaffolds exploitable in diverse biotechnological areas.

Conclusions: Our findings represent a significant advance in valorizing the lignin chemicals platform, enabling high yields of biologically active compounds with potential applications in, e.g., medicinal chemistry and polymer synthesis.

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ID415 | Affordable small molecules as promising fluorescent labels for biomolecules: application in RNA-FISH probes

Author: Raquel Eustáquio

1. Laboratório HERCULES, Instituto de Investigação e Formação Avançada, Universidade de Évora, 7000-809 Évora, Portugal

E-mail: raqueleustaquio1998@gmail.com

Other authors: Ana Teresa Caldeira1,2,3, António Candeias1,2,3, António Pereira1,2,3

1. Laboratório HERCULES, Instituto de Investigação e Formação Avançada, Universidade de Évora, 7000-809 Évora, Portugal. 2 Departamento de Química e Bioquímica, Escola de Ciências e Tecnologia, Universidade de Évora, 7000-671 Évora, Portugal. 3 City University of Macau, Faculty of Innovation and Design, Avenida Padre Tomás Pereira, Taipa, Macau.

Fluorescent labels play a vital role in a wide range of contemporary scientific techniques, including direct and indirect immunochemistry, fluorescence microscopy, histochemistry, flow cytometry, and fluorescence in situ hybridization (FISH). Small fluorescent labels provide substantial practical advantages by allowing multiple fluorophores to be conjugated to a single biomolecule, thereby enhancing fluorescence signal optimization. Widely used fluorescent labels are often too costly for routine applications and typically exhibit small Stokes shifts, limiting their practical utility. In this study, we report three novel small molecules as promising fluorescent labels for biomolecules, synthesized via an efficient, straightforward, and cost-effective approach. Additionally, we assess the fluorescence properties of the three new small molecules as potential fluorescent labels for biomolecules. Six novel fluorescent oligonucleotide probes were synthesized, including three targeting the rRNA region of eukaryotic cells (EUK516) and three targeting the rRNA region of prokaryotic cells (EUB338). The synthesized fluorescent probes were evaluated using microorganisms from the culture collection of the Laboratory of Biodegradation and Biotechnology at the HERCULES Laboratory, University of Évora. The probes demonstrated effective performance in RNA-FISH applications, highlighting the potential of these new small molecules for biomolecule labeling and bioimaging.

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ID457 | Enhancing Acidogenic Fermentation of Fruit Waste to Boost Sustainable Bioplastic Precursors

Author: Eliana C. Guarda

1 Associate Laboratory i4HB - Institute for Health and Bioeconomy, Caparica, Portugal; 2 UCIBIO – Applied Molecular Biosciences Unit, Caparica, Portugal

E-mail: e.guarda@fct.unl.pt

Other authors: Claudia Galinha3, Marisa Saloio1,2, João Pereira1,2, Marisa Cardoso1,2, Mariana Matos1,2, Nídia Lourenço1,2, Maria Reis1,2

1 Associate Laboratory i4HB - Institute for Health and Bioeconomy, Caparica, Portugal; 2 UCIBIO – Applied Molecular Biosciences Unit, Caparica, Portugal; 3 LAQV-REQUIMTE, Caparica, Portugal

Background: Polyhydroxyalkanoates (PHA) production using mixed microbial cultures (MMC) involves a 3-stage process: acidogenesis, culture selection and PHA accumulation. Acidogenesis aims at converting the organic waste into fermentation products (FP), whose composition and concentration depend on feedstock and process conditions. Current monitoring tools are mostly offline and slow, limiting real-time control and reducing process efficiency/yield. This study investigated the effect of pH on FP profiles and microbial community composition and evaluated the application of 2D fluorescence spectroscopy as a rapid, non-invasive tool to predict FP concentrations over time towards better monitoring/control of the process.

Methods: Fruit waste was fermented in a 54 L UASB reactor, with OLR and pH optimized to maximize PHA precursor-rich effluent production. Routine samples were analysed both conventionally and via 2D fluorescence, using an immersed optical probe, aiming at developing mathematical models through partial least squares (PLS) to predict FP concentrations.

Results: The UASB reactor showed stable performance from day 31, operating at a pH of 4.85 with ~75% acidification. The FP profile was dominated by acetate and butyrate, along with propionate and valerate - precursors for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)], with an estimated 3HV content of 8.3%. When the pH was increased to 5.16, the FP profile shifted towards caproate, butyrate, and lactate, acidification dropped to ~50%, and 3HV precursors were no longer detected - thus producing the homopolymer P(3HB). Preliminary results from 2D fluorescence show potential for real-time monitoring of the FP concentrations throughout UASB operation.

Conclusions: This study demonstrated the feasibility of producing FP from fruit waste, with pH acting as a key factor in the acidification degree, granules' microbiome, FP profiles, and subsequently, in the final polymer composition. 2D fluorescence-based models enable real-time data collection, supporting improved process control and optimization of PHA production steps.

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ID508 | Production of pigments using Rhodotorula glutinis cells and tomato waste hydrolysates as nutritional sources

Author: Helena Ribeiro

University of Coimbra, CERES, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790, Coimbra, Portugal.

E-mail: helenaribeiro270@gmail.com

Other authors: Boelo Schuur2, Jorge F. B. Pereira1

1 University of Coimbra, CERES, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790, Coimbra, Portugal. 2 University of Twente, Faculty of Science and Technology, Green Energy Initiative, Sustainable Process Technology Group, PO Box 217, 7500 AE Enschede, The Netherlands.

Background: Utilizing food waste in a biorefinery framework enhances the sustainability and competitiveness of the agro-food sector. Tomato processing by-products, including peels, pomace, and seeds, are rich sources of carotenoids, lipids, proteins, and carbohydrates, making them ideal for microbial fermentation processes. The microbial production of natural pigments is increasingly attractive to industry as an eco-friendly alternative to synthetic pigments, with Rhodotorula glutinis demonstrating particular promise due to its ability to produce carotenoids, lipids, and enzymes. Its metabolic flexibility and ability to grow on low-cost substrates position this yeast as a valuable candidate for sustainable bioprocess development.

Methods: Tomato waste biomass underwent acid hydrolysis with different sulfuric acid concentrations to generate nutrient-rich hydrolysates. These were characterized and evaluated as carbon and nitrogen sources for R. glutinis growth and carotenoid pigment production. Yeast cultivation and pigment synthesis took place under controlled conditions. Biomass yield, nutrient uptake, and pigment accumulation were measured.

Results: Acid hydrolysis of tomato waste produced nutrient extracts that effectively fostered R. glutinis growth and pigment production. The pigment amounts varied with hydrolysate composition, highlighting the influence of nutrient availability on microbial activity. Two carotenoids, β-carotene and torularhodin, were identified, with torularhodin being the dominant compound in all samples and the control. β-carotene levels in the hydrolysates ranged from 4.4 to 9.2 times higher than the control, while torularhodin increased by 3.8 to 7.3 times. These results provide valuable insights into how hydrolysate composition affects R. glutinis growth and carotenoid synthesis, affirming tomato waste hydrolysates as promising and efficient feedstocks for this purpose.

Conclusions: This research confirms that tomato waste can be used in microbial pigment production, with hydrolysates successfully promoting the growth and carotenoid production by R. glutinis. The composition of hydrolysates influences pigment yields, providing valuable insights into the biosynthesis of β-carotene and torularhodin. Overall, this method enhances food waste valorisation, facilitates the creation of sustainable biorefineries, and increases economic value.

ID537 | The pink secret of an inland portuguese saltern as a source of high-value biomolecules

Author: Joana Gomes

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

E-mail: joana.gomes@ceb.uminho.pt

Other authors: Ana Pereira1; Catarina Gonçalves1; Cátia Santos-Pereira1; Joana Sousa1; Yang He2,3; Marta Simões2,3; André Antunes4; Sara Silvério1,5; Lígia Rodrigues1,5

1 Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal; 2 State Key Laboratory of Lunar and Planetary Sciences, Macau University of Science and Technology (MUST), Macau SAR, China; 3 China National Space Administration (CNSA), Macau Center for Space Exploration and Science, Macau SAR, China; 4 Institute of Science and Environment, University of Saint Joseph, Macau SAR, China; 5 Associate Laboratory in Biotechnology and Bioengineering and Microelectromechanical Systems (LABBELS), University of Minho, Braga, Portugal

Background: Most industrial molecules are still obtained by chemical synthesis or plant extraction, processes often linked to environmental impacts and, in plant-based cases, low yields. Surfactants and pigments are notable examples. Surfactants, amphiphilic molecules that reduce surface tension and improve solubilization of hydrophobic compounds such as pigments, are widely applied across sectors. Thus, developing sustainable alternatives is crucial. Extremophilic microorganisms are promising sources of metabolites, including biosurfactants and natural pigments, with lower environmental impact and comparable or even superior performance. In this study, the Rio Maior (RM) Saltern, the only inland, non-marine saltern in the Iberian Peninsula, was explored for the isolation of microorganisms with potential for biosurfactant and pigment production.

Methods: A water sample from the Rio Maior Saltern (RM-A) was physicochemically characterized and used to isolate extremophilic microorganisms on agar plates with a salinity gradient, incubated at 37 °C. Isolates were screened for biosurfactant and pigment production in Marine Broth medium (15 %(m/v) salinity and 1%(v/v) n-hexadecane), assessed by the Victoria Pure Blue BO (VPBO) assay. Production was optimized by varying medium composition, salinity, temperature and agitation. Biosurfactant activity was validated through surface tension reduction, emulsion index and VPBO assay.

Results: RM-A water showed high salinity (30 %m/v) and elevated sulfate, calcium and manganese content. A promising pink-pigmented microorganism was isolated and had a positive response for biosurfactant production in the VPBO assay. It produced a cell-bound biosurfactant and an intracellular pink pigment when cultivated in Modified Growth medium (15% salinity, 1% n-hexadecane, 30 °C, 200 rpm). Biosurfactant and pigment were extracted with PBS 1x and organic solvents, respectively, and characterized by TLC.

Conclusions: These findings highlight the Rio Maior Saltern as a valuable source of extremophilic microorganisms with potential for sustainable biosurfactant and pigment production. Beyond their role as eco-friendly alternatives to conventional molecules, these compounds may also hold antimicrobial and antioxidant potential, enhancing their applicability in biotechnology and industry.

ID565 | Valorization of Eucalyptus Bark Hydrolysate for PHB Production by Burkholderia thailandensis DSM 13276

Author: Thomas Rodrigues

UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, MOVA School of Science and Technology, NOVA University Lisbon, 2829-516 Caparica, Portugal

E-mail: ta.rodrigues@campus.fct.unl.pt

The overuse of fossil-based plastics has led to significant environmental challenges, including their persistence in ecosystems and the proliferation of microplastic pollution. Polyhydroxyalkanoates (PHAs) have emerged as a biodegradable alternative; however, their high production costs have delayed large-scale commercialization. Renewable lignocellulosic biomass, such as eucalyptus bark, presents a sustainable solution aligned with circular bioeconomy principles. Burkholderia thailandensis DSM 13276 has demonstrated remarkable metabolic versatility in utilizing synthetic C5 and C6 sugars, achieving yields exceeding 60% in PHA accumulation. This makes it a highly promising candidate for sustainable biopolymer synthesis using C5 and C6 sugar-rich eucalyptus bark hydrolysates. This study aimed to evaluate the impact of substrate variability in eucalyptus bark enzymatic hydrolysates on the production of polyhydroxybutyrate (PHB) by B. thailandensis DSM 13276. Additionally, an enzymatic extraction method for PHB recovery was implemented as a sustainable alternative to traditional solvent-based methods. Eucalyptus bark was pretreated using steam explosion and enzymatic hydrolysis, generating three hydrolysates characterized for key properties (pH, sugars, ammonium, protein, and inhibitors such as furfural and 5-HMF). B. thailandensis was cultured in 2 L batch bioreactors under controlled conditions (30 ° C, pH 7.0, 30% dissolved oxygen), with periodic sampling for CDW, PHB content, sugars and ammonium consumption. PHB was extracted using Alcalase enzymatic digestion (pH 8.3, 50-55 °C) and compared to chloroform-based methods. Structural and thermal characterization was performed using FTIR, SEC, DSC, TGA, and XRD. Hydrolysate composition significantly impacted PHB production. High C/N ratios (160.7) and glucose concentrations (>80 g/L) favored cell growth (CDW = 9.48 g/L) but reduced PHB accumulation (19 wt%). Lower C/N ratios (41.5) improved PHB content (60 wt%) and productivity (1.36 g/L·day). Enzymatic extraction achieved comparable purity (100 ± 3%) and efficiency (96%) to solvent-based methods, demonstrating greater sustainability. PHB properties, including molecular weight (2.77 × 10^5 to 6.30 × 10^5 Da), melting temperature (170–175 °C), and thermal stability (Tdeg = 280–297 °C), were consistent across samples. Eucalyptus bark enzymatic hydrolysates offer a highly promising and sustainable feedstock for efficient PHB production by B. thailandensis DSM 13276

ID567 | Bacillus-driven bioprocessing for simultaneous extraction of chitosan, astaxanthin and protein hydrolysates from shrimp shell waste

Author: Ana Zamorano Sánchez

1 Centre of Biotecnology of Azores (CBA), Faculty of Science and technology, University of Azores

E-mail: zamoazores.ciencia@gmail.com

Other authors: Roger Artiola, Andreia Peixoto, Nelson Simões, Ana Casas, Hugo Almeida, Duarte Toubarro

1 Centre of Biotecnology of Azores (CBA), Faculty of Science and technology, University of Azores 2Associate Laboratory for Green Chemistry, REQUIMTE, Faculty of Sciences, University of Porto 3Mesosystem Investigação & Investimentos by Spinpark, Barco, 4805-017 Guimarães, Portugal.

Shrimp processing generates significant amounts of shell waste, currently underexploited despite being a rich source of high-value biomolecules such as chitin, astaxanthin, and proteins. Conventional extraction methods are mainly chemical, involving separate and environmentally hazardous steps to obtain each compound. Recent advances in microbial biotechnology open new opportunities to replace chemical approaches with sustainable processes aligned with the principles of circular bioeconomy.

A screening of Bacillus spp. isolates was performed and identify strains able to carry out two key bioconversion steps: deproteinization and deacetylation of chitin. Selected isolates were applied directly to shrimp shell residues to test their efficiency in a integrated bioprocess. The resulting fractions were analyzed for chitosan yield and purity, astaxanthin recovery, and the production of protein hydrolysates. Antioxidant activity was assessed using scavenging assays, and potential applications were explored.

Two Bacillus isolates exhibited the ability to simultaneously deproteinize shrimp shells and deacetylate chitin, effectively replacing conventional chemical treatments. This process resulted in the production of (i) chitosan, (ii) astaxanthin, and (iii) a low-molecular-weight protein hydrolysate with strong antioxidant activity. Chitosan and astaxanthin are being further developed for applications in health and cosmetics in collaboration with Mesosystem. The nutritional and probiotic potential of the protein hydrolysate is being evaluated in fish models.

This study demonstrates a novel biotechnological strategy for the integrated valorization of shrimp shell waste through microbial processing. By simultaneously extracting chitosan, astaxanthin, and antioxidant protein hydrolysates in a single eco-friendly step, the approach minimizes waste, reduces environmental impact, and generates multiple value chains, contributing to a circular and zero-waste bioeconomy.

ID593 | Design of Experiments as a Tool for Optimizing Batch Crystallization of β-Lactoglobulin

Author: Diana Machado

Centre of Biological Engineering, University of Minho, Braga, Portugal

E-mail: b13533@alunos.uminho.pt

Other authors: Jéssica Gomes 1; José António Teixeira 1,2; Filipa Castro 1,2

1 Centre of Biological Engineering, University of Minho, Braga, Portugal; 2 LABBELS - Associate Laboratory, Braga, Guimarães, Portugal

Background: Whey disposal presents serious environmental concerns and results in the loss of valuable proteins [1]. Conventional separation methods are limited by low throughput, poor scalability, high operational costs, and low purity [1,2]. Crystallization is a promising separation and purification technique because it is cost-effective and scalable [3]. This work aims to identify the critical process parameters for the batch crystallization of β-lactoglobulin (BLG) in an oscillatory flow reactor with smooth periodic constrictions (OFR-SPC), which offers enhanced hydrodynamic and transport properties [4].

Methods: BLG crystallization conditions screened via microbatch assays with ZnCln (precipitant agent) were transferred to a batch-operated OFR-SPC at 20 ºC. A Rotatable Central Composite Design (RCCD) was selected as the Design of Experiments (DoE) strategy to efficiently characterize the response surface and detect factor interactions. RCCD was conducted with BLG concentration, oscillation frequency and amplitude as factors, and induction time (tind) and mean crystal size as responses. tind was estimated from online turbidity measurements and mean crystal size (Feret diameter) was measured by image analysis (optical microscopy).

Results: Preliminary results confirm the influence of the studied factors on both crystallization kinetics (tind) and mean crystal size (Feret diameter).

Conclusion: DoE is a multivariate approach that yields more reliable and informative results per run than the One-Factor-At-a-Time (OFAT) method. In this study, DoE proved to be a valuable tool for the identification of the key process parameters in the crystallization of BLG in a patented OFR-SPC.

Acknowledgements:

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ID600 | Portoamides production: Downstream and Upstream process optimization in *Phormidium sp.* lege 05292

Author: Mariane Bittencourt Fagundes

ciimar – interdisciplinary centre of marine and environmental research, university of porto, terminal de cruzeiros do porto de leixões, avenida general norton de matos s/n, 4450-208 matosinhos, portugal

E-mail: mfagundes@ciimar.up.pt

Other authors: mariana miranda moutinho, marco preto, pedro nuno leão

1ciimar – interdisciplinary centre of marine and environmental research, university of porto, terminal de cruzeiros do porto de leixões, avenida general norton de matos s/n, 4450-208 matosinhos, portugal; 2 ciimar – interdisciplinary centre of marine and environmental research, university of porto, terminal de cruzeiros do porto de leixões, avenida general norton de matos s/n, 4450-208 matosinhos, portugal; 3 ciimar – interdisciplinary centre of marine and environmental research, university of porto, terminal de cruzeiros do porto de leixões, avenida general norton de matos s/n, 4450-208 matosinhos, portugal

Background: Portoamides were first identified in Phormidium sp. LEGE 05292, noted for their wide biological activities and potential in biotechnology, pharmacology, and antimicrobial development. Growing interest in these metabolites highlights the need for cultivation and extraction processes that ensure higher and sustainable yields. Methods: To address this, we herein applied an integrated optimization approach. Flat-Panel photobioreactors provided kinetic parameters, while a Multicultivator system assessed the effect of light intensity on biomass. Portoamides A and B were quantified by LC-MS/MS using external calibration. Optimization targeted two axes: downstreaming and upstreaming. Results: In the downstream axis, vortex and ultrasound-assisted miniaturized protocols increased selectivity and reduced solvent use. Comparative tests showed that miniaturized ethanol systems performed best, yielding about 1.5-fold higher portoamide A and 1.6-fold higher portoamide B than conventional methanol extraction. A replacement with ethanol proved a greener alternative by lowering toxicity. The A:B ratio remained stable (4–7), with reproducibility (RSD 8–15%) and recovery (8–15%, ~10–11%) supporting scalability with reduced environmental impact. In the upstream axis, biomass rose from 0.28 g/L at 30 µE to 1.06 g/L at 600 µE (3.7-fold increase). Portoamide accumulation, however, peaked at 300 µE, with a ~1.35-fold increase relative to 30 µE. Correlation analysis showed a positive but non-linear association ($r = 0.88$), indicating metabolite accumulation depends on specific conditions rather than simple growth. Based on the 300 µE optimum, a 3^3 factorial design under Quality by Design (QbD) principles evaluated nitrate, phosphate, and micronutrients. The model was significant ($p = 0.04$), with nitrate–phosphate interaction ($p = 0.002$) as the main determinant of biomass increase. Under optimal conditions, cultures reached 2.5 g/L biomass, corresponding to a 3.7-fold increase compared to basal conditions. Conclusions: The integration of greener, miniaturized extraction methods with optimized light and nutrient regimes demonstrates the feasibility of scaling up portoamide availability, establishing a foundation for sustainable industrial production of these high-value metabolites.

ID629 | Unveiling Antibody–Ligand Interactions on High-Capacity Protein A Resin Using SEM and SAXS

Author: Cristina Dias-Cabral

RISE-Health, Department of Chemistry, Faculty of Sciences, University of Beira Interior, Covilhã, Portugal.

E-mail: ccabral@fcsaude.ubi.pt

Other authors: Gonçalo Silva 1, Rupert Tscheliessnig 1, Jasek Plewka 1, Leo Jakob 1,2, Helga Lichtenegger 2, Alois Jungbauer 1,2

1 Austrian Centre of Industrial Biotechnology, Austria; 2 University of Natural Resources and Life Sciences, Austria

The process of purifying antibodies via Staphylococcal Protein A affinity chromatography serves as a critical stage in platform production procedures, facilitating the manufacturing of biopharmaceuticals. Throughout the years, there have been notable advancements in refining the structure of ligands, augmenting ligand density, and optimizing methods for immobilization, as well as enhancing bead properties and backbone composition. These efforts have been directed towards improving dynamic binding capacity (DBC) and operational efficiency. Notably, enhancements in DBC have been achieved by consolidating repetitive antibody binding domains into singular ligand structures and increasing the quantity of binding domains. Despite these advancements, there remains a significant gap in comprehending the interaction during chromatographic processes.

This study places significant emphasis on employing advanced techniques to elucidate the binding orientation of the antibody-staphylococcal protein A complex. Through a comprehensive analysis using adsorption isotherms, scanning electron microscopy (SEM), and small-angle X-ray scattering (SAXS), the spatial distribution, orientation, and stoichiometry of protein A to the resin TOYOPEARL® AF-rProtein A HC have been clarified. In-situ monitoring of chromatographic steps using SAXS further reveals the interaction between antibodies and the resin, with findings supported by adsorption isotherms. These techniques provide intricate insights into monolayer adsorption, stoichiometry, and reversible protein-protein interactions, shedding light on the spatial distribution of Protein A ligands and enhancing the understanding of antibody purification through protein A affinity chromatography.

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ID696 | Computational Discovery of Affinity Ligands for mAbs Purification

Author: Arménio J. M. Barbosa

1 UCIBIO, i4HB – Chemistry Department, NOVA School of Science and Technology, NOVA University Lisbon, Almada, Portugal

E-mail: aj.barbosa@fct.unl.pt

Other authors: Jéssica Rodrigues, Ana Cecília A. Roque

1 UCIBIO, i4HB – Chemistry Department, NOVA School of Science and Technology, NOVA University Lisbon, Almada, Portugal

Background: The application of atomic scale in silico techniques is important to design and discover ligands, giving insights on the molecular level interactions of affinity ligands to their targets and the influence of binding and elution buffers. Successful integration of molecular modeling has been described in the development of synthetic ligands for purification of: antibodies, chicken IgY, and antigen-binding fragments

(1); Human Serum Albumin (HSA) (2); and Viral spike protein (3). To streamline the discovery of new synthetic affinity ligands for purification, a High-Throughput Virtual Screening pipeline has been developed. Methods: A solid state virtual combinatorial library was generated using chemical fragments and filtered by the Petasis-Ugi scaffold reacting groups. The library was curated and screened using Vina, and the 1% top hits were evaluated with induced fit docking. Molecular dynamics simulations and binding free energy calculations using MM-PBSA were used to select the final ligands.

Results: The Petasis-Ugi virtual combinatorial library resulted in more than 15 million unique compounds, and simulations identified five potential synthetic affinity ligands for trastuzumab purification. Experimental validation of these ligands against trastuzumab is in development.

Conclusions: The developed ultra-large Petasis-Ugi library holds promise for applications in other biopharmaceutical targets. These findings represent a significant step toward more cost-effective antibody purification and affinity ligand discovery.

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ABSTRACTS

TOPIC #7: New and Emerging Technologies

ORAL COMMUNICATIONS

ID341 | Aiming at the Automation of Genome-wide Regulatory Model Inference and Metabolic Integration in Saccharomyces cerevisiae

Author: Diogo Alexandre Carracedo Couceiro

INESC-ID, Lisboa; iBB - Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa – Lisboa;

E-mail: d.alexandre963@gmail.com

Other authors: Carolina Parada 1,2, Tiago Val 1,2,3, Leonardo Paulo1,2,3, Luís Coutinho 2,3, Miguel Cacho Teixeira 2,3, Pedro Tiago Monteiro 1,4

1INESC-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

Metabolism is dynamic and in close liaison with the cell's environment, from reacting and adapting to stress, to preferentially consuming specific carbon sources. This dynamic nature stems from signaling/regulatory pathways whose major effectors are Transcription factors (TF) that regulate enzyme encoding genes - target metabolic genes (TMG) -, a phenomenon systematically ignored by metabolic models.

This work aims at developing methods for the automated inference and metabolic integration in Yeastract+ (containing around 200K TF-target associations for Saccharomyces cerevisiae) [Teixeira et al, NAR, 51: D785-D791, 2023] of Boolean regulatory models. Integration of TF-TMG regulations in the Yeast9.0.0 Saccharomyces cerevisiae model has already been performed with promising in silico results, although still under wet-lab validation. The next step is then the integration of TF inter-regulations (TF-TF) over these TF-TMG associations so to more closely describe the dynamics of regulatory-metabolic systems. Foremost in this work we propose an innovative approach to unify Boolean regulatory models and continuous metabolic models. This approach differentiates itself in that thoroughly justified biological meaning is attributed to concepts such as the regulatory models' Stable States, the notion of parsimony in a metabolic model's flux distribution, the idea that metabolic models can inform on the regulatory model's short-comings and incompleteness as well as the relative biological meanings of Boolean active and inactive states. Computational experiments, coherent in some aspects with existing literature, have already been successfully performed and promising candidates for wet-lab validation have already been identified – work still underway.

Furthermore, introducing quantitative expression data into Yeastract is ongoing, establishing the starting point for other newer and also ongoing efforts to create models that integrate quantitative regulatory data with Yeast9.0.0.

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ID363 | Cyanobacterial extracelular vesicles as vaccination platforms for fish

Author: Jorge Matinha-Cardoso

CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, 4450-208 Matosinhos, Portugal; i3S - Institute for Research and Innovation in Health from the University of Porto, 4200-135 Porto, Portugal; ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal

E-mail: jorgematinhacardoso@gmail.com

Other authors: Gabriela Gonçalves1,4, Filipe Coutinho1,4, Steeve Lima2,3, Beatriz Teixeira1,4, Aires Oliva-Teles1,4, Paula Tamagnini2,4, Cláudia Reis Serra1,4, Paulo Oliveira1,4

*1 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, 4450-208 Matosinhos, Portugal; 2 i3S - Institute for Research and Innovation in Health from the University of Porto, 4200-135 Porto, Portugal; 3 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal; Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal**Background: Bacterial extracellular vesicles (EVs) are now considered a cutting-edge technology for human and animal vaccination [1]. The conventional strategy involves the use of EVs derived from pathogenic bacteria, which naturally possess native immunogenic antigens. Nevertheless, inspired by synthetic biology, an alternative involves engineering non-pathogenic bacteria to express heterologous immunogenic antigens from pathogens and target them to EVs, which then serve as antigen carriers for immunization. Cyanobacteria, non-pathogenic and photosynthetic prokaryotes are emerging as sustainable microbial cell factories and producers of safe EVs [2, 3]. Previously we have genetically engineered the model cyanobacterium Synechocystis sp. PCC6803 for the expression of the reporter green fluorescent protein (GFP) and its targeting to EVs [3]. Here, envisioning cyanobacterial EVs as vaccination platforms and targeting the fastest growing food-industry sector – aquaculture – we demonstrate the capacity of EVs carrying GFP to modulate the fish immune system.*

Methods: Stability tests were firstly performed to assess EV integrity under various temperature storage conditions and after freeze-drying. Then, fish antibody production after intraperitoneal injection of EVs carrying GFP was assessed. Looking ahead to the aquaculture industry demand for oral vaccination, the intestinal uptake of EVs carrying GFP was investigated after their oral administration.

Results: Synechocystis sp. EVs are resilient nanostructures that promote the increase of antibody titters in fish after injection. Additionally, EV uptake by fish intestinal tissues could be observed upon oral administration.

Conclusions: This work unveils the potential of cyanobacterial EVs as stable and safe vaccination platforms for fish in aquaculture. Currently, immune responses in fish after the oral administration of EVs carrying GFP is being investigated. Furthermore, Synechocystis sp. mutants that produce vesicles containing immunogenic antigens from relevant fish pathogens are now being developed.

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ID375 | Botryosphaeriaceae early detection using a DNA-based Biosensing Platform

Author: Paula Martins-Lopes

1 DNA & RNA Sensing Lab, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal 2 Faculdade de Ciências, Universidade de Lisboa, BioISI – Instituto de Biossistemas e Ciências Integrativas, Lisboa, Portugal

E-mail: plopes@utad.pt

Other authors: Filipe Azevedo-Nogueira1,2, Cecília Rego3, Helena M.R. Gonçalves4

1 DNA & RNA Sensing Lab, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal 2 Faculdade de Ciências, Universidade de Lisboa, BioISI – Instituto de Biossistemas e Ciências Integrativas, Lisboa, Portugal 3 LEAF - Linking Landscape, Environment, Agriculture and Food-Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa, Portugal 4 LAQV, REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Background: Grape Trunk Diseases (GTDs) is a complex of diseases that affect grapevine production worldwide. Symptoms of GTD infection in vines are only visible after several years, and meanwhile the inoculum is spread throughout the vineyard. As there are no effective treatments, detecting plants infected by GTD in an early stage are the most effective way of reducing inoculum and yield losses. DNA-based biosensors can be used to detect small amounts of a given target in a reduced time under field conditions (Barrias et al., 2024). Our study aimed to develop a portable DNA-based biosensor platform capable of detecting GTD related pathogens without compromising the plant.

Methods: Probes were designed based on the Internal Transcribed Spacers locus (ITS) for GTD associated pathogens, namely Botryosphaeria dothidea and Neofusicoccum parvum, two species of the Botryosphaeriaceae family. The biosensor kits were produced by Biosensor NTech Nanotechonology Services, LDA (Lisbon, Portugal) according to a patented method (Duarte et al., 2023). Samples used were pure fungal cultures and sawdust samples from commercial vineyards.

Results: The analytical parameters of each probe were: LOD of 1.48 and 2.93 pM and Sensitivity of 45.5 and 78.9 pM, for B. dothidea and N. parvum, respectively. The biosensing platform allowed the discrimination between symptomatic and non-symptomatic plants using trunk sawdust obtained using 3 mm Ø drill.

Conclusion: This biosensor platform can be used by grape producers to detect early Botryosphaeriaceae infections, allowing a more efficient GTDs mitigating and avoiding yield losses.

Barrias S., Ibáñez J., Fernandes R., Martins-Lopes P. Trends in Food Science & Technology 145 (2024) 104350 Duarte, A., Gonçalves, H., Martins-Lopes, P. (2023) PCT/IB2022/056509. Internacional ref. WO 2023/007289. Funding: Fundação para a Ciência e Tecnologia: TrunkBioCode Project (http://doi. org/10.54499/PTDC/BAA-DIG/1079/2020); (http://doi.org/10.54499/2022.04199. CEECIND/CP1724/CT0008) and (https://doi.org/10.54499/2020.04459.BD) and BioISI center grant UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020).

ID405 | Network Dynamics as Fingerprints of Thermostability in an In Silico-Engineered DyP-type Peroxidase

Author: Carolina Ferro Rodrigues

ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal

E-mail: carolinafrodrigues@itqb.unl.pt

Other authors: Diogo Silva, Constança Lorena, Patrícia Borges, Laura Masgrau, Lígia Martins

1 ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal; 2 ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal; 3 ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal; 4 Department of Chemistry, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; 5 ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal

Background: Stabilising enzymes are essential for advancing sustainable and efficient bioprocesses. However, the molecular determinants underlying protein thermostability remain poorly understood. Dye-decolorising peroxidases (DyPs) are a promising class of enzymes for industrial applications; however, their inherent thermal sensitivity limits their broader use.

Methods: We used two open-source computational design platforms, PROSS and FireProt, to independently generate thermostable variants of the tetrameric PpyP from Pseudomonas putida [1-3]. The most promising designs were further optimised through recombination to reduce the number of mutations while maintaining or improving stability. To understand the structural basis of the observed thermostability, we performed detailed structural and dynamic analyses, including molecular dynamics simulations and protein correlation network analysis.

Results: The engineered DyP variants showed significantly improved thermal properties, with optimal activity temperatures of 65–70 °C and melting temperature (Tm) increases of 10 °C and 20 °C. Despite originating from different design algorithms, the variants shared convergent stabilising features, including greater compactness, increased rigidity, and a denser network of hydrogen bonds and hydrophobic interactions. Mutations clustered in similar structural regions despite differing in identity. Dynamic modelling and network analysis revealed a novel stabilisation fingerprint: highly connected internal networks with persistent interactions that promote coordinated motions across the tetramer. These dynamic features appear to help prevent local unfolding and subunit dissociation at high temperatures.

Conclusions: These findings highlight dynamic connectivity as a potential signature of thermostability that remains hidden in static structural analyses, offering insight beyond the traditional rigidity-focused view and pointing to an unexplored design principle in engineered proteins, particularly in engineered systems developed using computational tools.

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ID461 | From Images to Insights: Evaluating Cell Segmentation Strategies for Biofilm Analysis

Author: Arefeh Mazarei

LEPABE, ALiCE, Faculty of Engineering, University of Porto, Porto, Portugal

E-mail: arefeh.mazarei@fe.up.pt

Other authors: Albert Parker (2,3); Darla Goeres (1,2); Joana Vilas Boas (1); Jaime Santos Cardoso (4); Nuno Filipe Azevedo (1)

1 LEPABE, ALiCE, Faculty of Engineering, University of Porto, Porto, Portugal; 2 Center for Biofilm Engineering, Montana State University, Bozeman, USA; 3 Department of Mathematical Sciences, Montana State University, Bozeman, USA; 4 INESC TEC, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

Background: The structure of microbial biofilms shapes their ecological interactions, growth, and tolerance to antimicrobials. Three-dimensional (3D) confocal microscopy enables visualization at single-cell resolution. However, to translate images into quantifiable biological knowledge, individual cells can be identified through segmentation. Segmentation quality directly impacts biological readouts such as cell numbers, density, and spatial organization. Errors here can mislead interpretation and reduce reproducibility.

Methods: We compared five cell segmentation strategies, from conventional thresholding (Otsu´s method) to advanced deep learning models (BCM3D 2.0, StarDist OPP, DeepSeeded, Cellpose-SAM). 3D images of public biofilms (24 stacks) and in-house early-stage Pseudomonas aeruginosa biofilms (23 stacks, 512^2×70–90) were analyzed, and performance was evaluated with both object-level metrics and voxel-level metrics. Quantitative metrics were complemented by qualitative inspection.

Results: Otsu's method underperformed, with poor positive predictive value and accuracy, confirming its naivety. BCM3D 2.0 also showed limited applicability. DeepSeeded after retraining gave the best boundary accuracy (F1=0.58; Dice ≈0.80) and uniquely combined high sensitivity (0.96) with strong specificity (0.95). Cellpose-SAM, the only generalist deep learning segmentation method investigated, in its pretrained form provided the most stable and generalizable results, with the lowest cell counting error (≈16%) and consistent voxel-level performance (Dice 0.78–0.82; MCC ≈0.70–0.75). Its sensitivity (0.79) was slightly lower than DeepSeeded, while retrained Cellpose-SAM favored specificity (0.99) over sensitivity (0.59). Retraining improved voxel-level performance of StarDist OPP (Dice ≈0.75, sensitivity 0.68, specificity 0.98) but remained intermediate in object-level accuracy (F1 score ≈ 0.20 at IoU 0.5).

Conclusions: Cell segmentation is not just a technical step but the bridge between biofilm imaging and biological interpretation. Our analysis shows that pretrained Cellpose-SAM is the most reliable choice for reproducible out-of-the-box biofilm analysis, providing stable results with low errors and no need for retraining. DeepSeeded after retraining is the best option when precise separation of neighboring cells is required, offering high accuracy but with more variability. These results give practical guidance for selecting segmentation tools in 3D biofilm studies.

ID475 | Novel Phage-derived strategy for Early Detection of Paenibacillus larvae, the Etiological Agent of American Foulbrood

Author: Renato Veloso da Mota

CEB – Centro de Engenharia Biológica, Universidade do Minho, 4710-057 Gualtar, Braga, Portugal

E-mail: renatovelosomota@gmail.com

Other authors: Paulo Carvalho1, Diana Rodrigues1, Artur Ribeiro1,2, Sílvio Santos1,2 & Ana Oliveira1,2

1 CEB – Centro de Engenharia Biológica, Universidade do Minho, 4710-057 Gualtar, Braga, Portugal; 2 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Advanced biotechnological methods are increasingly shaping the field of pathogen detection, providing us new tools for sensitivity, specificity, and speed. In this study, we explore the use of phage-derived proteins to develop a novel detection approach for spore-forming bacteria. The case study addresses Paenibacillus larvae, the etiological agent of American Foulbrood (AFB), a devastating honeybee disease. AFB is caused by highly resistant spores that, once germinated in larvae, rapidly disseminate the infection. Because antibiotics use is forbidden, the incineration of colonies is mandatory. Current diagnostic methods are labor-intensive, need specialized equipment, and lack sensitivity at the asymptomatic stage of infection, which makes it impossible to monitor the hive's health in real time. Thus, early detection of spores—before clinical symptoms appear—is essential for AFB control.

Methods: Here we describe a novel two-step strategy that integrates magnetic spore capture with specific bioluminescent detection of P. larvae spores in honeybee samples. The method integrates two protein-based modules: (i) magnetic capture of spores using beads coated with γD-crystallin (MB-Cryst); and (ii) highly specific recognition of P. larvae via a bacteriophage cell wall-binding domain (PlyPl23CBD) fused to Nanoluciferase (NLuc-CBD). Following a short germination step, captured spores are detected by measuring light emission.

Results: MB-Cryst achieved spore capture efficiencies above 70%, enabling robust enrichment from complex biological matrices. NLuc-CBD demonstrated high specificity for P. larvae and produced strong luminescent signals after a short germination step. The combined workflow (MB-Cryst + NLuc-CBD) provided reliable detection of P. larvae spores with less than one working day, representing a substantial reduction compared to the standard microbiological method.

Conclusions: This study introduces the first bioluminescence-based detection system for P. larvae spores, offering a sensitive and rapid alternative to existing diagnostic approaches. Beyond AFB, the modularity of this detection strategy highlights its potential for broader applications in spore-forming pathogens.

ID526 | Exploring cellulolytic activity in compost-derived metagenomic libraries

Author: Joana Sousa

1 CEB - Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

E-mail: joana.sousa@ceb.uminho.pt

Other authors: Joana Gomes 1, Cátia Santos-Pereira 1,2, Ângela Costa 1,2, Andréia Santos 1,2, Susana Chaves 3,4, Ricardo FranconDuarte 3,4, João Linhares 5, Lígia Rodrigues 1,2, Sara Silvério 1,2

2 LABBELS - Associate Laboratory, Braga/Guimarães, Portugal; 3 CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710n057 Braga, Portugal; 4 IBnS - Institute of Science and Innovation for BionSustainability, University of Minho, Campus de Gualtar, 4710n057 Braga, Portugal; 5 Physics Center of Minho and Porto Universities (CFnUMnUP), Universidade do Minho, Campus de Gualtar, 4710n057 Braga, Portugal

Microorganisms are a promising source of bioactive metabolites, particularly enzymes, widely used in industrial and biotechnological processes. Cellulases play a key role in breaking down plant polysaccharides and are applied in diverse sectors, such as textiles, agriculture, pulp and paper, food and beverages, pharmaceuticals and biofuels. The cellulase system includes three main glycoside hydrolase (GH) groups: endo-1,4-β-D-glucanases, cellobiohydrolases (or β-1,4-exoglucanases) and β-glucosidases. Many cellulase-producing microorganisms remain unexplored due to limitations of conventional cultivation methods. Metagenomics, a culture-independent approach, has revealed the potential of uncultured microbes from diverse habitats, including composting and advanced cellulase bioprospecting. Sequence-based metagenomics, from shotgun to high-throughput sequencing, predicts gene functions via database comparison, while functional metagenomics enables the identification of new enzyme families whose activities cannot be predicted from DNA sequences alone.

In this work, three fosmid libraries prepared in Escherichia coli using DNA from compost samples collected at Portuguese composting units (Terra Fértil, Lipor and Gintegral) were screened through a two-step functional metagenomic approach to identify clones with endo-1,4-β-D-glucanase and/or β-glucosidase activities. Two substrates were used in both steps: carboxymethylcellulose for endo-1,4-β-D-glucanase and esculin for β-glucosidase. Over 1,500 clones were initially tested using a robotic screening system, and their enzymatic activity was evaluated after one week at 25 °C. Eight positive clones were further screened at different temperatures (25, 37, 45 and 55 °C), being the enzymatic activities monitored after 3, 5 and 7 days of incubation. Clear and brown halos indicated positive responses for endo-1,4-β-D-glucanase and β-glucosidase, respectively. The fastest and most promising clones were identified by automatic extraction of halo areas from color image analysis using MATLAB's in-house functions.

After three days at 25, 37 and 45 ºC, four promising clones for cellulolytic activity were selected: one clone exhibiting β-glucosidase, two clones showing endo-1,4-β-D-glucanase, and one clone with a strong response for both enzymatic activities. Sequencing analysis revealed a putative β-glucosidase with ~90% similarity to a GH3 β-glucosidase and a putative cellulase with ~100% similarity to a GH5 cellulase.

ID566 | Molecular Dynamics of CexA uncovers relevant citrate binding and recognition sites

Author: Francisca Ferreira

1Centre 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

E-mail: pg52236@alunos.uminho.pt

Other authors: João Alves, Margarida Casal, Isabel Soares-Silva

1Centre 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

Citric acid is the most widely used carboxylic acid, with a global market value reaching approximately 3.37 billion euros in 2024. The primary microbial cell factory for the industrial bioproduction is the filamentous fungus Aspergillus niger, well-known for its capacity to secrete citric acid. CexA, the main citric acid exporter in A. niger, has been functionally characterised and several amino acid residues have been identified as critical for citrate transport. However, a Steered Molecular Dynamics analysis of citrate moving through CexA revealed new substrate positions on the intracellular side of the protein, not revealed in the previous molecular docking analysis due to the static conformation of the CexA 3D structure prediction. The residues of this new position likely serve as targets for citrate binding and recognition. In this work the functional role of these residues in citric acid export capacity was studied.

Five amino acid residues were substituted with alanine by site-directed mutagenesis, and the resulting CexA mutant alleles were heterologously expressed in the Saccharomyces cerevisiae CEN.PK 113-5D strain for functional characterisation. The growth phenotypes of the engineered strains were assessed in media containing distinct carboxylic acids. Additionally, the subcellular localisation of the mutant proteins was evaluated by GFP-tagging of the respective alleles.

All five CexA mutations were viable in the S. cerevisiae CEN.PK 113-5D strain and subcellular localisation analysis confirmed that all mutant proteins were properly localised at the plasma membrane. In media containing mono- and dicarboxylates as sole carbon and energy source, three mutant alleles allowed an improved cell growth, compared to the strain expressing the native CexA transporter, while the remaining two exhibited a similar growth profile. Notably, none of the engineered strains grew in the citric-acid-containing media.

This study shows how Molecular Dynamics can uncover important details about membrane transporters and demonstrate its potential to enhance biotechnological processes.

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ID604 | Development and validation of a nanotechnology-based medical device for treating corneal diseases

Author: Patrícia Nápoles

1Department of Physics, Faculty of Sciences and Technology, University of Coimbra, Portugal

2FCTUC - Faculty of Sciences and Technology, University of Coimbra, Portugal

3CEReS – Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering,University of Coimbra, Portugal

E-mail: patricia.r.n.l.n@gmail.com

Other authors: Vítor Francisco 4 , Lino Ferreira 4,5 , Elisa Julião Campos 2,3,4

2FCTUC - Faculty of Sciences and Technology, University of Coimbra, Portugal

3 CEReS – Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering,University of Coimbra, Portugal

4 CNC – Center for Neuroscience and Cell Biology, CIBB – Centre for Innovative Biomedicine and Biotechnology,University of Coimbra, Portugal

5 Faculty of Medicine, University of Coimbra, Portugal

Background: The cornea is essential not only for light refraction but also as a key anatomical barrier. In the epithelium, the tight junctions preserve tissue integrity yet significantly restrict drug penetration. This barrier represents a major challenge in ocular therapy, particularly in keratoconus, where stromal delivery of riboflavin is required for effective cross-linking. In the gold standard epi-off protocol the epithelium is removed. While effective it is invasive, painful, and associated with risk of infection. Several epi-on strategies have been proposed; however, they consistently result in lower stromal riboflavin concentrations compared to epi-off procedures. This highlights the need for innovative approaches that enhance riboflavin delivery without compromising epithelial integrity.

Methods: This study validated a novel medical device as an epi-on strategy. The medical device is a nanoparticle-functionalized contact lens. Characterization included nanoparticle concentration, surface charge and morphology. Absorbed irradiance and surface temperature were analyzed during laser exposure. In the anterior segment, endothelial viability was assessed by Trypan Blue and Alizarin Red S staining, and the integrity of tight junctions was evaluated by immunofluorescence labeling. In the posterior segment, retinal morphology was examined using cresyl violet staining, and the retinal pigment epithelium (RPE) by phalloidin labeling. Surface temperature was monitored during the irradiation protocol. Riboflavin permeability was tested in an ex vivo cell insert model.

Results: Surface analyses confirmed successful functionalization of the medical device, establishing the basis for the intended photothermal responsiveness. Safety experiments demonstrated preserved endothelial viability and minimal retinal and RPE alterations. Permeability assays revealed riboflavin penetration levels with no statistical differences from the clinical epi-off standard. This effect was associated with reduced tight junction integrity.

Conclusions: This study demonstrates the potential of a nanotechnology-based device to enable effective riboflavin delivery under epi-on conditions. Achieving permeability, comparable to epi-off while maintaining ocular safety, represents a less invasive and clinically relevant alternative for keratoconus. Furthermore, modulation of epithelial tight junctions shows promise as a broader strategy to enhance corneal drug delivery in ocular therapy.

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ID623 | Microfluidic techniques for microbial manipulation: studies on enrichment and detection

Author: Violina Baranauskaite Barbosa

1LEPABE–Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering of University of Porto, Portugal. 3 ALiCE–Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

E-mail: violina.barbosa@gmail.com

Other authors: Laura Cerqueira 1,3, João Mário Miranda 2,3, Nuno Filipe Azevedo 1,3

1LEPABE–Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering of University of Porto, Portugal. 2 CEFT–Transport Phenomena Research Center, Faculty of Engineering of University of Porto, Portugal. 3 ALiCE–Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

Background: Standard microbial analysis relies on initial enrichment and detection with culture-dependent or - independent methods, often requiring several days. Alternatively, microfluidics enables enrichment of target microorganisms or integration with molecular methods for microbial detection. Therefore, we evaluated deterministic lateral displacement (DLD) for microbial enrichment and combined a filtration method with fluorescence in situ hybridization (FISH) for microbial detection.

Methods: Microfluidic platforms based on DLD and filtration were designed with AutoCAD® and fabricated in polydimethylsiloxane (PDMS) using soft-lithography. DLD arrays of varying lengths were designed at 3.36µm critical diameter (Dc). Then, enrichment in microchannels was evaluated using model microorganisms (E. coli and S. cerevisiae) suspended in sodium dodecyl sulfate (SDS) supplemented with dextran at 0.2µL/min. Microorganism trajectories were visualized through streak image photography, followed by quantification of displaced and non-displaced modes to evaluate separation. Filtration platform underwent computational fluid dynamics (CFD) to obtain velocity fields across the gaps. Then, the platform was used to trap C. tropicalis in artificial urine (AU) at 1µL/min. Then FISH, using a genera-specific peptide nucleic acid (PNA) probe, was applied onto immobilized C. tropicalis cells. Finally, fluorescence signal intensity of hybridized C. tropicalis was confirmed by epifluorescence microscopy.

Results: In the long DLD array, qualitative data indicate that E. coli were non-displaced, while S. cerevisiae were displaced, as predicted by theoretical Dc (3.36µm). Quantitative data from spatial distribution revealed a high recovery of S. cerevisiae (84.1%) in the long DLD array. CFD simulations showed similar velocity magnitudes across all gaps in the filtration section. Fluorescence quantification demonstrated significantly higher signal intensity of C. tropicalis hybridized with probe compared to cells subjected to hybridization solution alone, which corroborates with qualitative data of microfluidic PNA-FISH method.

Conclusions: Overall, this work demonstrated the potential of DLD to enrich yeast and that the filtration method coupled with FISH is a reliable approach for C. tropicalis detection in a microfluidics chip. Future efforts will focus on integrating DLD and filtration into a single platform for rapid microbial enrichment and detection in clinical or food safety

POSTERS

ID309 | Lignin extraction through acid-assisted deep eutectic solvents from steam-exploded Eucalyptus bark residue

Author: Michele Michelin

University of Minho

E-mail: mimichelin@ceb.uminho.pt

Other authors: José A. Teixeira1,2; Alexandre Rubira3,4 and Aloia Romani3,4

1 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal; 2 LABBELS–Associate Laboratory, Braga, Guimarães, Portugal; 3 Departamento de Enxeñaría Química, Unversidade de Vigo, Facultade de Ciencias, 32004 Ourense, Spain; 4 Instituto de Agroecoloxía e Alimentación. Universidade de Vigo – Campus Auga, 32004 Ourense, Spain.

Eucalyptus bark is an abundant forestry residue with high lignin content, offering significant potential for the production of bio-based chemicals and functional materials. However, efficient fractionation of its complex lignocellulosic structure remains a challenge. This study proposes an integrated pretreatment strategy combining steam explosion (SE) and deep eutectic solvents (DES) to achieve selective lignin extraction and fractionation.

SE (205 ºC for 10 min) was applied as the first step to disrupt the dense bark matrix, enhancing the accessibility of lignin and facilitating its solubilization in DES. Eutectic mixtures based on choline chloride and organic acids (formic and/or acetic acids) were employed for biomass fractionation (130 ºC for 2 h). Lignin fractions were characterized using FTIR, Py-GC/MS, TGA, DSC, total phenolics, and antioxidant capacity (DPPH).

The combined SE-DES process achieved a higher lignin recovery (26% yield) than DES pretreatment alone (17% yield). These lignins showed high purity (aprox. 90%) with tunable chemical and thermal properties, as verified by the H:G:S ratio (Py-GC/MS) and FTIR assignments, as well as the thermal decomposition temperature (TGA) and glass transition temperature (DSC). Furthermore, the combined SE-DES process yielded lignins with a higher total phenolic content and lower IC50.

This work highlights the synergy between SE and DES as a sustainable, low-toxicity, and versatile approach for biomass fractionation. The findings demonstrate the potential of this method to replace conventional harsh chemical treatments, aligning with the principles of green chemistry and circular bioeconomy.

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ID419 | PyHIV: A Python package for local HIV-1 sequence alignment, subtyping and gene splitting

Author: Ana Santos Pereira

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: anasantospereira@med.uminho.pt

Other authors: João Correia 3, Matheus Tavares 1,2, Nuno S. Osório 1,2

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3 Institute of Computing and Data Science, Universidade Católica Portuguesa, 4710-362 Braga, Portugal

Background: The growing availability of HIV-1 genomic data creates a major opportunity to study a virus that still imposes a substantial global health burden and to strengthen outbreak detection and response. Different subtypes and circulating recombinant forms (CRFs) exhibit distinct transmission and infection dynamics, accumulate different drug-resistance mutations, and differ in tropism. Therefore, accurate subtyping and gene-level analysis are essential. However, most HIV gene-cutting tools rely solely on the HXB2 reference and are usually web-based, as are many widely used subtyping tools, which requires sharing sequences and can compromise privacy.

Methods: We developed PyHIV, a local Python package that aligns user sequences against a curated Los Alamos (LANL) panel of subtype/CRF references using pyFAMSA, with GenBank-derived gene coordinates accessed via Biopython. For each query, PyHIV selects the best-scoring reference to (i) assign the subtype/CRF and (ii) split the sequence into gene-level regions according to that reference's coordinates. The pipeline is multi-threaded and local-only to maximize throughput and preserve data privacy.

Results: On LANL references, PyHIV reproduced curated annotations with 99.61% accuracy for pol and 99.81% for complete genomes, outperforming COMET (80.69%/80.50%) and REGA (17.37%/19.88%), two widely used and established subtyping tools. Using HXB2 as reference, 98.4% of PyHIV resultant pol segments matched Los Alamos Gene Cutter outputs. With the full reference set, 87.8% pol sequences were identical. The framework successfully partitioned all references into major structural genes.

Conclusions: PyHIV unifies accurate subtyping and gene splitting in a scalable, privacy,preserving package. By leveraging multiple references and local processing, it improves performance for diverse HIV,1 variants and facilitates robust studies of viral diversity, drug resistance, and vaccine design.

ID432 | Integrative OMICs Unlocks Hidden Cyanobacterial Diversity and Toxin Production

Author: Rita Cordeiro

CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning; UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, 9500-321 Ponta Delgada, Portugal

E-mail: rita.ip.cordeiro@uac.pt

Other authors: Muriel Gugger, Rúben Luz, Aniket Saraf, Petr Dvorak, Amélia Fonseca, Vítor Gonçalves

2 Institut Pasteur, Université Paris Cité, Collection of Cyanobacteria, F-75015 Paris, France ; 3 Department of Botany, Faculty of Science, Palacký University Olomouc, Slechtitel 27, CZ-783 71 Olomouc, Czech Republic; 4 Universidade dos Açores, Faculdade de Ciências e Tecnologia, 9500-321, Ponta Delgada, Portugal

Background: OMIC tools, including genomics and metabolomics, have expanded in recent years, enabling more comprehensive cyanobacteria studies of and providing deeper insights into taxonomy and secondary metabolites. Despite these advances, publicly available genomes remain scarce in many genera, such as Kamptonema. This recently established genus comprises several toxic species producer of cylindrospermopsin, anatoxin, and homoanatoxin. However, the full extent of its toxic potential is still poorly characterized.

Methods: In this study, we investigated 24 Kamptonema strains from various culture collections including the Bank of Algae and Cyanobacteria of the Azores (BACA), the Pasteur Culture Collection of Cyanobacteria (PCC) and the Culture Collection of Autotrophic Organisms (CCALA), covering diverse geographic origins. Genomes were sequenced through Illumina, and assembled and annotated following established pipelines. Genomic characterization was based on phylogenomics, average nucleotide identity (ANI) and biosynthetic gene clusters (BGCs) mining. Toxin production was assessed by ELISA and metabolomic analysis was done by ESI-HRLC-MS/MS, supported by curated databases.

Results: Twenty new draft genomes were assembled, including Kamptonema holotype K. animale CCALA 139. Genome mining revealed BGCs for cylindrospermopsin and homoanatoxin-a, with toxin production confirmed by ELISA and ESI-HR-LC-MS/MS, leading to the identification of new toxic strains. In addition, BGC analyses revealed novel proteusins (landormanides and kamptornamides) and geosmin. Phylogenomics together with 16S rRNA gene analyses and morphology assessment provided evidence for the description of at least one new species.

Conclusions: This study contributes with 20 new Kamptonema draft genomes, expanding genomic resources for the genus. Genomics and metabolomics unlocked the hidden diversity within Kamptonema, not evident with only single-gene phylogeny (e.g. 16S rRNA) or morphology alone, and highlights the link between taxonomy and the metabolic richness of Kamptonema, including the discovery of new toxic species.

ID437 | Unlocking Malaria Treatment Failure Prediction: An AI-Powered Analysis of Parasite Morphodynamics in Thick Blood Smears

Author: Ana Margarida Gonçalves

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: b14842@med.uminho.pt

Other authors: Ana Santos-Pereira 1,2; Maria Jardim 3,4; Beatriz Fonseca 1,2; Claudia Fançony 4; Nuno S. Osório 1,2; Maria Isabel Veiga 1,2

1 Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3 Clínica Multiperfil, Luanda, Angola; 4 Health Research Center of Angola (CISA), Caxito, Angola

Background. Malaria remains one of the most significant global health challenges. Microscopic examination of thick blood smears is the global standard for malaria diagnosis, yet its application is almost exclusively limited to parasite detection and quantification. This conventional approach overlooks a vast dataset hidden within the images: the dynamic morphological changes of parasites under drug pressure. This project moves beyond simple parasite counting to pioneer a new method, using artificial intelligence (AI) to unlock this hidden visual data and identify novel biomarkers predictive of treatment failure.

Methods. This work is centered on the development of a novel computational pipeline. We are using approximately 17,280 high-resolution images collected longitudinally from 108 patients during an artemether-lumefantrine (AL)—a first-line therapy for uncomplicated Plasmodium falciparum malaria—drug efficacy clinical trial (PACTR202405595426468). The core of our methodology is the creation of a user-friendly bioinformatic tool that allows expert microscopists to annotate not just parasites, but also subtle morphological features. This expert-labeled dataset will then train a sophisticated AI model designed to extract and quantify previously unrecorded visual features, such as stress-induced changes in parasite shape, texture, and clearance dynamics over the 28-day follow-up.

Results. The primary output will be a validated AI pipeline that transforms raw microscopic images into a rich, quantitative feature set of parasite morphodynamics. We expect this process to identify specific visual signatures—novel image-based biomarkers—that are highly correlated with AL treatment failure. The aim is to demonstrate that this AI-derived data provides a powerful, independent source of predictive information that is inaccessible through traditional microscopy.

Conclusions. This research establishes a new paradigm for the use of thick blood smears, evolving them from a simple diagnostic tool into a source of deep prognostic data. By developing an AI-driven method to quantify parasite morphological responses to treatment, this project aims to deliver a scalable, cost-effective technology that can serve as an early warning system for therapeutic failure, fundamentally enhancing disease surveillance and patient management in resource-limited settings.

ID504 | PyHIV-Resist: a reproducible Python tool for clinical resistance scoring of antiretroviral therapy from HIV sequences

Author: Matheus Tavares

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal. 2 ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: id11290@uminho.pt

Other authors: Ana Santos Pereira 1,2, João Correia 3, Nuno S. Osório 1,2

3 Institute of Computing and Data Science, Universidade Católica Portuguesa, 4710-362 Braga, Portugal

Background: Clinical interpretation of HIV-1 drug resistance relies on consistent mutation calling and standardized scoring. Web-only tools and single-reference workflows can limit privacy and robustness across diverse subtypes/CRFs. Extending PyHIV, a local package for alignment, subtyping, and gene splitting, we created and integrated PyHIV-Resist. This internal module performs local, rule-based clinical resistance scoring using the Stanford HIVdb rule set. Because HIVdb rules are defined on HXB2 amino-acid numbering, PyHIV-Resist explicitly translates coding regions and normalizes all variant calls to the HXB2 reference coordinate space to ensure rule compatibility.

Methods: PyHIV-Resist is invoked downstream of PyHIV. PyHIV performs local alignment (pyFAMSA) for each input sequence against a curated LANL panel, assigns subtype/CRF, and splits into gene regions using GenBank-derived coordinates. PyHIV-Resist then: (i) performs frame-aware translation of PR, RT, IN, and CA; (ii) maps coordinates to HXB2 AA numbering (reference-normalization), even when gene boundaries were selected from a non-HXB2 reference; (iii) identifies amino-acid mutations by comparison to the normalized HXB2 reference; and (iv) applies HIVdb rules to compute per-drug numeric scores and clinical categories (susceptible/low-level/intermediate/high-level). Outputs include per-sample/per-drug CSV tables and a PDF report. The workflow is local-only, multi-threaded, and preserves PyHIV's reference choice for accurate gene segmentation while guaranteeing HXB2-consistent scoring.

Results: For each query, the module returns a structured mutation list plus the HIVdb-based numeric score and categorical interpretation for clinically used antiretrovirals across PR, RT, IN, and CA. Tight integration with PyHIV ensures consistent gene boundaries before HXB2 normalization, avoiding HXB2-only assumptions when a different reference yields better alignment for a given subtype/CRF. By design, outputs are reproducible, privacy-preserving, and ready for benchmarking against laboratory reports or surveillance datasets.

Conclusions: PyHIV-Resist adds reliable HIVdb-based resistance scoring to the PyHIV toolkit, unifying sequence processing, mutation calling, and clinical interpretation in a single, reproducible pipeline.

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ID506 | Sustainable protein co-polymer for cosmetic innovation: combining silk, elastin and keratin

Author: Ana Mota

SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal

E-mail: amm@solfarcos.com

Other authors: Marta Caçador; José Pedro Carvalho; Ana Tinoco; André da Costa; Artur Ribeiro; Filipa Gonçalves; Artur Cavaco-Paulo

1 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal. 2 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal. 3 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; 4 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; 5 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal; 6 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; 7 SOLFARCOS, Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal; Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal; LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: The hair cosmetic industry is constantly searching for new ingredients and innovative materials that can enhance properties such as softness, shine, shape, and color. Conventional products and treatments often contain harmful chemicals that damage the hair. To overcome this problem, bio-based alternatives inspired in structural proteins such as silk, elastin, and keratin represent a promising strategy.

Methods: A recombinant protein co-polymer, designed as SELP::KP, and composed of consensus motifs from silk, elastin, and keratin, was expressed in Escherichia coli and purified using a non-chromatographic method to obtain high purity. Its structural properties were characterized by Fourier-transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). The cosmetic performance of SELP::KP was evaluated through application to virgin hair, followed by localization analysis on hair, mechanical tests, and assessment of perming and conditioning effects.

Results: SELP::KP demonstrated a ,-sheet-rich secondary structure and high thermal secondary stability. The protein is co-localized at both the cuticle and cortex of hair, indicating strong penetration capacity. Mechanical testing revealed a 34.9% increase in Young's modulus and a 12.4% increase in extensibility compared to untreated hair, demonstrating improved hair strength and elasticity. As a safer perming agent, SELP::KP maintained a curling effect similar to chemical treatments but with reduced damaging effects. Furthermore, hair treated with SELP::KP required only half the combing strength of untreated hair, confirming its efficacy as a conditioning agent.

Conclusions: SELP::KP is a promising bio-based protein co-polymer for hair cosmetics, providing multifunctional benefits such as strengthening, elasticity enhancement, conditioning, and safer perming. These properties support its potential use in leave-in treatments or hair masks aimed at improving both styling performance and hair health.

ID523 | Reconsidering Classical Disc Diffusion Antimicrobial Assays for Nanoparticles: A Kinetic Perspective on Silver Ions

Author: António Carrapiço

HERCULES Laboratory and IN2PAST, University of Évora, Portugal; Institute for Research and Advanced Training (IIFA), University of Évora, Portugal

E-mail: antonio.carrapico@fa.uevora.pt

Other authors: Manuel Pico 3; Elisabete P. Carreiro 4; Pedro Barrulas 1,3; José Mirão1,5; Ana Teresa Caldeira 1,3; Jorge Ginja Teixeira 1,3; Luís Dias 1,2; Maria Rosário Martins 1,6

1 HERCULES Laboratory and IN2PAST, University of Évora, Portugal; 2 Institute for Research and Advanced Training (IIFA), University of Évora, Portugal; 3 Department of Chemistry and Biochemistry, ECT, University of Évora, Portugal; 4 LAQV-REQUIMTE, IIFA, University of Évora, Portugal; 5 Department of Geosciences, ECT, University of Évora, Portugal; 6 Department of Medical Sciences and Health, ESDH, University of Évora, Portugal

Background: Nanoparticles are broadly studied as antimicrobial agents [1], yet their evaluation often relies on classical assays, such as disc diffusion, that were not designed for nanomaterials. Because nanomaterials act through distinct physicochemical mechanisms, these assays are often irreproducible and may misrepresent antimicrobial activity [2–4].

Methods: Antimicrobial activity of silver nanoparticles (AgNPs), biosynthesized using microbial culture supernatants, was evaluated against environmental bacterial and fungal strains using broth microdilution (MIC) and disc diffusion assays. A mechanistic model was applied to analyze the contribution of silver ion release kinetics to inhibition profiles.

Results: Disc diffusion produced inhibition zones that showed no correlation with nanoparticle concentration or MIC values. Kinetic modeling revealed that microbial growth inhibition in disc diffusion assays is dictated by the rate of silver ion release and diffusion through agar, rather than nanoparticle diffusion, helping explain inconsistencies reported in nanoparticle antimicrobial research [5].

Conclusions: Disc diffusion is not suitable for evaluating nanoparticle antimicrobials, as inhibition profiles are governed by ion release kinetics and microbial growth dynamics rather than direct nanoparticle action. This work highlights the need to adapt microbiological testing protocols to the specific properties of nanomaterials. This will improve the reliability and comparability of antimicrobial assays, with broad implications for microbiology and biotechnology.

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ID556 | Enhancing dyed hair color stability through formulations using recombinant proteins

Author: Marta Filipa Teles Caçador

Solfarcos – Soluções Farmacêuticas e Cosméticas, Lda., 4700-034 Braga, Portugal ; Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

E-mail: mfc@solfarcos.com

Other authors: Filipa Gonçalves1, André da Costa1, Artur Cavaco-Paulo1,2,3

1 SOLFARCOS – Soluções Farmacêuticas e Cosméticas, Lda., 4700-034, Braga, Portugal. ; 2 Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal; 3 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

Background: Thermal and environmental stressors remain major obstacles to the retention of color and structural integrity in dyed hair, driving innovation within the cosmetic industry. Our aim is to explore the use of recombinant proteins as novel cosmetic actives for the protection of dyed hair. The recombinant proteins can be formulated in aqueous, ethanolic and final commercial solutions and applied to human hair samples—including virgin and bleached types.

Methods: The recombinant proteins can be formulated in aqueous, ethanolic and final commercial solutions and applied to human hair samples, including virgin and bleached types. Rigorous evaluation methods, including protein solubility assays, circular dichroism spectroscopy, surface tension and contact angle measurements, fluorescence imaging, and mechanical and thermal property testing, are incorporated to elucidate how the proteins interact with hair and protect hair fibers under repeated heat exposure.

Results: By assessing these parameters, the work aimed to elucidate recombinant proteins potential for improving hair fiber robustness and maintaining color vibrancy in dyed hair and its potential to form a protective film around the hair shaft, enhancing barrier function and moisture retention—addressing not only immediate cosmetic effects but also long-term hair health.

Conclusions: Integrating advances from recombinant DNA technology into cosmetic formulations, we aim at creating promising, sustainable solutions for next-generation heat protectants and color stabilizers. The findings may stimulate interdisciplinary discussion around the development of protein-based cosmetics with enhanced efficacy, safety, and scalability, driving new standards in hair care innovation.

ID569 | Harnessing Bioinformatics Tools to Advance Corporate Sustainability

Author: Miguel Batalha

Universidade de Coimbra, Centro de Engenharia Mecânica, Materiais e Processos, ARISE, Departamento de Ciências da Vida, 3000-456 Coimbra, Portugal.

E-mail: miguel.batalha@uc.pt

Other authors: Ana Paula Chung; Paula Vasconcelos Morais

Universidade de Coimbra, Centro de Engenharia Mecânica, Materiais e Processos, ARISE, Departamento de Ciências da Vida, 3000-456 Coimbra, Portugal.

Background: The transition to a sustainable economy places microorganisms at the core, as renewable sources for bio-based products and as biological agents for environmental cleanup. Leveraging bioinformatics and expanding biological databases, genome mining has emerged as an efficient strategy for uncovering microbial functions and enzymes relevant to industrial sustainability. To address lipid-rich industrial wastewaters, whose lipid contaminants raise chemical oxygen demand (COD) and hinder treatment, we screened annotated bacterial genomes for lipase-encoding genes.

Methods: All the annotated genome data stored in the University of Coimbra Bacteria Culture Collection (UCCCB) was compiled into a local database. Genomes lacking functional annotation were processed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). To identify lipase,encoding genes, a custom Python script was developed to query and extract all gene sequences whose identifiers matched lipase,related keywords. To organize these findings, a second custom Python script parsed the output and generated a table listing each gene along with its source strain and accession number.

Results: Across the 172 genomes queried, a total of 1161 lipase and related genes were identified, spread across 131 bacterial strains. The top 10 strains encoded more than 20 lipase,associated genes. Three candidate strains (Collimonas pratensis ter91, Pseudomonas sp. M47T1 and Diaphorobacter sp. B2A2W2) were subjected to adaptive-cycle cultivation in wastewater-based media. Metagenomic analysis of those cultures showed that only the first two strains persisted, coinciding with a reduction in COD and suggesting a potential effect of the candidate strains on wastewater quality.

Conclusions: The application of this methodology was instrumental in streamlining the selection of candidate strains, whose activity may show promise for lowering COD in industrial effluents and offers a viable strategy for enhancing treatment processes and ensuring the environmentally safe discharge of process waters.

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ID639 | Scalable and green recovery of extracellular polymeric substances using hydrothermal extraction

Author: Ivana Rita da Silva Mendonça

1 Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal

E-mail: ivana.mendonca@staff.uma.pt

Other authors: Filipa Rodrigues 1,2,3, Marisa Faria 1,2 Juan Luis Gómez Pinchetti 4, Artur Ferreira 3, Nereida Cordeiro 1,2

1 Laboratory of Bioanalysis, Biomaterials and Biotechnology, Faculty of Sciences and Engineering, University of Madeira, 9020-105 Funchal, Portugal; 2 CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal; 3 CICECO - Aveiro Institute of Materials and Águeda School of Technology and Management, University of Aveiro, 3810-193 Aveiro, Portugal; 4 BEA - Spanish Bank of Algae, Institute of Oceanography and Global Change (IOCAG), University of Las Palmas de G.C, Muelle de Taliarte S/N, Telde, 35214 Las Palmas, Canary Islands, Spain

Background: Bound extracellular polymeric substances (B-EPS) are cell-surface polymers secreted by cyanobacteria and microalgae that form a protective matrix, enhancing stress tolerance and offering industrial potential through their stability and functional versatility. However, sustainable and scalable extraction methods remain challenging for large-scale applications.

Methods: This work optimized B-EPS extraction using a hydrothermal autoclave process, optimized via Response Surface Methodology (RSM). Three variables were investigated: biomass-to-solvent ratio (1:5

-1:20 w/v), temperature (108-134 °C), and extraction time (5-25 min). Model validation was performed using ANOVA, and the extracted polymers were compared with those obtained from conventional techniques in terms of chemical composition, functional groups, surface charge, and thermal stability. A solvent-free ultrafiltration (UF) step was integrated with the autoclave process to enhance recovery while minimizing chemical and energy consumption.

Results: Autoclave extraction produced polymers that closely resembled native capsular (or bound) material, achieving higher yields than conventional methods. Statistical analysis revealed that temperature and time were the main determinants of yield, whereas biomass-to-solvent ratio had a minor effect. 3D response surfaces indicated that simultaneous increases in temperature and time improved recovery. ANOVA confirmed high model accuracy ($F = 94.37$, $p < 0.0001$; $R^2 = 0.9659$) with no significant lack of fit. Optimal conditions (1:20 w/v, 130 °C, 16 min) yielded an extraction rate of 91%, closely matching the predicted value of 93%.

Conclusions: Coupling hydrothermal autoclaving with solvent-free UF eliminated hazardous reagents, reduced processing time and energy demand, and preserved polymer integrity, making the method sustainable, scalable, and industrially relevant. This green process aligns with circular bioeconomy principles and can support the replacement of fossil-derived plastics with B-EPS-based coatings, films, and functional biomaterials. Moreover, cultivating photosynthetic marine microorganisms in seawater provides additional benefits through biogenic carbon capture, advancing carbon neutrality goals, and enabling the development of carbon-negative material platforms.

ID662 | Unlocking biotechnological potential through OMICS tools in the BACA culture collection

Author: Rúben Luz

1 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning; UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, R. Mãe de Deus, 9500-321 Ponta Delgada, Portugal

E-mail: ruben.fs.luz@uac.pt

Other authors: Rita Cordeiro1, Inês Borges2, Amélia Fonseca1,2, Vítor Gonçalves1,2

1 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIOPOLIS Program in Genomics, Biodiversity and Land Planning; UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, Universidade dos Açores, R. Mãe de Deus, 9500-321 Ponta Delgada, Portugal; 2 Faculdade de Ciências e Tecnologia da Universidade dos Açores, 9501-801 Ponta Delgada, Portugal.

Background: The Bank of Algae and Cyanobacteria of the Azores (BACA) is a culture collection of microalgae and cyanobacteria, holding a diverse range of strains collected in the Azores archipelago. An effort has been made to characterize this diversity, with a particular focus on cyanobacteria, resulting in the description of five genera and over ten species from the strains maintained in the BACA culture collection, which were isolated from samples of freshwater and thermal habitats in the Azores. This high diversity of cyanobacteria, comprising many novel species, some of which are endemic to the Azores, represents significant potential for biotechnology and the exploration of novel resources and compounds for human applications.

Methods: The cyanobacteria were checked for contamination and grown under controlled conditions for three weeks. Biomass was collected, freeze-dried and extracted using commercial column kits. Sequencing was carried out by Illumina as a service provided by Novogene. Bioinformatics were performed in-house using a custom pipeline comprising SPAdes, CONCOCT, BUSCO and CheckM2, among others, to obtain high-quality genomes and explore strain potential through genomic exploration using antiSMASH.

Results: A total of 130 cyanobacteria strains from the BACA culture collection had their genome sequenced. This produced high-quality genomes (more than 95% complete and less than 5% contaminated), which, through genomic analysis using specialized software, allowed the identification of many strains with many unidentified biosynthetic gene clusters (BGCs), revealing high potential for biotechnological applications. Sequencing the cyanobacteria genomes also revealed the complexity of the cyanobacterial microbiome with many uncultured species, some of which have a high number of BGCs.

Conclusions: OMICs tools, such as genomics, provide a wealth of information and help to streamline work in biotechnological discovery by applying in silico methods, which could contribute to scientific advancement. These tools can reduce the time taken for the discovery of novel bioactive compounds by selecting only strains with the potential to produce them.

ABSTRACTS

TOPIC #8: From Science to Society and Business

ORAL COMMUNICATIONS

ID216 | Techno-economic analysis and Life Cycle Assessment of Bacterial Cellulose production: a comparative study with plant derived micro and nanocelluloses

Author: Fernando Dourado

Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

E-mail: fdourado@ceb.uminho.pt

Other authors: Ana Cristina Rodrigues1, Francisco Garrett, Miguel Gama1

1Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Background: The transition to sustainable materials is crucial for addressing global environmental challenges. Bacterial cellulose (BC), a high-purity biopolymer, has emerged as a promising alternative to plant-derived cellulose. However, its industrial adoption is limited by economic constraints. This study evaluates the techno-economic viability and environmental sustainability of BC production compared to plant-derived micro- and nanocellulose (PMNC), aiming to identify pathways for scalable, sustainable bio-based material solutions.

Methods: A techno-economic analysis (TEA) and life cycle assessment (LCA) were conducted using a process model (SuperPro Designer) for an industrial-scale BC production facility (264 ton/year, dry weight). Standardized LCA methodologies were applied to evaluate environmental impacts. Sensitivity analyses explored the effect of substituting conventional culture media with agro-industrial byproducts.

Results: TEA revealed BC production is economically feasible with a 4–5-year payback period, despite high capital and energy demands from fermentation/purification. Market analysis indicated BC struggles to compete with low-cost PMNC (e.g., MCC at €2–5/kg) in commodity markets but shows promise in high-value sectors (biomedical, cosmetics, advanced composites). LCA revealed BC has lower environmental impacts in some categories, but downstream processing (e.g., textile fibers) significantly increases its footprint due to high energy and chemical demands. Substituting culture media with byproducts could mitigate environmental impacts by over 60%.

Conclusions: This study highlights the need for process optimization, regulatory incentives, and strategic market alignment to enhance BC's economic and environmental viability. Leveraging sustainable feedstocks and addressing production bottlenecks can position BC as a key contributor to the bio-based economy. Circular economy approaches can significantly improve BC sustainability, supporting its adoption as a sustainable material.

ID448 | mosquitoWEB – A citizen science tool for arbovirus vector surveillance

Author: Luis Filipe Lopes

1Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal. cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal.

E-mail: filipe.lopes@ihmt.unl.pt

Other authors: Teresa Novo 2, Carla Sousa 2

2Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal.

Background: mosquitoWEB is a citizen science project, launched in 2015 by the Institute of Tropical Medicine and Hygiene, aiming to involve the public in monitoring and early detection of mosquito species of public health interest, such as Aedes albopictus.

The Asian tiger mosquito, Aedes albopictus, is a widely spread mosquito in Europe, being already established in most central and southern countries and as far north as Germany and Netherlands. In Portugal, Aedes albopictus was first detected 2017. This species is a vector of human pathogens, such as dengue, yellow fever, Zika and chikungunya viruses. The native species Culex pipiens, widely distributed in Portugal, is a vector of both West Nile and Usutu Viruses.

Methods: Mosquito photos or specimens are submitted to mosquitoWEB and mosquito identification is done by two entomologists. If a contact is provided feedback is given to the participants. Whenever an invasive species is detected in a new location, Health Authorities are informed. Outreach activities are held to enhance the visibility of the project.

Results: Since 2015 more than 880 submissions were received, which is a low level of participation, but has been increasing in recent years. Citizen engagement is clearly related to media exposure and mosquito nuisance. mosquitoWEB has received submissions compatible with Aedes albopictus mainly from the North and South regions. In 2023 mosquitoWEB was responsible for the first reports of the presence of this species in Lisbon and Oeiras municipalities. Recent submissions demonstrate the geographic expansion of this species to new areas. Culex pipiens is the most frequently submitted mosquito species.

Conclusions: Citizen science collaborative studies are a valuable complement to active surveillance systems implemented by Public Health authorities and can effectively detect the presence of invasive mosquitoes in new areas, earlier than active monitoring programs. This has been shown by mosquitoWEB and by similar projects in other European countries such as Spain and Germany. Furthermore, mosquitoWEB is also a vehicle for science dissemination, bringing information to the community and promoting public awareness of the importance of mosquito-borne diseases.

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ID564 | Biosensors in Practice: Socioeconomic Insights from Forensics and Food Safety Monitoring in Portugal

Author: Kristen Connor

Centre for Social Studies (CES), University of Coimbra, Colégio de S. Jerónimo Apartado, 3087 3000-995, Coimbra, Portugal

E-mail: kristenconnor@ces.uc.pt

Other authors: Joana Vaz Sousa 2 Filipe Santos 3 Gustavo García-López 4 Rita Campos 5

1, 2, 3, 4, 5 Centre for Social Studies (CES), University of Coimbra, Colégio de S. Jerónimo Apartado, 3087 3000-995,Coimbra, Portugal

Background: Based on recent fieldwork and stakeholder interviews, this presentation shares findings on the development and implementation of biosensors in forensic science and food safety monitoring in Portugal. While biosensor research often emphasizes proof-of-concept and technical performance, our study focused on how these tools are being integrated into real-world settings, and what barriers and opportunities shape their adoption. We explored how biosensors are imagined, designed, and evaluated by researchers, engineers, and end-users. Although many applications are technically promising, their impact is shaped by regulatory processes, institutional infrastructures, and questions around usability, accessibility, and relevance. We highlight how emerging biosensor technologies may influence social and economic relationships, depending on how they are implemented, scaled, and governed.

Methods: Our approach draws from qualitative research methods, including 21 semi-structured interviews, multi-sited participant observation, and thematic coding. Fieldwork included involvement in the BioAssembler consortium (Horizon Europe No 101070589), visits to partner laboratories, participation in international conferences, and interdisciplinary training courses. These encounters provided insights from researchers, engineers, and members of the public, some deeply embedded in biosensing work, others encountering it for the first time.

Results: Research, innovation, and investment in microbiotechnology such as biosensors are rapidly outpacing public understanding and preparedness. This disconnect may limit broader adoption beyond niche applications. In forensics, the European Network of Forensic Science Institutes (ENFSI) is a key gatekeeper for technological uptake. In Portugal, crime scene technicians expressed interest in point-of-need sensors for detecting fentanyl and other hazards. In food and environmental monitoring, biosensors are being explored for bivalve surveillance, aquaculture management, species identification, food fraud detection, and smart packaging.

Conclusions: Prevailing narratives around biosensor scalability often prioritize automation over local expertise, risking mismatches between design and practice. We argue biosensor innovation should involve grassroots producers to avoid reinforcing exclusionary systems. As biosensing remains an emergent field, its future offers opportunities for more inclusive, participatory models of microbiotechnology

POSTERS

ID357 | Enzymes Driving Sustainability in the Textile Value Chain

Author: Adelaide Braga

CITEVE—Technological Centre for Textile and Clothing of Portugal, Rua Fernando Mesquita, 2785, 4760-034 Vila Nova de Famalicão, Portugal

E-mail: abraga@citeve.pt

Other authors: Silva, Carla Joana

CITEVE—Technological Centre for Textile and Clothing of Portugal, Rua Fernando Mesquita, 2785, 4760-034 Vila Nova de Famalicão, Portugal

Background: The textile value chain faces growing challenges related to sustainability, environmental regulations, and consumer demand for eco-friendly products. Conventional processing relies on harsh chemicals, high water use, and energy-intensive operations, which contribute to pollution and reduce process efficiency. Biotechnology provides a sustainable alternative through enzyme-based processes that are cleaner, safer, and more efficient.

Methods: This study explored different enzymatic strategies for textile processing. Pectinases, xylanases, cellulases, and laccases were applied for degumming natural fibers. Cellulases were also used for bio-polishing and pilling reduction, while laccases were employed in enzymatic pre-treatments to improve subsequent functionalization steps. Enzyme-assisted methods for textile recycling were also considered, focusing on hydrolysis and selective fiber separation.

Results: The obtained results demonstrated that enzymatic treatments are effectively applied at multiple stages of textile processing to enhance fiber quality and functionality. In enzymatic linen degumming, combined pectinase and xylanase treatments increased cellulose content (>50%), while reducing hemicellulose and lignin. This process also improved fiber linear density, from 7.9 dTex to 5.6 dTex. At the finishing step, cellulase-based bio-polishing effectively reduced pilling and surface fibrillation in 100% hemp knitted fabrics, enhancing smoothness. Regarding functionalization, laccase-assisted cationization achieved results comparable to chemical mordanting, with hemp fabrics functionalized with bioactive gallnut extracts showing improved wash fastness, with only 6% of antioxidant activity loss and minimal color change ($\Delta E = 2$), outperforming conventional treatment. In textile recycling, cellulases enabled efficient separation of cotton from polyester and elastane blends, achieving up to 50 % (w/w) recovery of the cellulosic fraction. The recovered cellulose exhibited a degree of polymerization (DP) between 350–600, which is within the acceptable range for reuse as feedstock in new fiber production, including solvent-based regeneration routes such as the lyocell process.

Conclusions: Enzyme-based technologies represent a key step toward greener and more sustainable textile manufacturing. Their use reduces the environmental footprint of processing while aligning industrial practices with global sustainability goals and consumer expectations.

ID597 | Let the microorganisms speak: integrating scientific illustration and impact assessment in microbiology communication

Author: Ana Beatriz Neves

Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal

E-mail: anab.neves@ua.pt

Other authors: Carlos Torres 2, Rui Sousa 3, Yeva Poberezhna 2, Pedro Beça 4, Ana Cristina Esteves 1

2 Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal;

3 Departamento de Comunicação e Arte, Universidade de Aveiro, 3810-193 Aveiro, Portugal;

4 DigiMedia e Departamento de Comunicação e Arte, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Communicating science often fails to correctly define and connect to its public. These challenges are maximized in fields like microbiology, where concepts rely on abstraction, and where media representation often ends up perpetuating negative stereotypes. Comics can be a powerful tool to engage empathy with readers, as a mixed medium with particularities fit for science communication: visual metaphors, a condensed message and a narrative driven by two languages – the visual and the written.

The 'Let the microorganisms speak' project explores the intersection of creativity and rigor, introducing different forms of microorganisms with a young-adult non-specialist audience in mind. At the same time, it addresses a frequently overlooked dimension: impact evaluation.

The first phase of the project involved the conceptualization and development of a scientifically-based strip cartoon where an extraterrestrial microorganism, driven by loneliness, travels to Earth in search of a compatible microbial match via a dating application. Each microorganism introduced is accompanied by a brief and accessible scientific description, rich in metaphors and comparative language.

The second phase of the project focused on pilot-testing tools for evaluating the effectiveness of the initiative among its target public. These tools are expected to be applicable to other science communication initiatives for non-specialist adult publics.

Analysis of the answers of the 37 participants highlighted that, after reading the comic strip, they associated the concept of "microbial diversity" with more complex ideas and showed more comprehension of abstract concepts. In some cases, answers highlighted new empathy for microbial life, and a newfound or rediscovered sense of wonder towards microbiology. Most participants showed interest in reading further into the topic, provided the right tools.

The 'Let the microorganisms speak' project embraces collaboration across student profiles - from science to design - and proves that when biologists and media specialists join forces, the results can be unexpectedly lively. After all, if science depends on imagination, should not science communication and learning do so as well?

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ABSTRACTS

TOPIC #9: Biological Resources and Networks

ORAL COMMUNICATIONS

ID471 | 17 Windows to the Future: UCCCB and the Sustainable Development Goals

Author: Paula Vasconcelos Morais

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal

E-mail: pvmorais@uc.pt

Other authors: Tiago Manuel Henriques, Pedro Farias, Romeu Francisco, Rita Branco, Miguel Batalha, Ana Margarida Mota, and Ana Paula Chung

University of Coimbra, CEMMPRE, ARISE, Department of Life Sciences, 3000-456 Coimbra, Portugal

The UCCCB – University of Coimbra Bacteria Culture Collection – is the first Portuguese collection dedicated to bacteria, recognised by the World Federation of Culture Collections (WDCM 1179) and since 2024 by WIPO as an International Depository Authority under the Budapest Treaty. Beyond preservation, UCCCB integrates research, characterisation, and innovation, aligning its mission with the United Nations Sustainable Development Goals (SDGs). By supporting antimicrobial discovery, diagnostic tools and training programmes, UCCCB contributes to SDG 1 (No Poverty) and SDG 3 (Good Health and Well-being). Work on plant growth-promoting and nematocidal strains in projects such as SIRAM (PRIMA) enhances food security and resilience, addressing SDG 2 (Zero Hunger) and SDG 13 (Climate Action). By conserving microbial diversity worldwide, while safeguarding Portuguese microbial heritage, UCCCB underpins SDG 14 (Life below Water) and SDG 15 (Life on Land). Projects like AM2R (RRP – Recovery and Resilience Plan) and H2OforAll (Horizon Europe) advance SDG 6 (Clean Water and Sanitation) through water treatment and monitoring of disinfection by-products. Initiatives such as DUST+ (COMPETE 2020) and PHAsFilm (INOV C+), dedicated to bacterial polymers and bioplastics, reinforce SDG 7 (Affordable and Clean Energy) and SDG 12 (Responsible Consumption and Production). By engaging in bioremediation of mining and electronic wastes (BIORECOVER, Horizon 2020; REVIVING, ERA-MIN; Agenda Microelectrónica, RRP), UCCCB strengthens SDG 8 (Decent Work and Economic Growth) and SDG 9 (Industry, Innovation and Infrastructure). Training within undergraduate and postgraduate curricula promotes SDG 4 (Quality Education), while fostering inclusion, skills development and equal opportunities, thereby advancing SDG 5 (Gender Equality) and SDG 10 (Reduced Inequalities). Located in a region with below-average R&D investment, UCCCB acts as a hub for collaborations, exemplifying SDG 11 (Sustainable Cities and Communities). During the COVID-19 pandemic, UCCCB supported UV-based disinfection (UltraBot, SII&DT) and mask sanitisation, reinforcing SDG 16 (Peace, Justice and Strong Institutions) through science-driven resilience. Finally, its publicly accessible catalogue and international partnerships embody SDG 17 (Partnerships for the Goals). With its 17 “windows to the future”, UCCCB shows how a culture collection can drive science, innovation, and sustainability towards the 2030 Agenda.

ID697 | Transnational Access Opportunities to Researchers at MIRRI-ERIC: The Microbes-4-Climate Project

Author: Nelson Lima

1Micoteca da Universidade do Minho (MUM), Centre of Biological Engineering, University of Minho, Braga; LABBELS-Associate Laboratory, Braga/Guimarães; MIRRI-PT, Braga, Portugal

E-mail: nelson@ie.uminho.pt

Other authors: Laura Gómez 2,3, Aurora Zuzuarregui 3,4, Ana Mellado 5, Adrián Guerrero 5, Florent Massol 6, Youssef Haidala 7, Michel Boër 7, Heba Ibrahim 8, Roland Pieruschka 8, Rosa Aznar 2,3,4 and the M4C Consortium

2Department of Microbiology and Ecology, Universitat de València - Valencia, Spain

3MIRRI-ES, Valencia, Spain

4Spanish Type Culture Collection (CECT), Universitat de València - Valencia, Spain

5LifeWatch-ERIC, ICT Core, Plaza España SN, SECTOR II-III, 41071 Seville, Spain

6CNRS/CEREEP ECOTRON Ilede, France

7Analysis and Experimentation on Ecosystems - European Infrastructure Consortium

8Institute of Bio-and Geosciences IBG-2, Forschungszentrum Jülich GmbH, D-52425 Juelich, Germany

MIRRI-ERIC is a pan-European research infrastructure (RI) with 39 partners across Europe and over 185K microbial strains and plasmids catalogued, with the mission to facilitate compliant access to microbial resources, helping the bioscience and bioindustry communities thrive.

It is MIRRI-ERIC vision and ambition to become EU's primary hub for microbial resources and data, underpinned by values that include collaboration, sustainability, excellence, and societal impact.

All the services from MIRRI-ERIC are provided by several partners form 8 different EU countries: Portugal, Spain, Italy, France, Belgium, Greece, Romania and Latvia. Each one of these national nodes are in turn composed by several national partners. The Portuguese node in particular is composed by 6 partners providing different services and microbial resources from fungi, yeasts, bacteria and biotropical resources.

MIRRI_ERIC is also deeply involved in several EU funded projects both as partner and coordinator. MICROBES-4-CLIMATE (M4C), is a 5-years INFRA-SERV project funded by Horizon Europe, coordinated by MIRRI-ERIC, that started in February 2024, with a budget of 14.5M€, with a Consortium of 31 partners from all across Europe involving 5 RIs.

The main goal of M4C is to deepen the comprehension of the complex relationships among microbes, plants, soil and atmosphere within a dynamic environment in times of climate change, by offering access to advanced services of RIs through the Transnational Access (TNA) programme. TNA provides short-term access to cutting-edge laboratories, state-of-the-art equipment, and unique datasets across borders — onsite or remotely — empowering researchers to innovate and collaborate globally.

In this presentation a general overview of MIRRI-ERIC is going to be shown, and you will discover how TNA allows researchers to access cutting-edge scientific infrastructures, facilities and resources at leading European RIs.

The TNA catalogue will be presented offering 144 services covering areas such as biological resource supply and preservation, microbial isolation and characterization, access to microbial

discovery and phenotyping facilities, soil and plant analysis techniques, big data and machine learning tools, as well as remote consultancy. Results of the 1st TNA call will be presented and you will also receive step-by-step instructions on how to apply for the next TNA opportunities, including tips on eligibility and the application process.

Acknowledgements

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POSTERS

ID213 | CIIMAR Blue Biobank: A Repository of Marine Biological Resources with Biotechnological Potential

Author: Diogo A. M. Alexandrino

1CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto (Portugal); Department of Environmental Health, School of Health, P. Porto (Portugal)

E-mail: dalexandrino@ciimar.up.pt

Other authors: Ana P. Mucha 1,3, Rodrigo Ozório 1, Francisco Arenas 1, Joana R. Xavier 1, Maria de Fátima Carvalho 1,4, Iris Sampaio1, Vitor Vasconcelos 1,3

3 Department of Biology, Faculty of Sciences, University of Porto, Portugal;

4 ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Portugal

The Blue Hub Leixões (https://hubazulleixoes.com/), a node of the Portuguese Blue Hub network, is a consortium composed by R&D institutions (CIIMAR, INESC-TEC and INEGI), a local governmental entity (Matosinhos City Hall), a port authority (APDL) and a Blue Economy Cluster NGO (Fórum Oceano). This consortium is working to foster the regional development of the Blue Engineering and Biotechnology sectors in northern Portugal. In this context, CIIMAR is the strategic partner of the consortium responsible for the dynamization of the Blue Biotechnology vector and aims to achieve so through the establishment of the CIIMAR Blue Biobank (CBB).

CBB will be a centralized repository of marine biological resources that are part of CIIMAR's main culture collections: LEGE-CC (cyanobacteria and microalgae), CM2C (bacteria and fungi) and DEEP-Biobank (deep-sea marine invertebrates). This centralization effort will confer CBB a privileged positioning in the Blue Biotechnology ecosystem, as it will streamline the curation, biobanking, digitization and access to over 1500 microbial strains with a well-mapped biotechnological potential, as well as to hundreds of deep-sea corals and sponges which can also be made available for further biotechnological bioprospecting and valorization.

The core R&D strategy of CBB will rely on three key axes: (1) pilot scale cultivation of autotrophic and heterotrophic microorganisms; (2) development and optimization of bioprocesses for biodiscovery, biorefinery and bioremediation applications; (3) valorization of biomaterials sourced from deep-sea invertebrates for tissue engineering and biomimetic applications; and (4) a marine experimentation system, at the mesocosm scale, in close coordination with a scientific diving center. Thus, CBB will be outfitted with the necessary capabilities to provide R&D services that cover various technology readiness levels (TRL) across the Blue Biotechnology value chain, spanning from bioprospecting (TRL 1) to pilot scale cultivation and mesocosm experimentation (TRL 4). These capabilities will significantly narrow the academia-industry gap and enhance the functionalization of the large selection of marine biological resources that CIIMAR is currently curating.

ID253 | Exploring Marine Microbial Resources for Sustainable Biotechnological Applications: A Multi-Functional Screening

Author: Ana Rita Bragança

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

3 Aquatic Research Network (ARNET) Associate Laboratory

E-mail: anaritabrag@hotmail.com

Other authors: Mariana Rodrigues 1,2,3, Tiago Cunha 1,2,3, João Gomes 1,2,3, Ana Rita Silva 1,2,3 Tony Collins 1,2,3, Margarida Casal 1,2,3, Isabel Soares-Silva 1,2,3, Raul Machado 1,2,3

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

3 Aquatic Research Network (ARNET) Associate Laboratory

The ocean constitutes the largest ecosystem on Earth, yet it remains one of the least explored. Subjected to extreme environmental conditions — temperature, salinity, and pressure — marine habitats are further impacted by climate change and anthropogenic stressors, such as oil contamination and plastic waste pollution. These combined pressures have driven marine microorganisms to develop physiological plasticity and functional diversity, making them valuable sources for the discovery of novel bioactive compounds and metabolic functions.

Our group established a biobank of marine microorganisms, which was investigated for the production of high-value bioproducts and bioprocesses with strong biotechnological potential, including enzymes, biosurfactants, and bioremediation strategies.

Enzymatic screenings using organic and polymeric substrates identified several isolates as producers of hydrolytic enzymes such as esterases, proteases, pectinases, xylanases and cellulases, indicating strong potential for industrial and environmental applications. Of the 84 bacteria and 13 yeasts tested, only 6 bacteria did not demonstrate any enzymatic activity. Among the tested bacteria, 14 species demonstrated the ability to degrade at least 5 of the 9 substrates used. Notably, all clear-halo assays were conducted under conditions supplemented with 15 g/L of Sea Salts and an incubation temperature of 15 °C. These results highlight the capacity of marine microorganisms to produce active enzymes under high-salinity and low-temperature conditions — characteristics of significant interest for industrial applications that require robust biocatalysts in extreme environments. The hydrocarbon degradation potential was also assessed using artificial seawater supplemented with hexadecane as the sole carbon source. Growth assays at 20 ºC revealed that 27 isolates from distinct species (25 bacteria and 2 yeasts) hold significant hydrocarbon-degrading capabilities. In addition, 21 species were found to produce biosurfactants, as demonstrated by surface tension and emulsification analyses, indicating their potential applicability in the decontamination of oil-polluted environments.

This study highlights the untapped potential of marine microorganisms as sources of bioactive compounds and metabolic capabilities. Their multifunctionality supports their integration into sustainable industrial and environmental technologies.

ID296 | Uncovering the potential of Bacillus amyloliquefaciens 14C2 against plant-parasitic nematodes

Author: Cláudia Vicente

MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, University of Évora, Portugal

E-mail: cvicente@uevora.pt

Other authors: João Gordo1, Pedro Barbosa 2, Margarida Espada 2, Cláudia Vicente 2

MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE Global Change and Sustainability Institute, University of Évora, Portugal

Plant-parasitic nematodes (PPN) are major constraints to agriculture worldwide, affecting nearly all cultivated crops. The sustainable control of these PPN is currently focused on biological strategies with low impact on the environmental and human health, such as biological control. The genus Bacillus is one of the most studied bacterial genera expressing antagonistic potential against several important PPN. Their nematicidal effect is usually linked to the production of several toxic molecules (e.g., shingosine produced by B. cereus S2 or secreted proteases of endophytic B. cereus BCM2, both against the PPN Meloidogyne incognita; or other proteases of B. cereus i.24 and Lysobacter capsica i.17 against the root-lesion nematode Pratylenchus penetrans). Previously, we have evaluated the nematicidal potential of endophytic and soil-inhabiting PGPR from genus Bacillus against P. penetrans. In this work, we analysed the complete genome sequence of B. amyloliquefaciens (= B. velezensis) 14C2 in search of potential proteases to be explored as natural nematicides. The genome size of B. amyloliquefaciens 14C2 is 3,528,213 bp with an average of GC content of 46.5% and consists of one chromosome of 3.9 Mbp encoding for 3935 protein-coding and 131 RNA genes. Several serine proteases (C2_GM001392, C2_GM001840, C2_GM001358, C2_GM003264 and C2_GM004060), known to be related with nematicidal effect in other bacteria, have been identified and validated. Moreover, 247 proteins involved in biosynthesis of secondary metabolites pathways, also a source of nematoxic molecules, were identified. The B. amyloliquefaciens 14C2 genome has shown to be a potential source for the development of new biotechnological nematicides.

ID385 | The IST-Yeasts Culture Collection: a microbiological resource of Blue Yeasts with biotechnological potential

Author: Mónica A. Fernandes

iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal; Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

E-mail: monica.a.fernandes@tecnico.ulisboa.pt

Other authors: Madalena Matos 1,2 and Isabel Sá-Correia1,2,3

1 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 Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

The IST-Yeasts Culture Collection (IST-Yeasts CC) is a microbiological resource located in the Biological Sciences Research Group (BSRG) of the Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico (IST) University of Lisbon. The IST-Yeasts CC is a recent collection of nonconventional Blue Yeasts, started during 2023, in the framework of the “Algae Vertical” and the “Portuguese Blue BioBank” work-programs of the PBA (Pacto de bioeconomia Azul) Project. It is a Culture Collection with a focus on yeasts isolated, following an isolation protocol developed to detect this low-abundance population, mostly in association with industrial cultures of microalgae and macroalgae in estuarine and marine environments of Olhão and Aveiro region. Most of them are from Necton S.A. and ALGAplus. The yeast isolates were molecularly identified, and the IST-Yeasts CC comprises 115 yeast strains, most of them belonging to the phylum Basidiomycota (92%) and to the genus Rhodotorula (R. mucilaginosa, R. diobovata, R. sphaerocarpa, R. taiwanensis) (69%). Other Basidiomycota species present are Cystobasidium minutum and C. slooffiae, Vishniacozyma carnescens, Moesziomyces aphidis, Sporobolomyces roseus, S. salmonicolor, and Naganishia diffluens. The Ascomycota species present are Meyerozyma guilliermondii, [Candida] atlantica and [Candida] vartiovaarae. Most of the yeast strains gathered in the IST-Yeasts CC have potential for the (co)production of added-value bioproducts in the context of a Circular Biobased Economy. There are strains capable of using a very wide range of carbon sources and of co-producing biotechnologically relevant lipids and biosurfactants/bioemulsifiers. The red yeasts of the Rhodotorula genus also produce economically relevant carotenoid pigments, particularly torulene and torularhodin. The capacity of many isolates to co-produce those promising compounds, as well as to produce enzymes and exopolysaccharides, among other relevant bioproducts, based on cultivation on a wide range of carbon sources, makes them very attractive as microbial cell factories in the scope of a sustainable circular bioeconomy. Some of these yeast isolates are also capable of significant auxin production, their use being promising for enhancing algal growth and metabolite production and as probiotics in aquaculture.

In the framework of the Blue Bioeconomy Pact Project (Blue Portuguese Biobank).

ID426 | Biobanking for Antimicrobial Resistance Surveillance: The Strategic Role of the BIOTROP Infrastructure (IHMT NOVA)

Author: Ana Paula Arez

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

E-mail: APArez@ihmt.unl.pt

Other authors: Jorge Ramos 1, Alexandra Santos 1, Isabel Couto 1, Liliana Rodrigues 1, Sofia Santos Costa 1, Sofia Gonçalves Seabra 1, Miguel Viveiros 1,

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Antimicrobial resistance (AMR) is currently a significant challenge to global public health. It requires a more comprehensive response, not only in terms of diagnostics and surveillance, but also ensuring long-term quality access to microbial resources. Biobanks have emerged as key scientific infrastructures for this purpose, offering standardized, traceable, and ethically governed collections.

This overview aims to highlight the strategic role of BIOTROP Biobank (Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa - IHMT NOVA) as a facilitator of biomedical research and AMR surveillance through reliable, compliant, and regulated access to microbial resources relevant to infectious disease control.

BIOTROP curates and maintains collections of biological specimens, such as multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis, Mycobacterium avium, Staphylococcus aureus, Neisseria gonorrhoeae and environmental specimens with relevance to AMR monitoring, among others. These resources have been employed in phenotypic and genotypic resistance analyses, diagnostics optimization and epidemiological research. Specimens are collected, processed and stored following validated procedures and harmonized SOPs, according to international standards (ISO 20387: 2018) and aligned with ISBER and OECD best practices guidelines. Genetic, phenotypic, and epidemiological metadata are integrated into a digital Laboratory Information Management System (LIMS) for specimen traceability and quality assurance, following the principles of FAIR data and complying with ethical and legal requirements (GDPR, Nagoya Protocol). BIOTROP is part of the Biobanco.pt Consortium and MIRRI-ERIC, which enhances national and international interoperability and resource sharing, respectively. BIOTROP also fosters collaboration and capacity building in Lusophone Africa and Brazil through the development of the Lusophone Network of Biobanks.

To conclude, BIOTROP showcases the potential value that microbial biobanks hold to improve readiness against AMR threats through strategically curated collections aligned with international standards. As an infrastructure for public health and translational science, BIOTROP actively fortifies its pivotal position in the One Health scientific ecosystem by providing a scalable model for governance efficacy in AMR surveillance, innovation in diagnostics, and collaborative initiatives in global public health.

ID436 | Unravelling the microbial diversity of CM2C (CIIMAR microbial culture collection) for the development of biotechnology

Author: Joana P. Fernandes

1 CIIMAR/CIIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal; 2 FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

E-mail: jfernandes@ciimar.up.pt

Other authors: Inês Ribeiro1, Ana Matos1, Olga Lage1,2, Vítor Vasconcelos1,2, Maria F. Carvalho1,3, Ana P. Mucha1,2

1CIIMAR/CIIMAR LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal;

2 FCUP – Faculty of Sciences, University of Porto, Porto, Portugal;

3 ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

CIIMAR Microbial Culture Collection (CM2C) is a georeferenced biobank hosted at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR). CM2C collection comprises non-photosynthetic aquatic microorganisms (bacteria and fungi), isolated from several environmental sources (e.g., seawater, sediment, salterns, macroalgae, sponges), collected in the Atlantic coast, deep-sea and inland waters from Portugal (including Azores and Madeira). CM2C microbial strains have been explored for their potential to produce new bioactive molecules with medical or industrial application. In fact, several CM2C strains belonging to the phyla Actinomycetota and Planctomycetota exhibited capacity to produce bioactive molecules with antimicrobial, anti-inflammatory, anticancer, antifungal, anti-obesity and pro-osteogenic activity [1-3]. Moreover, CM2C microbial strains have also been explored for the development of bioremediation tools for the degradation of different pollutants. CM2C strains from the Pseudomonadota, Actinomycetota and Bacteroidota phyla have been associated with the degradation of pesticides, pharmaceuticals and hydrocarbons [4-6]. CM2C collection is registered in the World Federation for Culture Collections (WFCC), in the World Data Centre for Microorganisms (WDCM), and is a member of the European Culture Collections' Organisation (ECCO), with the guaranty of full traceability of the samples, in compliance with the Access and Benefit Sharing regulation associated with the Nagoya Protocol.

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ID505 | A biological collection being established at the University of Cabo Verde: A Lusophone node e to strengthen research capacity in Cabo Verde

Author: Alexandra dos Santos

Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LAnREAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349n008 Lisbon, Portugal.

E-mail: a21002297@ihmt.unl.pt

Other authors: Sofia Santos Costa1, Walter Gomes Vieira2, Sofia Seabra1, Kleidi dos Santos Monteiro2, Jorge Ramos1 , Maria do Rosário Oliveira Martins1, Maria do Céu Teixeira2, Isabel Inês Araújo1,2,3, Ana Paula Arez1

1 Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LAnREAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349n008 Lisbon, Portugal. 2 Faculdade de Ciências e Tecnologia, Universidade de Cabo Verde, Praia, Cabo Verde 3 oNe hEalth reSearch cenTer – Cabo Verde, Universidade de Cabo Verde, Praia, Cabo Verde

Biobanks and Biological Resource Centres are critical infrastructures for biomedical, biotechnological and environmental research, yet they remain unevenly distributed worldwide. Portuguese-speaking African countries face gaps in infrastructure, regulation, governance and specialized human resources to preserve and share biological specimens. To address this asymmetry, a Lusophone Network of Biobanks and Biological Collections is being structured to promote capacity building, standardization and equitable benefit-sharing across Lusophone institutions.

Within this framework, a biological collection is being established at the University of Cabo Verde (Uni-CV) with blood samples and nasal swabs collected within the scope of RESPIRA-CV, assessing the prevalence of asthma and atopic diseases in children of the island of Santiago, in Cabo Verde. This collection will serve as Cabo Verde's reference initiative for the curation of human, microbial and environmental samples. The initiative follows international biobanking standards and ethical best practices, while considering the specific challenges and opportunities of biobanking in low resource settings. Despite the existence of the National Data Protection Commission and the National Ethics Committee for Health Research, Cabo Verde still lacks specific regulations to govern biobanks and ensure fair use and benefit-sharing. By offering harmonized SOPs, interoperable information systems and transparent access policies, this initiative will enable responsible sharing of specimens and metadata, facilitating multi-centre, trans-biobank studies.

The initiative aims to catalyse national partnerships with public health institutes, hospitals, universities and research centres—turning dispersed collections into a coherent, governable and sustainable national asset. For Cabo Verde, expected benefits include faster outbreak investigation, context-specific diagnostic and vaccine research and strengthened research sovereignty. For the wider Lusophone space, integration into a network will enhance data management, standardize operations, expand training opportunities and foster South–South–Europe collaboration.

By positioning Cabo Verde within a Lusophone infrastructure, the biological collection at Uni-CV will help convert biological resources into shared scientific value, reinforcing preparedness, innovation and equitable participation of Portuguese-speaking countries in global health research.

ID515 | VIASEF – In Vivo Arthropod Security Facility

Author: Luis Filipe Lopes

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal. cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal.

E-mail: filipe.lopes@ihmt.unl.pt

Other authors: Carla Sousa

Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal.

Background: VIASEF – In Vivo Arthropod Security Facility – is hosted at the Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa, and is part of the National Research Infrastructure Roadmap. It is a high-security facility adapted for rearing and maintaining invasive arthropod species, transgenic strains, and specimens infected with human pathogens. VIASEF also provides safe laboratory conditions for projects involving human pathogens (vector-borne and others) classified as biosafety level 3 (e.g. Mycobacterium tuberculosis strains). Access is open to academic, scientific, and industrial communities, fostering scientific excellence and technological innovation nationally and internationally.

Methods: The arthropod containment level 3 facility includes 5 insectaries with security levels 2 and 3, an ACL-3 unit to harbour arthropods infected with human pathogens, a BSL-3 laboratory, and a bioassay laboratory. VIASEF offers access to specific model organisms (e.g. live/dead mosquitoes) and biological material (e.g. dissected organs, sporogonic stages of parasites), as well as bioassays with autochthonous, exotic, and invasive arthropods, and the establishment of extrinsic development cycles of human vector-borne pathogens. Insectaries and laboratories are also available for rental, with or without technical support. Results: VIASEF has supported MSc and PhD projects, promoting researcher mobility and exchange programmes, including training within the Ciência-LP programme. It has established strong partnerships with industry by providing SMEs – often lacking autonomous research capacity – with infrastructure, equipment, know-how, and standardized experimental procedures for product testing and development. VIASEF has supported innovation in insecticides, repellents, and personal protection tools. Current research focuses on invasive arthropod bionomics, host–pathogen interactions, and vector competence. The facility has also contributed to work on non-vector pathogens, including national efforts against the SARS-CoV-2 pandemic.

Conclusions: VIASEF has strengthened research capacity in Portuguese public and private institutions, enabling projects requiring in vivo testing of vector-borne pathogens, previously unfeasible due to the absence of suitable infrastructure.

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ID670 | Biotechnology for Healthier and More Resilient Azorean Grapevines

Author: Marina Domingos-Lopes

CBA-Biotechnology Centre of Azores, University of the Azores, Angra do Heroísmo, Azores, Portugal

E-mail: marina.fp.lopes@uac.pt

Other authors: Maria Susana Lopes1; Artur da Câmara Machado1 and Duarte Mendonça1

CBA-Biotechnology Centre of Azores, University of the Azores, Angra do Heroísmo, Azores, Portugal

Background:Grapevine (Vitis vinifera) is a high-value crop of major interest and economic value worldwide, and holds significant historical, cultural, and economic importance in the Azores Archipelago, where traditional viticulture, particularly on the island like Pico, where UNESCO-listed vineyards are a symbol of traditional viticulture.

However, the productivity and longevity of local grapevine varieties are significantly affected due to persistent viral infections, including Grapevine leafroll-associated viruses (GLRaVs), Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), among others, that compromise potential yield, fruit quality, vine vigor and lifespan, leading to consequent economic losses.

These challenges are further intensified by the growing impacts of climate change, including shifts in temperature and humidity that can exacerbate virus transmission and symptom expression, while increasing vine sensitivity to stress.

Methods:These viruses are often latent and systemically infect the plant, making conventional control methods ineffective. To overcome these constrains, biotechnological approaches are being used to improve the health and resilience of three traditional Azorean grapevine cultivars – Verdelho, Arinto dos Açores, and Terrantêz do Pico – and rootstocks, well-suited to the regional conditions. Several clones of these cultivars and rootstocks were genetically characterized, established in vitro, surveyed for viruses, and subjected to virus eradication through in vitro thermotherapy and meristem culture.

Results:The application of thermotherapy (37 °C for 8 weeks), combined with meristem culture, has shown high success rates in obtaining virus-free grapevine plants, as confirmed by RT-PCR using virus-specific primers. We present results on the diversity of the viruses present in Azorean grapevine cultivars, highlighting viruses elimination rates and regeneration success achieved in the sanitation trials.

Conclusions:The implementation of virus sanitation contributes to the preservation of Azorean grapevine germplasm by ensuring the production of virus-free planting material, which is essential for the sustainability and resilience of viticulture in island ecosystems. This is particularly relevant in the context of climate change, which exacerbates pathogen spread and increases plant sensitivity to biotic and abiotic stress. Moreover, virus sanitation supports certified plant production in alignment with EU phytosanitary standards

ID685 | From Repository to Application: The Microbial Collection of the Centre for Biotechnology of the Azores

Author: Nelson Simões

Centro de Biotecnologia dos Açores (CBA) & Faculdade de Ciências e Tecnologia, Universidade dos Açores

E-mail: nelson.jo.simoes@uac.pt

Other authors: Carla Cabral and Duarte Toubarro

Centro de Biotecnologia dos Açores (CBA) & Faculdade de Ciências e Tecnologia, Universidade dos Açores

To explore and valorize the unique genetic resources of the Azores, we established a comprehensive repository of microorganisms isolated from diverse habitats, including soils, volcanic environments, and marine ecosystems. The collection currently comprises more than 3,000 bacterial isolates from soil, around

250 symbionts from marine organisms, and several insect pathogens. Complementary, genetic and chemical libraries have also been developed to expand the biotechnological potential of these resources. This repository is already contributing to research, education, and innovation, fostering academic networks and collaborations with industry. In this presentation, we will highlight both the achievements and limitations encountered in applying this collection to diverse fields and discuss its role as a platform for future discovery and sustainable bioprospecting.

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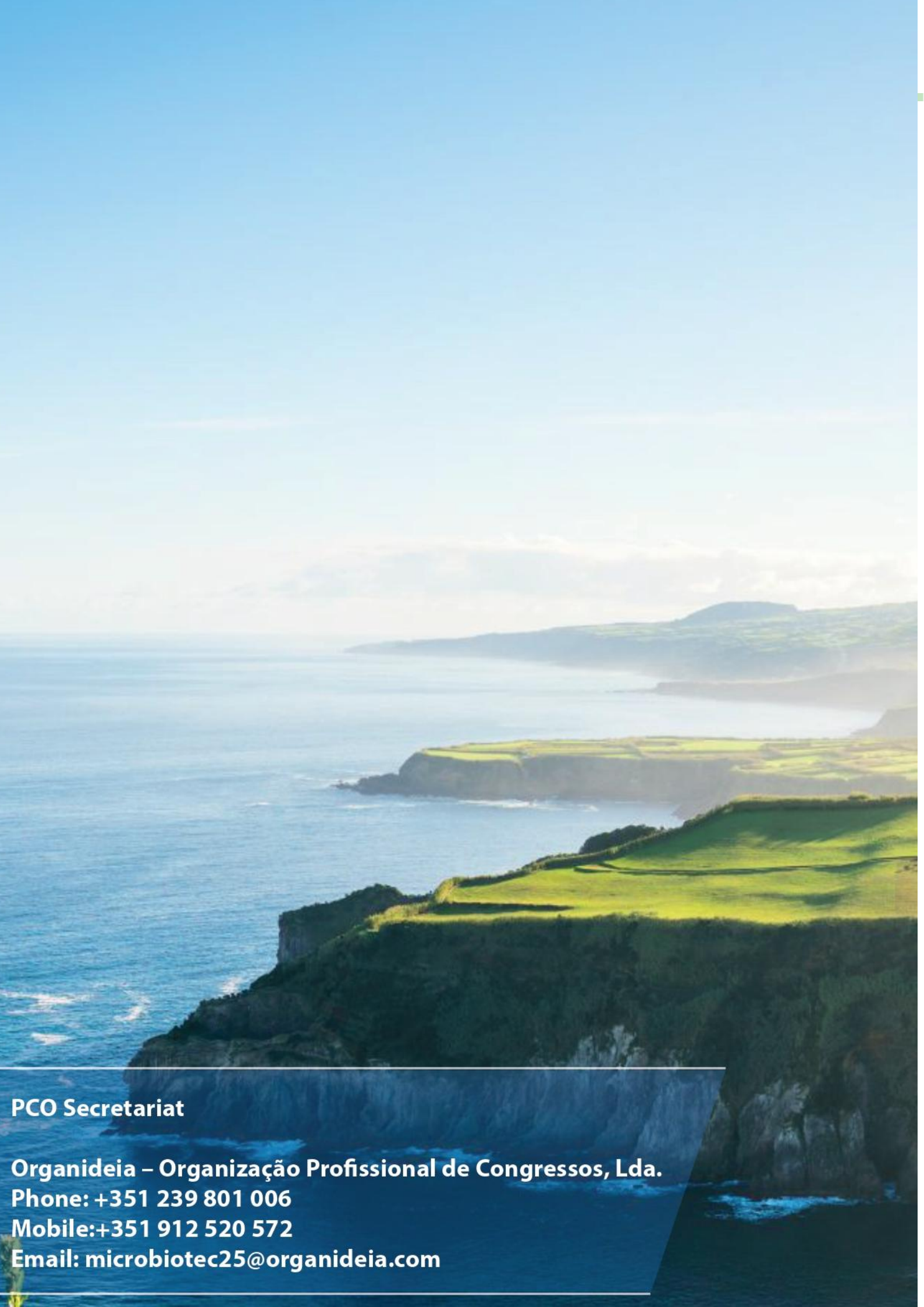

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