# XXIII Jornadas de Biologia de Leveduras

## Professor Nicolau van Uden

# ABSTRACTS BOOK

9–10 OCTOBER 2026

HOTEL TERMAS DA CURIA · ANADIA



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# WELCOME MESSAGE

Dear Colleagues,

The Organising Committee of the **XXIII Jornadas de Biologia de Leveduras Professor Nicolau van Uden**, are excited to bring together the fungal research community.

In this edition, we are broadening the meeting's scope to include our colleagues working on fungi other than yeasts.

We intend to create new synergies, exchange ideas, and share the latest knowledge across the following topics:

- Genomics and Evolution
- Biotechnology and Synthetic Biology
- Health
- Fermented Foods and Beverages
- Drug and Stress Resistance
- Adaptation, Cell Death and Ageing

We hope these two days will foster fruitful discussions, collaborations, and innovations that will enrich our community and inspire fungal research among early scientists and students.

*The Organizing Committee*

# COMMITTEES

# Honour Committee



| Amândio Madeira Lopes | Cândida Lucas | Cecília Leão | Célia Pais | Helena Cardoso |
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| Isabel Sá-Correia | José Martinez Peinado | Manuela Côrte-Real | Maria Conceição Loureiro Dias | Virgílio Loureiro |
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| IST, ULisboa | UCM | UMinho | ULisboa | ULisboa |

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Isabel Soares-Silva



Sandra Paiva



Paula Ludovico

XXIII Jornadas de Biologia de Leveduras
Professor Nicolau van Uden

# PROGRAM

# Friday, 9th October

09:00 **Registration**

10:00 **Welcome coffee**

10:30 **Welcome Session Professor Nicolau van Uden**
**Isabel Miranda** - On behalf of the Organizing Committee
**Isabel Sá-Correia**, iBB, i4HB, IST | Yeast Diversity for Circular Bioeconomy and Environmental Sustainability

11:10 **Plenary lecture**
Chair: Nuno Mira
**Kyle Cunningham**, Johns Hopkins University, Baltimore, Maryland, USA | Rethinking yeast genetics in the post-genome era, again

11:50 **Session I | Biotechnology and Synthetic Biology**
Chairs: Isabel João Silva | Nuno Mira

11:50 **Keynote Lecture**
**Cristina Pereira**, ITQB NOVA, UNL | Endohyphal Bacterial Symbionts in *Aspergillus fumigatus*: Expanding the Biology of a Pathogen

12:15 **Oral Communications**

12:15 **Diogo Couceiro** | A New Pipeline for Meta Transcriptomic Data Integration in the Metabolic Model Yeast 9.0.2 for *Saccharomyces cerevisiae*: Ethanol Production Optimization as a Case-Study

12:25 **Francisca Ferreira** | Mechanistic Insights into Citrate Export by the CexA Transporter from *Aspergillus niger*

12:35 **Andreia Pedras** | Coupling Computational Design with Yeast Display: a High-Throughput Pipeline for Antiviral Discovery

12:45 **Lunch**

14:00 **MIA Portugal Lecture**
Chair: Paula Ludovico
**Jeffrey Smith**, Univ. of Virginia School of Medicine, USA | Anti-aging properties of ketone bodies in budding yeast

14:40 **Session II | Adaptation, Cell Death and Ageing**
Chairs: Vítor Teixeira | Susana Chaves

14:40 **Keynote Lecture**
**Paula Ludovico**, UM | Living, Dying and Ageing in Yeast

15:05 **Oral Communications**

15:05 **Miguel Correia** | Yeast Profilin(g): New Insights into the Yeast Cytoskeleton-Mitochondria Crosstalk

15:15 **João Cardoso** | Bioactive Metabolites Drive Lifespan Extension in a Yeast Model of Niemann-Pick type C1

15:25 **Session III | Health**
Chairs: Teresa Gonçalves | Paula Sampaio

15:25 **Keynote Lecture**
**Agostinho Carvalho**, UM | Host Endotypes in Aspergillosis: Bridging Immune Mechanisms to Actionable Biomarkers and Therapies

15:50 **Oral Communications**

15:50 **Carolina Mariano** | Iron Compromises Fluconazole Activity in *Candida glabrata*

16:00 **Daniela Calheiros** | Characterization and Immunomodulatory Effect of *Alternaria alternata* Extracellular Vesicles in Host-Pathogen Interactions

16:10 **Joana Costa** | Chitosan-Coated Alginate Capsules for Delivery of 2'-O-MethylRNA EFG1 Oligomers Targeting *Candida albicans* Filamentation

16:20 **Lisa Rodrigues** | Obesity-Associated Gut *Candida* and *Penicillium* Dysbiosis is Linked to Purinergic Receptor Polymorphisms

16:30 **Mariana Fernandes** | MycoSifter - A Consensus-Driven, Multi-Threshold Pipeline for Species-Level Environmental Contamination Removal in Clinical Mycobiome Studies

16:40 **Isabel Oliveira** | Proteomic and Ultrastructural Dissection of N88S Seipin Inclusion Bodies Reveals Conserved Chaperone Defenses Against Motor Neuron Diseases

16:50 **Francielly Dias Pereira** | Production and Evaluation of a Novel Yeast-Derived Recombinant Iduronate-2-Sulfatase as a Potential Therapeutic Alternative for Hunter Syndrome

17:00 **Coffee break + Poster session**

18:20 **Conference "Jovem Investigador" | Tribute to Professor Isabel Spencer Martins**

**Sponsored by Sociedade Portuguesa de Microbiologia**

Chair: Isabel Miranda

**Andreia Pedras** | Bound to Iron: How Caspofungin Loses Antifungal Activity and Finds Its Way into the Cell

20:00 **Dinner**

# Saturday, 10th October

08:30 **Registration**

09:00 **Plenary lecture**

Chair: Sofia Costa de Oliveira

**Geraldine Butler**, University College Dublin, Ireland | Evolutionary Perspectives from Fungal Genomes

09:40 **Session IV | Genomics and Evolution**

Chairs: Paula Gonçalves | Artur Alves

09:40 **Keynote Lecture**

**Carla Gonçalves**, FCT Nova | Lose, Gain, Thrive: Genome Remodeling as a Driver of Adaptation in a Floral Yeast Clade

10:05 **Oral Communications**

10:05 **Luís Coutinho** | YEASTRACT+ Upgrades to a Quantitative Regulatory Resource: Transcriptome Integration for Genome-Scale Metabolic Modeling in Yeasts

10:15 **Mélissa José** | Characterization of a Novel Aphelid, a Fungal-Related Parasitoid, Infecting Industrial *Nannochloropsis* Cultures

10:25 **Coffee break + Poster session**

11:45 **Session V | Fermented Foods and Beverages**

Chairs: Maria João Sousa | Catarina Prista

11:45 **Keynote Lecture**
**Alexandra Mendes Ferreira**, UTAD | Wine Yeasts: Addressing Old and New Winemaking Challenges

12:10 **Oral Communications**

12:10 **Bruna Oliveira** | Exploring Glucose Transport to Improve the Fermentative Performance of *Torulaspora delbrueckii*

12:20 **Carolina Osório** | Genomic and Phenotypic Screening of Non-Saccharomyces Yeasts for Climate-Resilient Winemaking

12:30 **Diogo Santos** | Genomic and Transcriptomic Profiling of *Hanseniaspora guilliermondii*'s Ethanol Stress Response

12:40 **Lunch**

14:00 **Plenary lecture**
Chair: Teresa Gonçalves
**Márcio Rodrigues**, UFRJ, Brazil | Exploring the Extracellular Biology of Yeast-Like Pathogens

14:40 **Session VI | Drug and stress resistance**
Chairs: Catarina Pimentel | Miguel Teixeira

14:40 **Keynote Lecture**
**Ana Rita Bezerra**, UA | From Genetic Variation to Translational Regulation: Emerging Determinants of Antifungal and Stress Resistance in *Candida albicans*

15:05 **Oral Communications**

15:05 **Bárbara Coelho** | OMICS Analyses to Disclose Tolerance Mechanisms to the Antimicrobial SO2 in the Wine Spoilage Yeast *Saccharomycodes ludwigii*

15:15 **Débora Marques** | Drug Repurposing as a Strategy for Antifungal Adjuvant Development and Efflux Modulation in *Candida albicans*

15:25 **Inês Correia** | From CUG Decoding Ambiguity to Proteome-Wide Mistranslation in Clinical Isolates of *Candida albicans*

15:35 **Maria Inês Pacheco** | Exploring the Antifungal Potential of Novel 2-Aminoimidazole Azo Dyes Against *Candida krusei* and *Candida auris*

15:45 **Nuno Reisinho** | From Urban Water to Clinical Settings: Hidden Reservoirs of Opportunistic Antifungal-Resistant Yeasts

15:55 **Patrícia Serra** | Overcoming Fluconazole Resistance in *Candida glabrata* via PDR1-Targeted Antisense Oligomers

16:05 **Rute Chitas** | Exploring the Potential of Molecular Glues for the Development of Novel Antifungals Against *Candida albicans*

16:15 **Teresa Pissarro** | Pentabromopseudilin: a Marine Bacteria-Derived Compound with Potent Antifungal Activity Against Pathogenic Fungi

16:25 **Closing Session/ Farewell Coffee**

# WELCOME SESSION

## Yeast diversity for circular bioeconomy and environmental sustainability

Isabel Sá-Correia

iBB-Institute for Bioengineering and Biosciences and Associate Laboratory i4HB-Institute for Health and Bioeconomy, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

The transition towards a circular bioeconomy requires robust microbial platforms capable of converting renewable feedstocks and agro-industrial residues into valuable products. Owing to their metabolic versatility, physiological diversity, and adaptability to industrial environments, yeasts are particularly attractive cell factories. Although *Saccharomyces cerevisiae* remains the dominant industrial and model yeast system, growing attention is being devoted to yeast biodiversity as a source of naturally robust strains with unique catabolic and biosynthetic capabilities.

This talk will highlight the exploitation of non-conventional yeasts for the valorisation of lignocellulosic residues, addressing challenges posed by inhibitory compounds, including acetic and formic acids, furfural, and 5-hydroxymethylfurfural, as well as other industrial stresses such as high temperature. The oleaginous yeast *Rhodotorula toruloides* will be presented as a promising platform for the production of microbial oils for sustainable aviation fuels (SAF) and carotenoids from lignocellulosic hydrolysates. Recent insights into the molecular basis of multi-stress tolerance, obtained through adaptive laboratory evolution and genome-wide analyses, will be discussed, with particular emphasis on the role of the cell wall in yeast robustness.

The IST Yeasts Culture Collection (https://blueyeastscc.tecnico.ulisboa.pt/) preserving yeasts isolated from large-scale marine micro- and macroalgae cultivation systems, will also be presented. These strains are valuable producers of lipids, biosurfactants, pigments, and auxins, illustrating how yeast biodiversity can expand the toolbox for sustainable bioprocesses and environmental sustainability.

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# PLENARY LECTURES

## Rethinking yeast genetics in the post-genome era, again

Kyle Cunningham

Department of Biology, Johns Hopkins University, Baltimore, Maryland, USA

Without a doubt, the last 50 years of intensive research on model yeasts has yielded profound insights into a vast array of biological processes that are conserved in all eukaryotes, including humans. Many amazing "omics" technologies that have been developed in these model yeasts. Even so, functional genomics has proven to be the most enlightening because it directly links phenotypes and behaviors to individual genes. Research in non-model organisms has been hindered by lack of genomic tools. However, transposon mutagenesis coupled with deep sequencing (Tn-seq) has emerged as a facile and powerful new tool for generating barcoded gene knockout collections in non-model yeasts that can be easily screened for phenotypes of interest. My team has successfully implemented Tn-seq in the opportunistic pathogen *Candida glabrata* to investigate aspects of its biology that cannot be studied reliably in model yeasts, such as virulence and pathogenesis mechanisms. While investigating its mechanisms of resistance to antifungals, we have already overturned dogmas and invalidated widely held assumptions in the field. Some of the findings are immediately actionable in the treatment of fungal diseases. These early successes in *C. glabrata* highlight the vastness of the new opportunities that are now available to you by leveraging Tn-seq in combination with prior knowledge. A new "golden era" of yeast genetics with broader representation of clinical and industrial strains/species is just beginning.

## Anti-aging properties of ketone bodies in budding yeast

Swarup Mishra[1,4], Elisa Enriquez-Hesles[1], Connor Oakes[1], Christopher T. Letai[1], James Bain[5], Eyleen J. O'Rourke[2,3,4], and Jeffrey S. Smith[1]

[1]Department of Biochemistry and Molecular Genetics, [2]Department of Cell Biology, [3]Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine; [4]Department of Biology, College of Arts and Sciences, University of Virginia, Charlottesville, VA, USA; [5]Department of Medicine, Duke Molecular Physiology Institute, Duke University, Durham, North Caroline, USA

Caloric restriction (CR) extends lifespan and healthspan for most model organisms tested, including the budding yeast, *Saccharomyces cerevisiae*. Underlying conserved mechanisms remain under investigation. The CR regimen for yeast is typically glucose restriction, which robustly extends both the replicative lifespan (RLS) and chronological lifespan (CLS), which is defined as the number of days that cells remain viable after entering stationary phase in liquid cultures. To gain new insights about the mechanisms underlying CR in the yeast system, we performed untargeted metabolomics to identify CR-induced metabolites that contribute to enhanced quiescence and longevity during chronological aging. In stationary phase cells, CR significantly induced metabolites associated with flux through the polyamine biosynthesis and the methionine salvage pathway, including methylthioadenosine (MTA), adenine, and spermidine, consistent with the conserved lifespan extension induced by spermidine supplementation. The ketone body b-hydroxybutyrate (BHB) was also significantly elevated by CR, specifically as cells enter the diauxic shift and stationary phase. This was surprising, as *S. cerevisiae* was not previously reported to synthesize BHB. Acetoacetate supplementation also dramatically elevated BHB levels, pointing to the existence of an endogenous b-hydroxybutyrate dehydrogenase (BDH) activity. This was validated by metabolic tracing showing robust intracellular conversion of supplemented $^{13}$C-acetoacetate into $^{13}$C-BHB. The reverse reaction was also observed but less efficient. CR-induced BHB enrichment was fully dependent on the acetyl-CoA synthetase Acs1, but not the canonical fatty acid b-oxidation pathway, implying a novel pathway to ketone body synthesis. Interestingly, BHB supplementation induced a transcriptional pattern mimicking methionine restriction and the unfolded protein response, conserved pathways strongly linked to longevity. Accordingly, BHB was shown to significantly extend CLS, thus establishing budding yeast as a novel and unexpected model for investigating the effects of endogenous and exogenous ketone bodies on aging.

## Evolutionary perspectives from fungal genomics

Geraldine Butler

School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin, Ireland

Our contributions to fungal genomics centre mostly on the fungal pathogen *Candida parapsilosis*, a yeast that is an increasing cause of drug-resistant infection. Fluconazole-resistant isolates are associated with outbreaks of infection across the world. We used genome sequencing to identify novel mechanisms of resistance. One mechanism associated with a species-specific genome organisation of a member of the CDR family of drug transporters will be discussed. We also used comparative genomics to address fundamental biological questions, such as the structure and function of centromeres, the sites of attachment for spindle bundles during cell division. Centromeres in *C. parapsilosis* (and other *Candida* species) are very different to centromeres in *Saccharomyces* and related species, yet their function remains the same. We used experimental and bioinformatic analysis to characterise centromeres across several orders of budding yeasts. We found that the location of centromeres have remained constant over long stretches of evolutionary time. However, the structures vary tremendously, from sequence-defined short regions, to Inverted Repeats, presence of Ty transposon elements, and some with no obvious structure. Potential evolutionary origins will be discussed.

## Exploring the extracellular biology of yeast-like pathogens

Márcio Rodrigues

Carlos Chagas Institute of the Oswaldo Cruz Foundation (Fiocruz, Curitiba, Brazil) and Microbiology Institute of the Federal University of Rio de Janeiro, Brazil

The extracellular space is a major site of interaction between fungal pathogens, neighboring cells, and the host, yet many of the mechanisms governing extracellular communication remain poorly understood. Studies of fungal extracellular vesicles (EVs) have revealed a complex system of molecular export that contributes to virulence, intercellular communication, and modulation of host responses. In the yeast pathogen Cryptococcus neoformans, EVs transport diverse cargoes, including polysaccharides and bioactive small molecules, raising new questions about the role of extracellular components in fungal pathogenesis. The identification of an EV associated tripeptide with protective activity against cryptococcosis has uncovered unexpected links between fungal pathogenesis and dipeptidyl peptidase 4 (DPP4) biology. Emerging evidence further suggests that host metabolic pathways involving DPP4, GLP1 signaling, and insulin responsiveness influence cryptococcal physiology and disease outcome. Together, these observations support a model in which the fungal extracellular environment functions as a dynamic regulatory interface integrating microbial communication, host sensing, and virulence. Understanding this extracellular biology may reveal new opportunities for therapeutic intervention against fungal diseases.

# KEYNOTE LECTURES

# Endohyphal bacterial symbionts in *Aspergillus fumigatus*: expanding the biology of a pathogen

Cristina Silva Pereira

Instituto de Tecnologias Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA)

*Aspergillus fumigatus*, a WHO fungal priority pathogen responsible for approximately 1.3 million deaths annually from invasive aspergillosis, may harbour a previously overlooked community of endohyphal bacteria that influences fungal physiology and pathogenicity. Earlier metagenomic and microscopy studies identified core bacterial associates across nearly 50 clinical and environmental *A. fumigatus* strains, supporting their intracellular localization. Current work moves beyond classification towards mechanistic investigation of these fungal–bacterial interactions. It integrates bacterial monocultures, fungal–endobacterial co-cultures, untargeted metabolomics, transcriptomics and infection assays. Endobacterial DNA is consistently detected at low abundance within fungal material, and five bacterial associates have been axenically cultured from a reference clinical isolate and subjected to whole-genome sequencing. The cultured bacteria include *Paraburkholderia fungorum*, whereas others remain unresolved at species level and may represent novel taxa, including within the genus *Enterovirga*. Targeted 16S-FISH probes are being developed to confirm endohyphal localization of both cultivated and abundant uncultured bacterial partners. Genome mining indicates substantial biosynthetic potential, including pathways potentially involved in bacterial communication, microbial competition, stress tolerance and iron uptake. Several isolates also contain prophage or phage-derived mobile elements, which may offer future opportunities to perturb bacterial populations and examine their functional contribution to the fungal host. Together, these findings position the *A. fumigatus*–endobacterial system as a promising model for investigating how hidden bacterial partners may shape the biology and virulence-associated traits of opportunistic fungal pathogens.

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## Yeast as a cellular platform to explore longevity, stress responses and disease mechanisms

Paula Ludovico

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho

The budding yeast Saccharomyces cerevisiae is more than a classical microorganism of fermentation and biotechnology. Its simplicity, experimental flexibility and conservation of core eukaryotic pathways make it an exceptional system for addressing fundamental questions in cell biology, particularly those related to ageing and stress adaptation. In this presentation, yeast will be explored as a cellular platform to investigate how metabolic state, nutrient availability and environmental challenges shape lifespan. Special focus will be given to chronological ageing and to the way cells respond to energetic limitation, oxidative imbalance and mitochondrial alterations. These processes provide insight into how protective stress responses can promote survival and delay functional decline. The talk will also discuss how yeast contributes to the study of disease-associated cellular dysfunction. Models expressing proteins linked to neurodegeneration, such as α-synuclein, allow the dissection of events connected with proteotoxicity, mitochondrial impairment, defective quality-control pathways and genome instability. In this context, yeast offers a valuable framework to connect molecular damage with premature ageing phenotypes. Another topic to be addressed is the influence of amino acid balance on cellular fitness and longevity. Changes in specific amino acid groups can affect nutrient signalling, intracellular pH regulation, mitochondrial performance and degradative pathways such as autophagy and mitophagy. These findings highlight the importance of metabolic homeostasis in the control of ageing-related phenotypes. Finally, the presentation will consider how compounds commonly perceived as metabolically inert, including non-caloric sweeteners, may influence yeast lifespan by affecting osmotic balance, redox state and nutrient-responsive signalling networks. Overall, this work illustrates how S. cerevisiae can be used as a powerful and versatile model to connect metabolism, stress resistance, protein quality control and ageing, providing concepts with broader relevance for understanding cellular decline and age associated disease.

## Host endotypes in aspergillosis: bridging immune mechanisms to actionable biomarkers and therapies

Agostinho Carvalho

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

The host genetic background is a determining factor of susceptibility to invasive pulmonary aspergillosis (IPA). Upon exposure to Aspergillus, innate immune cells rely on a coordinated network of pattern-recognition receptors and cytokine circuits to mount effective antifungal responses, including pathogen recognition, phagocytosis, and fungal killing. Genetic variation across these pathways can compromise the efficiency of these effector functions, creating distinct host endotypes that predispose specific patient subgroups to IPA and worse clinical outcomes. We have identified genetic variants in innate immune genes that act as predictors of both susceptibility and mortality in patients with IPA, linking specific genotypes to defective antifungal effector responses at the cellular level. In parallel, patient cohorts with sampling across defined clinical timepoints have proven valuable in capturing immune dynamics at earlier, more actionable stages of infection, revealing biomarker signatures that precede clinical deterioration. As one example of this endotype-driven approach, we have shown that a genetic variant in LGALS3, encoding galectin-3, disrupts protein expression and impairs the phagocytic and fungicidal activity of macrophages against *Aspergillus fumigatus*, increasing susceptibility to infection and offering a mechanistic link between genotype and clinical risk. An improved understanding of how host genetic and immune profiles shape susceptibility to and outcome of IPA is ultimately expected to pave the way toward precision medicine strategies, including risk-adapted antifungal prophylaxis, personalized diagnostics, and host-directed therapies tailored to a patient's underlying immunogenetic architecture.

## Lose, gain, thrive: genome remodeling as a driver of adaptation in a floral yeast clade

Carla Gonçalves

FCT Nova, Lisbon, Portugal

The *Wickerhamiella*/*Starmerella* yeast clade currently comprises around 100 described species, most of which are associated with bees and their surrounding environments. Over the past ~10 years, we have studied this clade extensively because of its unusual physiological and genomic characteristics, including the preference of most species for fructose as their sole carbon and energy source and the presence of genes acquired by horizontal gene transfer (HGT). Our studies have revealed that an extensive history of HGT has profoundly reshaped the metabolism of these yeasts. Newly emerging clues suggest that this extraordinary history of gene acquisition in the *Wickerhamiella*/*Starmerella* clade may be rooted in a combination of intrinsic and extrinsic factors. Together, these factors may have shaped a lineage that represents a remarkable case of metabolic innovation and horizontal gene transfer, and probably one of the most unusual evolutionary stories yet uncovered among fungi.

## Wine yeasts: addressing old and new winemaking challenges

Alexandra Mendes Ferreira

WM&B—Laboratory of Wine Microbiology & Biotechnology, Department of Biology and Environment, Universidade de Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal
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

Wine yeasts have traditionally been selected for their ability to ensure efficient and reliable alcoholic fermentation, with *Saccharomyces cerevisiae* playing a central role in this process. Over the last decades, however, our understanding of wine fermentation has changed considerably. Advances in yeast ecology, physiology and biodiversity have broadened the way we look at their role in winemaking, while highlighting the potential of many other yeast species for new and diverse applications in wine production. While classical challenges such as the need for robust fermentations, consistent wine quality and effective control of spoilage microorganisms remain highly relevant, new demands have emerged in response to climate change, sustainability goals and evolving consumer expectations. These include the management of acidity and ethanol levels, the modulation of aroma and mouthfeel, the reduction of chemical inputs and the search of alternative strategies for microbial control. This talk will present some examples of how wine yeast research is contributing to addressing old and new winemaking challenges. Particular attention will be given to the exploitation of yeast biodiversity, including non-*Saccharomyces* species, and to the expanding use of yeasts as tools for targeted applications beyond fermentation, from modulating wine composition and sensory properties to biocontrol.

# From genetic variation to translational regulation: emerging determinants of antifungal and stress resistance in *Candida albicans*

Ana Rita Bezerra

Institute of Biomedicine – iBiMED, Department of Medical Sciences, University of Aveiro

Antifungal resistance in *Candida albicans* is a complex phenotype arising from the interplay between genetic variation and dynamic cellular responses to environmental stress. Although alterations in well-established resistance genes can explain resistance in some isolates, substantial phenotypic variation remains unexplained, highlighting the need to explore additional genetic and regulatory mechanisms. Population genomic analyses of Portuguese *C. albicans* isolates from the iBiMED collection have been used to investigate the genetic basis of antifungal resistance and stress-related phenotypes. Genome-wide analyses identified candidate single-nucleotide variants and copy-number changes associated with echinocandin resistance. Among the emerging candidates are genes involved in cell wall biology as well as genes related to tRNA modifications. These modifications, catalyzed by tRNA-modifying enzymes, are essential for translation efficiency and accuracy, providing an additional layer of gene expression regulation. These findings have prompted the investigation of the *C. albicans* tRNA epitranscriptome as an emerging regulatory layer of stress adaptation. Analyses of clinical and environmental isolates reveal substantial variation in tRNA modification profiles, including differences in wobble uridine 2-thiolation. Moreover, changes in tRNA modifications and tRNA-derived fragments under thermal stress, together with stress-dependent regulation of tRNA-modifying enzymes, indicate that the tRNA landscape is dynamically remodelled in response to environmental conditions. Together, these studies support a multilayered view of antifungal and stress adaptation in *C. albicans*, in which genetic variation, gene regulation and translational control contribute to phenotypic diversity. Linking genomic variation to the tRNA epitranscriptome may uncover previously overlooked mechanisms underlying fungal adaptation and provide new perspectives on antifungal resistance.

# ORAL COMMUNICATIONS

# Session I. Biotechnology and Synthetic Biology

# A new pipeline for meta transcriptomic data integration in the metabolic model yeast9.0.2 for *Saccharomyces cerevisiae*: ethanol production optimization as a case-study

Diogo Couceiro[1]; Leonardo Paulo[1,2]; Tiago Val[1, 2]; Luís Coutinho2, 3; Pedro Monteiro[1,4]; Miguel Cacho Teixeira[2,3]

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[4]Department of Computer Science and Engineering, Instituto Superior Técnico, Universidade de Lisboa – Lisboa

In an age of Climate Change the need for alternative biotechnological processes for producing added value compounds such as bioethanol as a biofuel is significant and pressing. Saccharomyces cerevisiae as for long been used as a cell factory for the production of such bio-industrial relevant compounds. Metabolic models have for long been useful and reliable tools for guiding wet-lab pipelines such as strain design or the study of pathogenicity. However they are fundamental incomplete as they disregard any kind of regulatory information which could render them more dynamic and a more truthful and complete representation of reality. This work takes advantage of a recent and significant update to the rich Saccharomyces cerevisiae regulatory information database Yeastract+. This update introduced vast datasets of quantitative transcriptomic data. This allowed for the development of a pipeline for the integration of large scale quantitative regulatory information integration into the metabolic model Yeat9.0.2. This pipeline allows for the construction of several normalized quantitative relative TF and enzyme encoding genes expression variations to be introduced and modelled on the metabolic model by being translated into relative reaction bound changes – constructing a series of newly restricted and modified metabolic model considering specific mutations of interest.

This pipeline generated a series of TF encoding genes such as CAT8 validated in existing literature as leading to increased ethanol production establishing this method as a promising new tool for large scale regulatory-metabolic mixed models refinement raising them to an even more reliable and truthful tool for the future.

# Mechanistic insights into citrate export by the CexA transporter from *Aspergillus niger*

Francisca Ferreira[1]; João Alves[1,2]; Margarida Casal[1,2]; Isabel Soares-Silva[1,2]

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Citric acid is one of two major carboxylic acids produced industrially by microbial cell factories, particularly by the filamentous fungus *Aspergillus niger*. This microorganism is characterised by its high citrate secretion capacity, which is attributed to the citrate exporter CexA. This transporter has been structurally and functionally characterised by our group, which identified key amino acid residues involved in citric acid transport. Subsequently, Molecular Dynamics simulations of citrate molecules moving through the AlphaFold model of CexA, revealed a putative citrate-binding site on the intracellular side of the transporter. Based on these findings, the residues S178, R181, V185, K380, and S450 were selected for site-directed mutagenesis, generating 14 mutant alleles that were heterologously expressed in Saccharomyces cerevisiae CEN.PK 113-5D. The effects of these mutations were evaluated through GFP-tagging to assess protein localisation and spot assays to evaluate cell growth on media containing different carboxylic acids as the sole carbon source. One mutation impaired the correct localisation of the transporter at the plasma membrane. Eleven mutant alleles promoted improved growth on carboxylic acids compared with cells expressing the native transporter, suggesting a decreased export activity, while the remaining mutants exhibited growth profiles similar to those of wild-type CexA. Together, these findings provide new mechanistic insights into citrate export by CexA and establish a framework for the rational engineering of membrane transporters to improve industrial carboxylic acid bioproduction.

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# Coupling computational design with yeast display: a high-throughput pipeline for antiviral discovery

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Viral diseases are continuously emerging, and pandemics are predicted to arise more often. Antiviral drugs are essential to reduce morbidity and mortality when vaccines are not available or have limited efficacy, most critically in high-risk populations, but only few are approved for clinical use. To expand these therapeutic options, monobodies offer a powerful solution for antiviral drug development against a broad range of viruses. These small proteins, derived from the human fibronectin type 3 domain (FN3), are highly stable and can be engineered to target virtually any protein with high specificity and affinity. In this work, we used a computational pipeline for the de novo design of monobodies and validated it against the pathogenic virus SARS-CoV-2. Monobodies were based on a highly thermostable FN3 scaffold, and their loop region, equivalent to antibody CDRs, was designed with variable length. Generated monobodies were filtered using confidence metrics, resulting in a large number of designs. Because computational pipelines generate thousands of potential candidates, we leveraged yeast display — a powerful high-throughput system that enables the screening of large libraries of computational designs simultaneously. In this method, monobodies are expressed in Saccharomyces cerevisiae cells and displayed on the surface by attachment to the cell wall. Upon incubation of cells with the viral target, and subsequent immunofluorescence labelling, binding is assessed by FACS. We successfully identified monobodies that bound to SARS-CoV-2 RBD, demonstrating the potential of our computational pipeline coupled with yeast display as a promising tool for the development of therapeutics against viral threats.

# Session II. Adaptation, Cell Death and Ageing

# Yeast profilin(g): new insights into the yeast cytoskeleton-mitochondria crosstalk

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The actin cytoskeleton is crucial for cellular homeostasis, including organelle dynamics, intracellular transport and mechanical force generation. Accumulating evidence suggests disruption in actin cytoskeleton dynamics as a driver of senescence and aging. Moreover, cytoskeletal compromise recapitulates core age-related phenotypes, namely mitochondrial dysfunction and impaired proteostasis. Notably, profilin (Pfy1 in yeast), a conserved actin-binding protein and a regulator of cytoskeletal architecture and remodeling, exhibits one of the most pronounced transcriptional declines during human aging. Thus, we aimed to investigate the role of profilin in actin regulation, mitochondrial homeostasis, and longevity using Saccharomyces cerevisiae as a model organism. To overcome long-standing challenges associated with tagging Pfy1, we systematically iterated distinct combinations of linkers and tags to label Pfy1 and successfully identified functional constructs with minimal impact on growth, establishing improved variants for in vivo applications. Using live-cell imaging of cells expressing Pfy1-yeGFP, our results show that Pfy1 is predominantly cytosolic with a detectable nuclear pool. Moreover, Pfy1 abundance tends to decline during yeast chronological aging, highlighting this protein as a potential target for longevity-promoting interventions. Since PFY1 is an essential gene, we implemented a repressible Tet-Off expression system to achieve controlled Pfy1 downregulation. Upon partial depletion of Pfy1, cells exhibited compromised growth and reduced viability, concomitant with pronounced mitochondrial fragmentation, membrane hyperpolarization, increased mitochondrial mass, and elevated levels of reactive oxygen species, suggesting profound changes in mitochondrial function and bioenergetics. Collectively, these findings highlight profilin as a regulatory node at the intersection of cytoskeletal dynamics, mitochondrial function, and lifespan regulation.

## Bioactive metabolites drive lifespan extension in a yeast model of Niemann-Pick type C1

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Niemann-Pick type C (NPC) is an autosomal recessive neurodegenerative disorder caused by loss-of-function mutations in lysosomal proteins NPC1 or NPC2, leading to defective intracellular cholesterol trafficking, lipid imbalance, and impaired mitochondrial function, autophagy and stress responses. We have previously shown that yeast lacking Ncr1p (NPC1 homolog) recapitulate features of NPC cells, including sphingolipid accumulation, iron overload, and mitochondrial dysfunction, leading to a reduced chronological lifespan (CLS). In this study, we used mass spectrometry-based untargeted metabolomics to obtain the metabolomic profile of ncr1Δ cells. KEGG analysis revealed ten significantly enriched pathways in the mutant, including those related to the metabolism of aminoacids, C5-branched dibasic acid, nicotinate and nicotinamide, 2-oxocarboxylic acid, thiamine and carbon. Several metabolites altered in ncr1Δ cells have been implicated in epigenetic regulation of aging but the deletion of selected histone-modifying enzymes (previously linked to aging) failed to extend the CLS of ncr1Δ cells, and no changes in total histone H3 levels were observed across strains. However, the supplementation of specific metabolites that were downregulated in ncr1Δ cells reduced intracellular oxidation and extended CLS of this mutant strain, although it did not rescue its autophagic and respiratory growth defects. Collectively, these findings identified compound-mediated lifespan extension in ncr1Δ cells that occurs independently of autophagy or respiratory function, suggesting alternative mechanisms of action in the yeast model of NPC1.

# Session III. Health

## Ion compromises fluconazole activity in *Candida glabrata*

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Fluconazole is the most widely prescribed antifungal agent worldwide. The drug targets Erg11, an enzyme involved in the biosynthesis of ergosterol, a key component of the fungal cell membrane. However, its fungistatic activity and extensive use have driven the emergence of fluconazole-resistant fungal pathogens. Recently, we found that iron loading conditions decrease fluconazole activity against *Candida glabrata*, an opportunistic yeast, that can cause life-threatening infections. The antagonism between the drug and the metal is evident across a wide range of concentrations. Iron upregulates genes crucial for fluconazole detoxification, namely the ABC transporters Cdr1 and Cdr2, and their regulator, the transcription factor Pdr1. Consistent with these findings, intracellular fluconazole levels are lower under co-treatment conditions and ergosterol depletion is less pronounced than under treatment with fluconazole alone.

The reduced activity of fluconazole under iron loading conditions may have important clinical implications, as this phenotype would not be captured by standard antifungal susceptibility assays, highlighting the need to consider the host environment when selecting antifungal treatment strategies.

# Characterization and immunomodulatory effect of *Alternaria alternata* extracellular vesicles in host-pathogen interactions

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*Alternaria* are melanized 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. Previously, we characterized the production of extracellular vesicles (EVs) by *Alternaria infectoria*; now we aimed to fully characterize the EVs cargo produced by *Alternaria alternata* (AaEVs), the most common Alternaria species involved in human disease(s), and their impact on host cells. Proteomic analysis, performed using RP-LC-MS/MS and DIA as previously described for the characterization of the *A. alternata* spore and hyphal proteome, identified over 1400 proteins in AaEVs, including allergens. The proteins were categorized based on function, and, excluding uncharacterized proteins, the proteome was distributed as follows: 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%). The MS data indicated that a putative sialidase was the most abundant protein in AaEVs. TEM showed double-membrane vesicles with an average diameter of 115 nm, containing melanin. AaEVs induced neutrophil extracellular trap formation and were internalized by macrophages. Sera from patients with respiratory allergies contained IgE that recognized AaEVs, indicating that these structures are immunomodulatory and potential carriers of allergens. The AaEVs proteome provided insights into the possible role of these structures in the physiopathology of respiratory diseases, in particular in allergy. The results indicate that AaEVS are key players in the early interaction with the human host cells (e.g. sialidase) and in the progression to allergic reactions (e.g. allergens).

# Chitosan-coated alginate capsules for delivery of 2'-o-methylrna EFG1 oligomers targeting *Candida albicans* filamentation

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Vulvovaginal candidiasis (VVC) is a fungal infection with significant clinical, social, and economic implications. Misuse of antifungal agents has contributed to the emergence of multidrug-resistant fungi, including *Candida albicans*, whose pathogenicity is closely linked to its ability to transition from a yeast to a filamentous form, facilitating the invasion of VV tissues and resistance to antifungal therapies. Antisense therapy has emerged as a promising approach to inhibit *C. albicans* filamentation, particularly using the 2'-OMethylRNA EFG1 oligomers targeting EFG1 and their delivery via lipoplex complexes. Vaginal drug delivery in gel capsules ensures a local action and allows controlled drug release. This research focused on developing vaginal gel capsules incorporating the 2'-OMethylRNA EFG1 oligomer designed to inhibit *C. albicans* filamentation into chitosan-coated alginate capsules to treat VVC. The chitosan-coated capsules were prepared by ionotropic gelation with an average size of 1.59 mm and weight of 1.85 mg. A physicochemical characterization of the capsules was performed through stability and swelling assays, morphological analyses, FTIR and DSC. The results confirmed a porous surface, which would enable gradual release of the oligomers. Capsules loaded with free oligomers showed an improved release at 6 and 24 hours, while oligomers lipoplex showed enhanced release at 24 and 48 hours. *In vitro* assays confirmed a significant reduction in *C. albicans* filamentation for up to 48 hours. Moreover, no significant signals of toxicity were observed. Overall, this delivery system represents a safe and effective alternative to treat of VVC, offering a novel strategy to control the escalation of *C. albicans* infections.

# Obesity-associated gut *Candida* and *Penicillium* dysbiosis is linked to purinergic receptor polymorphisms

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Obesity results from complex interactions between host genetics, environmental factors, and lifestyle. While the gut microbiota contributes to metabolic regulation, the fungal component (mycobiota), particularly yeasts, remains comparatively understudied. Purinergic signaling pathways mediated by the adenosine A2A receptor (A2AR) and ATP P2X7 receptor (P2X7R) regulate inflammation, immunity, and metabolism, but their influence on gut microbial communities is poorly understood. This study investigated whether genetic variation in A2AR and P2X7R is associated with alterations in gut microbial composition, with a particular focus on yeast communities, in obesity. Adult obese individuals and healthy controls were recruited, and clinical, anthropometric, and lifestyle data were collected alongside blood and fecal samples. Genotyping was performed for ADORA2A rs2298383 and P2RX7 rs208294 polymorphisms. Gut fungal communities were characterized by ITS2 sequencing, while bacterial microbiota composition was also assessed. Differences in genotype distribution were observed between groups, with the ADORA2A rs2298383 CT genotype more frequent among obese individuals and the P2RX7 rs208294 CC genotype associated with higher body weight and body mass index. Mycobiota analysis revealed reduced fungal diversity in obesity and a shift toward yeast and fungal taxa frequently linked to inflammatory conditions, including *Candida* and *Aspergillus*, whereas *Saccharomyces* was more prevalent in healthy controls. Bacterial community profiles were consistent with previously described obesity-associated dysbiosis. These findings suggest that purinergic receptor polymorphisms are associated with gut fungal community structure and metabolic imbalance, supporting the existence of a microbiota–purinergic signaling axis with potential relevance for obesity pathogenesis and future therapeutic interventions.

# MycoSifter - a consensus-driven, multi-threshold pipeline for species-level environmental contamination removal in clinical mycobiome studies

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Low-biomass clinical samples are highly susceptible to environmental fungal carryover, often misguiding host-microbiome associations. Current decontamination tools typically rely on a single DNA-concentration cut-off or prevalence filters, which can struggle when contaminants are sporadic or host biomass varies widely. To address these challenges, we developed MycoSifter, an automated R pipeline that identifies potential environmental contaminants using a consensus-driven, multi-threshold strategy. The tool sequentially bins samples across a user-defined DNA-load gradient (e.g., 10–50 ng/µL), calculating fold-change (FC, default >2) and Wilcoxon p-values (0.95) across all tested stringencies. By combining an adjustable multi-threshold consensus with ecological context, MycoSifter provides a flexible, reproducible framework to help researchers evaluate potential fungal contaminants and refine host-microbiome findings in low-biomass clinical settings.

# Proteomic and ultrastructural dissection of N88S Seipin inclusion bodies reveals conserved chaperone defenses against motor neuron diseases

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*Saccharomyces cerevisiae* is a powerful model to dissect the proteostasis networks that handle misfolded proteins linked to human disease. Seipinopathies are autosomal dominant motor neuron diseases caused by toxic gain-of-function mutations (N88S and S90L) in BSCL2/Seipin. We previously showed that, in yeast, mutant N88S Seipin forms highly insoluble cytosolic inclusion bodies (IBs), is cleared through ER-associated degradation (ERAD), and co-localizes with the Hsp70 chaperone BiP/Kar2 and the disaggregase Hsp104. Systematic genetic array (SGA) analysis uncovered synthetic sick interactions between N88S Seipin and cytosolic Hsp40s (Ydj1, Hsp31), Hsp70s (Ssa2, Apj1, Rot1), and Hsp104, while loss of early-acting chaperones (Ssa3, Sba1, Hsp26) increased misfolding, implicating a layered chaperone network in IB control. Here, we characterized the composition and ultrastructure of N88S Seipin IBs. Stress-responsive PACE- and CCAAT-driven mCherry reporters confirmed significant proteotoxic stress in mutant cells. Quantitative proteomics comparing wild-type (WT) and N88S strains, with or without a functional proteasome, revealed increased levels of Ssa2, Apj1, Rot1, and the ERAD component Dfm1, but reduced Ssa3 and Hsp26 levels. Hsp104-myc nanotrap pull-downs combined with mass spectrometry identified IB-associated proteins, including Apj1, mitochondrial translation and respiratory chain components, but not ubiquitin-conjugating enzymes Ubc5 and Ubc13. Transmission electron microscopy with Hsp104 immunogold labelling further showed larger lipid droplets, increased ER stacking, and significant redistribution of aggregates within the cell. This work delineates the chaperone machinery yeast cells deploy to cope with misfolding of N88S Seipin, offering a tractable platform to identify conserved and therapeutic nodes in proteostasis networks relevant to seipinopathies and motor neurodegeneration.

# Production and evaluation of a novel yeast-derived recombinant iduronate-2-sulfatase as a potential therapeutic alternative for Hunter syndrome

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Mucopolysaccharidosis type II (MPS II), or Hunter syndrome, is a rare lysosomal disorder caused by iduronate-2-sulfatase deficiency, leading to glycosaminoglycan (GAG) accumulation and multisystemic impairment. Although enzyme replacement therapy is available, high production costs limit access. This study evaluated a yeast-derived recombinant I2S enzyme, prIDS, as a potential alternative therapeutic approach. prIDS was produced and purified from Komagataella phaffii (DOI: 10.1002/bab.1660), yielding 2.5 mg of protein with enzymatic activity of 5.3 nmol/h/mg. Functional assays were performed in HEK293 wild-type and IDS-knockout cells treated with prIDS or idursulfase at 50 nM. IDS activity, GAG levels, lysosomal mass, and mannose-6-phosphate (M6P) receptor-mediated uptake were assessed. In vivo biodistribution was evaluated in male C57BL/6 wild-type and IDS-knockout mice after intravenous administration of prIDS or idursulfase, followed by IDS activity analysis in organs (n = 3). Statistical analyses included Kruskal–Wallis/Dunn's and ANOVA/Bonferroni tests. IDS activity was significantly reduced in MPS II cells compared with wild-type cells ($p < 0.0001$). prIDS significantly increased IDS activity ($p < 0.0001$), partially reduced GAG levels ($p < 0.05$), and normalized lysosomal content ($p < 0.01$). M6P competition markedly inhibited prIDS uptake, indicating M6P receptor-mediated internalization. At 48 h, prIDS showed higher activity in brain, kidney, spleen, and lung, whereas idursulfase showed higher activity in heart and liver (*$p < 0.05$; ***$p < 0.001$). prIDS restored key cellular alterations associated with MPS II and demonstrated a distinct biodistribution profile compared with idursulfase, supporting its potential as an alternative enzyme replacement therapy for Hunter syndrome.

# Session IV. Genomics and Evolution

# YEASTRACT+ upgrades to a quantitative regulatory resource: transcriptome integration for genome-scale metabolic modeling in yeasts

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The YEAst Search for Transcriptional Regulators And Consensus Tracking (YEASTRACT+) database has supported the study of transcriptional regulation in yeasts for 20 years through the curation of experimentally validated transcription factor (TF)–target gene (TG) associations. However, its application in quantitative systems biology has been limited by the predominantly qualitative nature of available regulatory information. To address this limitation, we are expanding YEASTRACT+ through the systematic integration of transcriptomics data. This effort includes the processing of more than 10 TB of publicly available RNA-seq datasets and the review of over 270 publications reporting microarray-based transcriptional profiling experiments. Nearly 500 TFs have been curated across Saccharomyces cerevisiae, non-conventional yeasts of biotechnological relevance, and pathogenic yeast species, resulting in more than 1.33 million statistically significant quantitative TF–TG associations linked to standardised experimental conditions. The resulting regulatory layer is being integrated with yeast genome-scale metabolic models (GEMs) to generate TF mutant-specific models. For each TF perturbation, transcriptomics-derived expression changes are translated into proportional adjustments of reaction flux bounds relative to the wild-type model. The resulting constrained GEM is analysed using parsimonious Flux Balance Analysis (pFBA), yielding flux distributions that capture the metabolic consequences of TF perturbation while minimising total network flux. This framework enables the prediction of strain-specific metabolic phenotypes and the identification of TF engineering targets predicted to enhance the production of industrially relevant metabolites. These developments establish YEASTRACT+ as a quantitative resource for regulatory-metabolic model integration and rational strain engineering in yeasts.

# Characterization of a novel aphelid, a fungal-related parasitoid, infecting industrial nannochloropsis cultures

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Aphelids are intracellular parasitoids of algae that represent one of the closest known relatives of fungi. Although they share a common ancestor with fungi, aphelids have retained phagotrophic feeding, providing valuable insight into the early evolution of Holomycota. Despite their evolutionary importance, aphelids remain poorly characterized, particularly in marine environments, and little is known about their diversity or their impact on industrial microalgal production. Here, we describe the morphological and molecular characterization of a marine aphelid responsible for the collapse of industrial cultures of *Nannochloropsis sp*. Contaminated cultures were maintained under laboratory conditions and examined by light and electron microscopy to document the parasite life cycle. Molecular characterization was performed through amplification and phylogenetic analyses of the 18S and ITS rDNA regions. Microscopy revealed several developmental stages, including a presumptive zoospore with multiple elongate projections but lacking a flagellum, an infective cyst attached to the host cell wall, and intracellular consumption of the host cytoplasm, leaving a characteristic residual body. No evidence of parasite reproduction within *Nannochloropsis* cells was observed. Phylogenetic analyses placed the organism within Aphelida but outside all currently described genera, indicating that it represents a distinct evolutionary lineage. These findings expand the known diversity of marine aphelids and contribute to our understanding of the evolution of fungal-related lineages. Furthermore, they highlight the significance of aphelids as overlooked biological contaminants capable of threatening the stability of industrial microalgal cultures.

# Session V. Fermented Food and Beverages

# Exploring glucose transport to improve the fermentative performance of *Torulaspora delbrueckii*

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Climate change has intensified sugar accumulation in grapes, disrupting wine composition and fermentation efficiency. Non-Saccharomyces yeasts such as *Torulaspora delbrueckii* can help modulate fermentation profiles under high-sugar conditions by reducing ethanol production, increasing glycerol and reducing off-flavor formation, and improving aroma complexity. However, *T. delbrueckii* fermentative performance remains less efficient than that of *Saccharomyces cerevisiae*. Since glucose transport is a rate-limiting step in sugar metabolism, reduced transport efficiency can compromise fermentation capacity. In *T. delbrueckii*, this limitation can also be aggravated by the ethanol sensitivity of its transport system, restricting its performance as ethanol accumulates. Therefore, we aimed to investigate the glucose transporter repertoire of *T. delbrueckii*, providing insight into its genetic basis and identifying targets to improve fermentative efficiency while preserving its aromatic contribution. For this purpose, a bioinformatic pipeline was used to analyze the genomes of 94 strains and identify candidate transporter genes. Additionally, we studied the expression of glucose transporters throughout fermentation to assess their contribution to sugar uptake. In parallel, we are evaluating whether ethanol-resistant S. cerevisiae glucose transporters can enhance the *T. delbrueckii* fermentative capacity. Results show that HXT1 expression can enhance *T. delbrueckii* specific growth rate under higher sugar conditions, but not under ethanol stress.

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# Genomic and phenotypic screening of non-*Saccharomyces* yeasts for climate-resilient winemaking

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The Portuguese viticulture industry faces severe threats from climate change. Rising temperatures cause earlier grape maturation, leading to higher sugar concentrations, reduced acidity, and altered aromatic profiles. Utilizing non-Saccharomyces yeasts during fermentation offers a promising biological approach to mitigate these effects, modulate ethanol content, and enhance sensory complexity. To explore this potential, we combined in silico and in vitro strategies. First, we bioinformatically analysed 1,197 genomes from 228 wine-associated yeast species. Evaluating carbohydrate-active enzymes, glycoside hydrolases (GH), and metabolic pathways revealed that Saccharomycotina yeasts possess robust metabolic machinery, while Basidiomycota show greater diversity in GH3, GH5, GH16, and GH51 families. The top five species scoring highest for oenological potential were Saccharomyces cerevisiae, as would be expected, *Wickerhamomyces anomalus*, *Cyberlindnera maritima*, *Nakazawaea ishiwadae*, and *Nakazawaea pomicola*. Next, we evaluated 40 non-*Saccharomyces* yeasts isolates through phenotypic assays under simulated grape must fermentation conditions. Screenings assessed thermal tolerance, carbon assimilation, environmental stress resistance, nitrogen preferences, and β-glucosidase activity. Fermentative metabolites were subsequently profiled and quantified using high-performance liquid chromatography. Ultimately, integrating predictive genomic tools with comprehensive in vitro validation provides a robust framework for discovering novel non-Saccharomyces starters. This approach aims to uncover unexploited yeast diversity, identifying promising candidates to mitigate climate change impacts, tailor wine profiles, and drive oenological innovation.

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# Genomic and transcriptomic profiling of *Hanseniaspora guilliermondii's* ethanol stress response

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*Hanseniaspora guilliermondii* (Hg) is a non-conventional yeast with recognized potential as a bioflavorant, particularly in winemaking. Nonetheless, its low ethanol tolerance is a recognized "Achilles-heel" that reduces its competitiveness during fermentation and limits its aromatic contribution. Leveraging the previous disclosure of the first Hg genomic sequence (UTAD222 strain), we explored transcriptomics, chemogenomics and comparative genomics to disclose the molecular mechanisms by which this species responds to ethanol stress. RNA sequencing performed on ethanol-stressed HgUTAD222 cells (exposed to 6%v/v for 1 hour) uncovered massive alterations in the transcriptome of this yeast, with 305 up- and 392 down-regulated genes, compared to non-stressed transcript levels. A considerable portion of the differently transcribed genes play a role in lipid metabolism, pointing to substantial membrane restructuring in response to ethanol toxicity. Results from phenotyping of a mutant library obtained by chemical mutagenesis of HgUTAD222 with EMS (ethyl methanesulfonate) corroborated this idea, being identified mutant strains hyper-susceptible to ethanol that harbor disruptive modifications in UFD2, a gene encoding a putative ubiquitin ligase predicted to regulate unsaturated fatty acid biosynthesis. Mutant strains with higher ethanol tolerance were also identified and will be additionally discussed. This is the first reported study so far to examine stress tolerance in Hg, which remains poorly characterized especially from a molecular point-of-view. The results elucidate essential aspects of Hg's physiology and stress response mechanisms, unlocking its potential in the industry as a bioflavorant and as a cell factory for production of add-value molecules where low ethanol tolerance can be a bottleneck.

# Session VI. Drug and Stress Resistance

# Omics analyses to disclose tolerance mechanisms to the antimicrobial $SO_2$ in the wine spoilage yeast S*accharomycodes ludwigii*

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In winemaking, sulphur dioxide ($SO_2$) stands as the most commonly used antimicrobial to prevent wine spoilage, given its capability to inhibit growth of contaminating microorganisms. However, some species can still tolerate this compound and thrive in sulphited wines. This is the case of the spoilage yeast *Saccharomycodes ludwigii*. *S. ludwigii* is a non-conventional yeast belonging to the poorly characterized Saccharomyceadea family, sister to the much more studied Saccharomycetacea to which the paradigmatic *Saccharomyces cerevisiae* belongs to. Our laboratory has been involved in genomic and phenotypic characterization of Saccharomycodeacea yeasts, having provided the first genome sequence of a *S. ludwigii* wine strain, UTAD17. This resource opened the door to genome-wide analyses that may help to elucidate different aspects of the biology of this organism, including its molecular responses to $SO_2$. It is with that principle that in this work it will be discussed the results obtained in comparative transcriptomic analyses conducted under $SO_2$ stress with two differently tolerant *S. ludwigii* strains. These results will be put in connection with detailed physiological analyses relevant under acid stress including cell wall structure, control of internal pH and availability of different amino acids whose abundance appears to modulate $SO_2$ tolerance. Overall, we expect that studies may shed light into the molecular mechanisms mediating the $SO_2$ tolerant phenotype of *S. ludwigii* strains, contributing to the design of more efficient wine preservation strategies.

# Drug repurposing as a strategy for antifungal adjuvant development and efflux modulation in *Candida albicans*

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*Candida albicans* is a leading cause of nosocomial fungal infections, associated with high morbidity and mortality. Resistance to antifungal drugs, particularly azoles, is increasing, highlighting the need for new therapeutic strategies. A key resistance mechanism involves the overexpression of efflux pumps, which reduce intracellular drug accumulation. Efflux inhibitors may help restore antifungal efficacy. Drug repurposing provides a cost-effective approach to identify novel antifungal adjuvants active against *C. albicans*. Approved drugs targeting membrane transport proteins in *C. albicans* were identified using an *in silico* repurposing strategy. A diverse subset was selected for in vitro testing. Minimum inhibitory concentrations (MICs) were determined by microdilution against *C. albicans* ATCC 90028. Potential adjuvant effects were assessed with fluconazole, and efflux modulation was evaluated by fluorometric measurement of ethidium bromide (EtBr) accumulation. Time-kill and mutant selection assays are currently ongoing to further characterize antifungal dynamics over time and the potential for resistance emergence under drug pressure. *In silico* analysis identified 245 membrane transport targets associated with 607 approved drugs. Fifty-nine drugs were screened. Miltefosine showed the lowest MIC (2 mg/L), while amlodipine and procainamide reduced fluconazole MIC by ≥4-fold. Fluorometric assays identified amlodipine, fluvoxamine and fluoxetine as potential efflux modulators in *C. albicans*. Repurposed drugs with adjuvant and efflux modulator activity show potential to enhance antifungal efficacy and overcome resistance, supporting development of novel therapeutic strategies against *C. albicans*.

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# From CUG decoding ambiguity to proteome-wide mistranslation in clinical isolates of *Candida albicans*

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*Candida albicans* is an opportunistic human fungal pathogen with remarkable genotypic and phenotypic plasticity, enabling adaptation to diverse host niches and environmental conditions. One potential source of this diversity is translational ambiguity arising from dual decoding of the CUG codon as serine or leucine. Although artificially increasing leucine incorporation at CUG sites (CUG mistranslation) is known to affect virulence-related traits, its contribution to phenotypic diversity in the clinical setting remains unclear. We characterized a collection of clinical *C. albicans* isolates regarding their genotype, stress responses, morphological transitions, and antifungal susceptibility, and observed substantial phenotypic variability that could not be fully explained by genetic differences, suggesting that additional mechanisms contribute to adaptation and pathogenicity. Using a GFP-based reporter system and flow cytometry, we quantified CUG mistranslation and found that it varies among isolates, indicating that translational ambiguity differs across natural clinical strains and may underlie proteomic, thus phenotypic heterogeneity. To investigate the extent of these events, we developed a mass spectrometry-based proteogenomic pipeline capable of detecting and quantifying rare amino acid substitutions across the proteome. This analysis showed that mistranslation in *C. albicans* is not restricted to CUG codons, with non-canonical amino acid substitutions occurring at multiple codon sites, a phenomenon associated with adaptive advantages under stress in other microorganisms. We are now applying this pipeline to clinical isolates to define mistranslation landscapes and determine whether isolate-specific signatures contribute to virulence. Together, these findings support loss of translational accuracy as an important and underappreciated source of diversity, adaptation, and pathogenicity in *C. albicans*.

# Exploring the antifungal potential of novel 2-aminoimidazole azo dyes against *Candida krusei* and *Candida auris*

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Invasive infections caused by *Candida* spp. represent a major healthcare challenge due to the emergence of non-*Candida albicans* species such as *C. krusei* and *C. auris*, which are WHO priority pathogens associated with antifungal resistance and biofilm formation. The antimicrobial properties of the imidazole moiety make 2-aminoimidazole azo dyes promising candidates for antifungal development. In this work, four newly synthesized azo dyes were obtained through the reaction of imidazole-based amidrazone precursors with secondary amines using an optimized synthetic procedure. Antifungal activity against *C. krusei* and *C. auris* was evaluated by Minimum Inhibitory Concentration (MIC) determination and the interaction between the most promising compounds was assessed using a checkerboard microdilution assay. Their ability to prevent biofilm formation was evaluated after 24 h by colony-forming units (CFUs) enumeration. Compounds A-D, differing only in their N-1 substituents, were successfully obtained as dark-purple solids. MIC values ranged from 2 to > 32 µg/mL, with *C. auris* generally showing greater susceptibility than *C. krusei*. Compound B exhibited the strongest antifungal activity, presenting the lowest MIC geometric mean across both species, while Compound D showed the weakest activity. Based on the antifungal profiles and close structural similarity, Compounds A and B were further evaluated in combination, revealing concentration-dependent interactions suggestive of synergistic effects. These findings guided the selection of both compounds for antibiofilm evaluation, individually and in combination. Our results highlight the potential of 2-aminoimidazole azo dyes as promising antifungal candidates and support their further evaluation against some of the most problematic *Candida* spp. biofilms.

# From urban water to clinical settings: hidden reservoirs of opportunistic antifungal-resistant yeasts

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Urban freshwater systems are increasingly recognized as environmental interfaces linking human activities, wildlife, and microbial communities. However, their role as reservoirs of clinically relevant yeasts remains less understood. In this study, we investigated the diversity, antifungal susceptibility, and biofilm-forming ability of yeast-like fungi recovered from an artificial lake system in Aveiro, Portugal, within a One Health framework. Water samples were collected during 4 campaigns between June 2025 and March 2026, and yeast-like isolates were cultured at 37 °C. In total, 290 isolates were recovered, grouped by MSP-PCR fingerprinting, and identified by ITS and 28S rRNA gene sequencing. A total of 27 species were identified, including clinically relevant taxa such as *Candida parapsilosis*, *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*, as well as uncommon taxa such as *Meyerozyma guilliermondii*, *Magnusiomyces capitatus*, and *Candida palmioleophila*. Alpha diversity did not differ significantly across sites or campaigns, whereas beta diversity did vary significantly among campaigns. Susceptibility testing of 86 representative isolates against fluconazole, voriconazole, posaconazole, anidulafungin, amphotericin B, and micafungin showed heterogeneous MIC profiles. Nineteen *Candida* isolates were classified as resistant to at least one antifungal agent according to the available EUCAST clinical breakpoints, with some showing resistance to multiple agents. Resistance was most frequently associated with echinocandins, particularly anidulafungin, although azole and amphotericin B resistance were also observed. All resistant isolates formed metabolically active biofilms. These findings suggest that urban freshwater systems can harbor clinically relevant fungi with antifungal resistance and biofilm-associated phenotypes, supporting their inclusion in One Health-oriented environmental surveillance.

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# Overcoming fluconazole resistance in *Candida glabrata* via PDR1-targeted antisense oligomers

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*Candida glabrata* (now *Nakaseomyces glabratus*) infections pose a global healthcare concern due to inherent and acquired resistance to azoles, predominantly driven by PDR1 transcription factor. Antisense oligomers (ASOs) offer a potential solution by silencing target genes at mRNA level. This study evaluates the efficacy of a designed ASO targeting PDR1 to inhibit growth and overcome fluconazole (FLU) resistance. *In vitro* ASO efficacy, alone or with FLU, was assessed in planktonic and biofilm states by evaluating fungal growth, PDR1 downregulation, biofilm biomass, and cell viability. ASO stability was tested in simulated human biological fluids, and toxicity assessed in human cell lines and *Galleria mellonella* model. In addition, the efficacy of lipid-encapsulated ASOs (LipoASOs) controlling *C. glabrata* infections was investigated in *G. mellonella*. *In vitro*, ASO alone reduced planktonic growth by 20% and PDR1 expression by 30%. The ASO–FLU combination enhanced FLU activity by over 30%, halving its MIC50 compared to FLU alone, and reducing gene expression by 40%. ASO retained stability and efficacy in simulated human fluids. Moreover, it showed no cytotoxicity in human cell lines and *G. mellonella* model. In biofilms, FLU showed minimal activity, declining efficacy over time; however, combined with ASO, inhibition was sustained, reducing total biomass by up to 30% and significantly the viable cells. In vivo, while FLU alone failed to improve *G. mellonella* infection outcomes, co-treatment with LipoASOs significantly increased larval survival by up to 32%. Overall, anti-PDR1 LipoASOs represents a safe, stable, and synergistic adjuvant therapy that effectively restores susceptibility in resistant *C. glabrata*.

# Exploring the potential of molecular glues for the development of novel antifungals against *Candida albicans*

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*Candida albicans* is an opportunistic yeast commonly responsible for the development of hospital-acquired infections, especially in immunocompromised patients1. The morphological plasticity of *C. albicans* is important for its pathogenicity, particularly the yeast-to-hypha transition, fundamental for biofilm formation, tissue penetration and immune system evasion2. The activation of this morphological switch is mediated by the cAMP and MAPK signaling pathways, which are regulated by Ras-like protein 1 (Ras1) and cell division control protein 25 (Cdc25)3. Ras1, a GTP-binding protein, is generally found in an GDP-bound inactive form, whose activation is mediated by the C-terminal Ras-guanine nucleotide exchange factor (GEF) domain of Cdc254. Therefore, the interaction between Ras1 and Cdc25 is a potential target to counteract C. albicans pathogenicity. We recently uncovered the existence of an auto-inhibitory region in Cdc25, a flexible lid that surrounds a druggable pocket in the catalytic domain of Cdc25. A synthetic peptide comprising the sequence of that auto-inhibitory region hampered the binding of Cdc25 to Ras14. As such, in this work we aimed at studying compounds targeting this druggable pocket to identify those that can act as molecular glues and modulate the Ras1/Cdc25 interaction. For that, a large-scale docking-based virtual screening was performed and nine compounds were selected for further trials. All of them were tested on a nucleotide exchange activity assay, where two compounds exhibited modulatory effects on the Ras1/CaCdc25 interaction. These compounds acted as molecular glues confirming their potential for the development of novel antifungal agents targeting *C. albicans*.

1. Lopes et al. Virulence (2022)
2. Prasad et al. Current Genetics (2023)
3. Pentland et al. Microbial Cel l(2017)
4. Manso et al. mBIO (2023)

## Pentabromopseudilin: a marine bacteria-derived compound with potent antifungal activity against pathogenic fungi

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There is a growing demand for novel antifungal agents with new mechanisms of actions effective against the increasing threat of antifungal resistance, recently highlighted by the World Health Organization (WHO). In this context, the microbiome of marine organisms has attracted considerable interest because, when cultivable, represents a sustainable and cost-effective source of bioactive compounds with therapeutic potential. In this work, a bacterial library isolated from the microbiomes of 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) associated with the production of bioactive secondary metabolites. Among the 39 bacterial isolates with antifungal activity, 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 decreased the intracellular pH after treatment, acting as a protonophore. It also showed strong activity against other fungi listed in the WHO critical priority group, such as *Candida auris*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. PBP impacts two of the most important virulence factors of *C. neoformans*, decreasing the polysaccharide capsule size by 60% and showing a significant reduction in melanin production. Cytotoxicity evaluation in fibroblasts revealed PBP is toxic at low concentrations, whereas pseudilin is non-toxic. To address this issue, we are developing PBP-inspired derivatives to boost potency and reduce toxicity.

# POSTERS PRESENTATIONS

**PO01 - Biotechnological potential of killer yeasts isolated from raw milk against mastitis-associated *Staphylococci***

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Killer yeasts are recognized for their ability to produce extracellular proteinaceous toxins and other bioactive compounds that confer competitive advantages within microbial ecosystems. Due to their antimicrobial properties, these microorganisms have attracted increasing attention as promising biotechnological tools for applications in food preservation, agriculture, and animal health. Raw bovine milk represents a diverse microbial ecosystem and a potential source of yeast strains with antagonistic activity against undesirable microorganisms. The present study aimed to evaluate the biotechnological potential of killer yeasts isolated from raw bovine milk through the assessment of their antagonistic activity against *Staphylococcus* spp isolates associated with bovine mastitis. A total of 11 yeast isolates were screened against 13 staphylococcal targets using cross-reaction double-layer agar assays. To simulate the physiological and pathological variations of the bovine mammary gland, these inhibition experiments were performed under distinct environmental combinations, including pH values of 4.88, 6.90, and 7.50, and incubation temperatures of 20 °C, 25 °C, and 30 °C. Several yeast isolates exhibited pronounced killer activity, with *Cyberlindnera jadinii* and *Kluyveromyces marxianus* demonstrating the strongest inhibitory effects against mastitis-associated staphylococci. Antimicrobial activity was influenced by environmental conditions, with the highest inhibition generally observed at 25°C and pH 6.90. The observed antagonistic activity demonstrates that raw milk constitutes a valuable source of killer yeasts with potential biotechnological relevance. These findings support further investigation of milk-derived killer yeasts as natural biocontrol agents and highlight their potential as complementary tools for controlling bacterial pathogens of veterinary importance in dairy production systems.

**PO02 - Development of a molecular diagnostic assay for rapid detection of *Botrytis cinerea* spores**

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Fungi are among agriculture's most persistent threats, spreading rapidly, establishing asymptomatic infections, and driving major outbreaks in high-value crops. Airborne spores from fungal pathogens such as *Botrytis cinerea* reduce yields, accelerate post-harvest spoilage, and compromise food quality and safety, contributing to losses that cost billions annually. Climate change is extending infection windows and accelerating the spread of more aggressive and fungicide- resistant strains. Despite these challenges, disease management remains fragmented, reactive and largely confined to in-field interventions. To support earlier and more accessible detection of *Botrytis cinerea*, we developed a loop-mediated isothermal amplification (LAMP) assay capable of detecting as few as 20 DNA copies within 30 minutes. We will further develop the assay to enable the direct detection of spores without the need for DNA extraction, thereby reducing the time to result. The assay will be integrated into a device currently being developed as part of the EU-funded SENTRY project, enabling near-source screening for *Botrytis cinerea* in propagation facilities, packing houses, and logistics hubs, supporting earlier disease detection and management.

**PO03 - Advancing the use of the non-conventional yeast *Pichia kudriavzevii* in valorisation of low-value feedstocks/residues into the high added value chemical itaconic acid**

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Itaconic acid is an unsaturated dicarboxylic acid widely used as a co-monomer in the synthesis of acrylic polymers and rubbers. Its versatile chemical structure makes it a promising alternative to petroleum-derived compounds (like acrylic acid) to be used in various catalytic and chemical synthesis routes. The itaconic acid available in the market today is largely produced by fermentation undertaken by *Aspergillus terreus* which produces this acid at high yields. Due to its pathogenic potential, handling of *A. terreus* in Europe requires higher safety facilities, prompting the search for alternative microbial hosts. *Pichia kudriavzevii* has emerged as a promising alternative host and has previously been genetically engineered to produce itaconic acid through the heterologous expression of the cis-aconitic acid decarboxylase gene (AtCadA) from *A. terreus*. Among other traits, *P. kudriavzevii* exhibits high tolerance to organic acids, bypassing the toxic effects imposed by the accumulation of itaconic acid in the broth, thus enabling its production under acidic conditions. This trait greatly facilitates subsequent downstream processing. In this study, key parameters and operation modes influencing *P. kudriavzevii*-mediated itaconic acid production from different feedstocks were investigated, with particular emphasis on the use of low-cost feedstocks (residues) obtained from agro-food industries. It is expected that these results may foster the utilization of *P. kudriavzevii* as a key player in the reuse of residues obtained from the agro-food industry, contributing to enable a circular bioeconomy in the sector.

## PO04 - Rational engineering of the Dct-02 transporter for enhanced dicarboxylic acid bioproduction

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The production of succinic (SA) and malic (MA) acids still relies largely on petrochemical processes, creating a need for more sustainable and efficient microbial production strategies. The Dct-02 transporter from *Aspergillus niger*, of the Tellurite-resistance/Dicarboxylate Transporter (TDT) family, promotes SA and MA secretion when heterologously expressed in *Saccharomyces cerevisiae*. Therefore, Dct-02 was engineered to improve dicarboxylic acid bioproduction. Conserved amino acid residues were identified by BLAST analysis, while the AlphaFold model of Dct-02 was used for molecular docking with dicarboxylic acids to identify putative substrate-binding residues. Thirty-one mutant alleles were generated by site-directed mutagenesis and expressed in *S. cerevisiae* CEN.PK 113-5D and W303-*1A jen1Δ ady2Δ* strains. GFP-tagging revealed that nine alleles were not properly located at the plasma membrane. The remaining mutants were characterised by spot assays and transport assays to evaluate cell growth on carboxylic acids and SA and MA import/export, respectively. Eleven mutant alleles promoted improved growth on carboxylic acids, indicating a decrease of transporter activity, whereas three mutants displayed poorer growth, suggesting increased export capacity. The quantification of extracellular organic acids identified one mutant allele with increased SA and MA secretion compared to the wild-type Dct-02. The mutant alleles had no impact in the import of radiolabelled dicarboxylates. Overall, these findings confirmed that Dct-02 acts as a SA and MA exporter and identified an engineered mutant with enhanced export capacity for microbial succinate bioproduction.

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## PO05 - Integrated discovery and engineering of chitinases for antifungal applications and chitin valorization

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Chitinases are attractive biocatalysts for both sustainable chitin valorization and the development of alternative antifungal strategies, owing to their ability to degrade chitin, a key structural component of fungal cell walls. This work presents an integrated approach combining chitinase screening, recombinant production, and protein engineering. A high-throughput screening platform identified Saccharomyces cerevisiae PYCC4072 and Candida albicans SC5314 as capable of growing with colloidal chitin as the sole carbon source, suggesting extracellular chitinolytic activity. Recombinant production of the *C. albicans* chitinase Cht3 yielded an active enzyme capable of inhibiting the growth of *Aspergillus niger* and *Diplodia corticola*, demonstrating its antifungal potential. To further improve enzyme performance, Cht3 is currently being engineered through fusion with chitin-binding domains and expressed in a high-copy *S. cerevisiae* system to enhance substrate affinity while maintaining enzyme stability. The optimized enzymes will ultimately be applied to the production of bioactive chitooligosaccharides from chitin-rich waste, contributing to the development of sustainable biotechnological processes with applications in agriculture, environmental biotechnology, and health.

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## PO06 - Efficacy assessment of a combination of wavelengths of blue light against healthcare-related *Candida* species

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Fungal infections within healthcare are increasing alongside with antifungal resistance, resulting in more difficult diagnosis and therapeutic options. The presence of fungi in healthcare settings is a risk factor for infection, as these microorganisms may act as reservoirs on surfaces and medical equipment surrounding patients. Consequently, efforts in infection prevention and control, such as disinfection, must be taken into serious account. Antimicrobial blue light (aBL) has been the focus of extensive research due to its potential for reducing bacterial contamination. However, recent studies have also demonstrated the efficacy of this non-touch disinfection method in inactivating *Candida* species. Most studies have focused on a single wavelength of 405 nm; however, a combination of wavelengths has been shown to have a more extensive spectrum of activity. The present study evaluated the effect of a combination of wavelengths of aBL upon *Candida albicans*, *Candida auris*, *Candida parapsilosis* and *C. glabrata* during eight hours of exposure. The most susceptible species was *C. glabrata*, with a sub-lethal dose of 5.5 hours for the reference strain and six hours for the clinical strain. *C. parapsilosis* and *C. auris* demonstrated a similar behaviour, with a mean death rate constant (k) of 0.35. Interestingly, *C. albicans* was demonstrated to be the most tolerant species. Overall, the antifungal effect of aBL appears to be a dose-dependent mechanism. These findings indicate that aBL disinfection may represent a viable option for the decontamination of *Candida* due to its practical use.

**PO07 - *Candida* species and hydrogen peroxide: effect following repeated exposure**

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Invasive fungal infections represent a significant healthcare problem, as *Candida* spp. belong to a top public health priority of the WHO. This problem is associated with the emergence of antifungal resistance, the lack of diagnostic tools and limited treatment options. To address the increasing presence of fungi in healthcare settings, the use of infection prevention and control strategies is essential. Hydrogen Peroxide ($H_2O_2$) is a well-known method of healthcare disinfection, with proven efficacy even in short cycles. However, when applied repeatedly at low concentrations, it might result in a selective pressure that could change several microbial characteristics. The present study aims to evaluate whether repeated exposure of *Candida* spp. to $H_2O_2$ affects antifungal susceptibility and biofilm formation. For 30 days, *Candida albicans*, *Candida parapsilosis* and *Candida auris* were exposed to different concentrations of $H_2O_2$ related to their minimum inhibitory concentration. The antifungal susceptibility testing was performed by broth dilution at each time point (days 0, 10, 20 and 30), as well as the evaluation of biofilm formation, using a crystal violet staining assay. Repeated exposure to the disinfectant did not affect the antifungal susceptibility of *C. auris*. However, minor variations in the profiles of *C. albicans* and *C. parapsilosis* were registered. No variations were identified in the capacity to form biofilm. In summary, the repeated exposure to $H_2O_2$ does not interact with the antifungal susceptibility profile of the tested *Candida* strains nor affect biofilm formation. Such findings support the use of $H_2O_2$ as an agent for healthcare settings disinfection, even for short cycles.

**PO08 - Adenosine A2A receptor deficiency reshapes gut microbiota, inducing an enrichment in *Saccharomyces* spp.**

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Adenosine A2A receptors (A2AR) are pharmacological targets in neurological and cardiovascular diseases, increasingly recognized as modulators of immune responses and intestinal barrier integrity. Before we described that inflammation due to gut *Candida albicans* dysbiosis is ameliorated by A2AR[1], but it remains largely unknown whether A2AR activity shapes the gut microbiota and how gut microorganisms can influence adenosine metabolism and consequently A2AR signaling. We hypothesized that the absence of A2AR promotes microbial dysbiosis associated with mucosal inflammation. To test this hypothesis, fecal mycobiota and bacteriota from adult wildtype (WT) and global A2AR knockout (A2ARKO) mice were characterized by ITS and 16S rRNA gene sequencing, respectively. Amplicon sequence variants were inferred using DADA2; diversity, community structure, and differential taxa were assessed using phyloseq, PERMANOVA, and LEfSe. Predicted microbial functions were inferred with PICRUSt2 based on KEGG pathways. A2ARKO mice displayed alterations in gut microbiota compared with WT controls. We identified specific microbial signatures of dysbiosis in the A2AR KO group, involving loss of homeostatic symbionts, such as *Dubosiela*, *Gordonibacter*, as well as the proliferation of pro-inflammatory pathobionts, such as *Mucispirillum*, *Acetatifactor*, *Anaerovorax*, *Desulfovibrio*, *Helicobacter*, and *Candida*. On the other hand, some potentially beneficial taxa, such as *Blautia*, *Roseburia*, and particularly *Saccharomyces*, appeared enriched in the A2ARKO group, suggesting an attempt to counterbalance the dysbiosis caused by A2AR impairment. Functional predictions revealed genotype-specific metabolic signatures, including enrichment of purine nucleotide degradation and pyrimidine biosynthesis. These findings demonstrate a previously underappreciated link between purinergic signaling, the gut microbiome, and intestinal homeostasis.

[1] Rodrigues et al. (2016) Oncotarget 7:62862–62872.

**PO09 - *Aspergillus fumigatus* and cryptic species exhibit distinct strategies for adaptation to host-relevant conditions**

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*Aspergillus fumigatus* is the leading cause of invasive aspergillosis however, cryptic species within section *Fumigati* are emerging pathogens due to antifungal resistance and misidentification in routine clinical practice. We investigated whether divergence in virulence-associated proteins accompanies phenotypic diversification across section *Fumigati*. Forty virulence-associated proteins were analyzed across 72 genomes (42 cryptic species and 30 *A. fumigatus*), while 16 random representative isolates were evaluated under host-relevant conditions. Protein-based multidimensional scaling revealed species-specific divergence in virulence-associated proteins, most prominently for host interactions (DHN-melanin, CatA, Fos1), and secondary metabolism (LaeA, GliP, HvlA). Phenotype-based principal component analysis reproduced the species-level clustering observed in protein-based analyses, clearly separating *A. fumigatus* from cryptic species. Cryptic species displayed enhanced metabolic activity and biomass production on alternative respiratory carbon sources (mannitol and lactose), reduced macrophage damage measured by lactate dehydrogenase release, and higher frequency of pan-azole resistance. *A. fumigatus* exhibited faster germination, greater metabolic activity and biomass under thermal, osmotic, cell wall and oxidative stress, and enhanced fungal persistence following macrophage interaction, reflected by higher post-interaction metabolic activity. Category-specific protein phylogenies and phenotypic heatmaps demonstrated broad genotype-phenotype concordance. Together, these findings demonstrate that divergence in virulence-associated proteins accompanies species-specific metabolic, stress-responses and host-pathogen adaptations across section *Fumigati*.

**PO10 - Exploring *Alternaria alternata* sialidase as a novel target for antifungal therapeutics**

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*Alternaria alternata* is one of the most important sources of fungal allergens worldwide and is frequently associated with severe asthma. A proteomic analysis of *A. alternata* revealed high expression levels of sialidase, an enzyme that cleaves terminal sialic acids from glycoconjugates and plays a key role in the pathogenicity of several microorganisms. The abundance of sialylated glycoconjugates in the human airway epithelium makes this enzyme important for host–pathogen interactions, namely being used as a therapeutic target for antivirals. With this work, we aim to characterize *A. alternata* sialidase by investigating its localization, activity, and structure, and to design specific inhibitors as a strategy to find novel antifungal therapeutics. To quantitatively assess enzyme activity in the form of free sialic acid, the Svennerholm periodate–resorcinol assay was used and optimized to improve the method's efficiency. Free sialic acid release was quantified using a calibration curve generated with N-acetylneuraminic acid (Neu5Ac). Sialidase activity was detected in both spores and hyphae, with significantly higher activity in spores and in intracellular extracts compared to extracellular fractions. Notably, AlphaFold-based structural prediction revealed an unusual seven-bladed β-propeller fold, in contrast to the canonical six-bladed β-propeller architecture reported for most sialidases. In parallel, the chemical synthesis of the classical sialidase inhibitors 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (Neu5Ac2en) and 2,3-dideoxy-2,3-dideoxy-D-glycero-D-galacto-nonulosonic acid (KDN2en) is currently underway, beginning with the protection of the hydroxyl groups of Neu5Ac. Ongoing studies aim to characterize this enzyme further and develop specific inhibitors as potential tools to mitigate *A. alternata*-associated diseases.

**PO11 - Towards the identification of new molecules with potential to control the activity of pathogenic *Candida* yeasts**

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The increase in infections caused by the genus *Candida* and the rise in resistance to currently available antifungal agents highlight the urgency of identifying new molecules with activity against species of this genus. In this work we will discuss the efforts that our lab has been putting in this matter, identifying molecules with potential to inhibit growth and pathogenesis of *Candida glabrata*, *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*, the most prominent causative agents of candidiasis. These putative candidate molecules with anti-Candida activity have been searched in natural extracts obtained from the tree Sorbus aucuparia (or TRAMAZEIRA), in the scope of the funded research project TRAMONTE. Similarly, we will show the results obtained using antagonistic assays between the selected target yeast species and a range of bacteria and yeasts isolated from the soil that have been collected during the execution of the science citizen project, “Hoje, o Biocientista sou eu!”. On the overall, this work is expected to boost the development of new antifungal therapies leveraging the rich diversity of molecules taken from plants and resulting from the microbiologial “warfare”.

## PO12 - Integrated gut mycobiome–urinary metabolome signatures in aortic stenosis patients after aortic valve replacement

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Severe aortic stenosis (AS) is the most common valvular heart disease requiring surgical replacement. The gut mycobiome and its association with urinary metabolome in AS remain poorly characterised. Surgical aortic valve replacement (SAVR) provides a longitudinal within-subject model to investigate postoperative recovery. In this exploratory pilot study, 105 patients undergoing SAVR were prospectively enrolled. Stool and urine samples were collected before surgery (V1) and approximately 4 months after SAVR (V2). The gut mycobiome was characterised by internal transcribed spacer amplicon sequencing and urinary volatile organic compounds (VOCs) by gas chromatography–mass spectrometry. Longitudinal paired analyses were performed using paired tests, participant-stratified PERMANOVA, repeated-measures ANCOM-BC2, and exploratory multilevel sparse partial least squares (sPLS) integration. Multiple testing was corrected within each analysis family (Benjamini–Hochberg; exploratory FDR < 0.10). No evidence of longitudinal changes in fungal α-diversity (Shannon: estimate = −0.005, 95% CI −0.299 to 0.284; P = 0.969) was observed, nor in community composition ($R^2$ = 0.021, P = 0.18). Meyerozyma (15.3% prevalence) was the only differentially abundant genus (log2FC = 0.83, 95% CI 0.37–1.29; FDR = 0.071). Five urinary VOCs changed despite a small overall volatilome shift ($R^2$ = 0.016, P = 0.003). Multilevel sPLS identified fungal genera and VOCs contributing to shared covariance between datasets, with Saccharomyces loading in the opposite direction to monoterpenes. This study identified coordinated fungal–metabolite signatures following SAVR. Given the single-arm before–after design, sample size, and fungal microbiome variability, these findings should be considered hypothesis-generating and warrant validation in larger longitudinal studies.

## PO13 - Characterization of household mould exposure in patients with fibrotic ILDs

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Fibrotic progression in interstitial lung diseases (ILDs) derives from complex interactions between genetic susceptibility and environmental exposures. Hypersensitivity pneumonitis (HP), the most common fibrotic ILD in our cohort, is frequently difficult to distinguish from idiopathic pulmonary fibrosis (IPF) due to overlapping clinical features. We previously showed that a mixture of fungal antigens collected from HP patients' homes elicited a strong T-cell response, supporting its potential as a complementary diagnostic tool. This study aimed to characterize household mould exposure in patients with pulmonary fibrosis through home inspection and air sampling. Duplicate indoor and outdoor air samples were collected from the homes of 62 patients with HP, IPF, and other ILDs in northern Portugal. Fungal concentrations were quantified and species identified by morphological analysis. Signs of water damage or visible mould were observed in 29% of homes, while 76% showed abnormal fungal exposure, defined as airborne fungal concentrations >500 CFU/m³, higher indoor than outdoor counts, or toxigenic species >12 CFU/m³. Cladosporium spp. and Penicillium spp. showed the highest prevalence across all diagnoses, whereas Rhodotorula spp. was significantly more frequent in IPF homes. Aspergillus fumigatus and Aspergillus niger were more concentrated indoors than outdoors. IPF patients were exposed to higher overall mould and toxigenic fungal concentrations than HP patients. These findings highlight underrecognized household mould exposure, particularly in IPF, and suggest that differences in fungal species may contribute to distinct clinical phenotypes and prognosis.

## PO14 - Host-derived polyols as metabolic regulators of macrophage function during fungal infection

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Invasive pulmonary aspergillosis (IPA) is a life-threatening fungal disease and a major cause of morbidity and mortality in immunocompromised individuals, including patients with hematological malignancies and recipients of solid-organ or hematopoietic stem-cell transplants. Each year, approximately 2 million people develop IPA, with mortality remaining unacceptably high due to limited diagnostic tools and therapeutic options. Recent evidence indicates that metabolic reprogramming is a key mechanism by which immune cells respond to infection. Targeted metabolomics analyses of Aspergillus-infected macrophages have revealed marked modulation of the polyol pathway during fungal infection. This metabolic pathway converts glucose into sorbitol through the activity of aldose reductase, after which sorbitol is oxidized to fructose by sorbitol dehydrogenase. In addition to its role in glucose metabolism, the polyol pathway can influence oxidative stress, inflammatory signaling, and nutrient availability, all of which may affect pathogen survival and host immune responses. We therefore hypothesize that this pathway plays a critical role in antifungal immunity. We are currently investigating how the polyol pathway shapes host–fungus interactions and influences susceptibility to IPA using advanced preclinical models and studies in human patients. Ultimately, our goal is to determine whether targeting this metabolic pathway represents a novel host-directed therapeutic strategy for IPA.

**PO15 - Antifungal activity of natural extracts against *Candida* spp.: from chemical characterization to synergistic interactions**

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The emergence of antifungal resistance highlights the need for new bioactive compounds and alternative strategies for the management of yeast infections. In this study, a sequential extraction approach was applied to obtain bioactive fractions from orange pomace. Volatile compounds were obtained by microwave-assisted hydrodistillation (MAHD), while the residual biomass was subsequently processed by microwave-assisted subcritical water extraction (MASWE) to obtain polyphenol-rich extracts. The chemical composition of the resulting extracts was characterized, followed by the evaluation of their safety in HaCat cell line and their antifungal activity against clinically relevant *Candida* spp. Antifungal activity was assessed by determining minimum inhibitory concentrations (MICS) and minimum lethal concentrations (MLCs), allowing the characterization of both inhibitory and fungicidal effects. The extracts displayed variable activity against the tested *Candida* strains, with differences in susceptibility observed between species, depending on the extract. Checkerboard assays were subsequently performed to investigate interactions between the most active extracts and conventional antifungal agents. Fractional inhibitory concentration indices (FICIs) revealed distinct interaction profiles, including synergistic, additive and indifferent effects, depending on the extract-antifungal combination and *Candida* species. Overall, these findings highlight the antifungal potential of the investigated natural extracts and their possible application as standalone agents or in combination with conventional antifungal drugs.

**PO16 - Species-dependent phenotypic adaptation of vaginal yeast isolates from asymptomatic women to vaginal-like acidic conditions**

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The healthy vaginal environment is characterized by an acidic pH maintained by lactic acid-producing *Lactobacillus* spp., yet vaginal yeasts frequently colonize asymptomatic women. As most phenotypic studies are performed under standard laboratory conditions, they may not accurately reflect the vaginal niche. This study investigated the influence of lactate-conditioned vaginal-like acidic conditions on antifungal susceptibility and biofilm-associated phenotypes of vaginal yeast isolates recovered from asymptomatic women. Twelve vaginal yeast isolates from asymptomatic women of reproductive age were identified by MALDI-TOF MS and/or molecular methods, comprising *Candida albicans* (n=6), *C. parapsilosis* (n=2), *C. guilliermondii* (n=2), *C. glabrata* (n=1) and *C. africana* (n=1). Minimum inhibitory concentrations (MICs) of representatives of four antifungal classes were determined under standard laboratory conditions and lactate-conditioned vaginal-like acidic conditions. Biofilm biomass and metabolic activity were evaluated using crystal violet (CV) and MTT assays, respectively. Preliminary results showed a marked increase in fluconazole MICs under lactate-conditioned vaginal-like acidic conditions in most isolates, whereas micafungin and flucytosine susceptibility remained largely unchanged. Biofilm biomass was maintained or increased under these conditions, while metabolic activity decreased markedly in most isolates. In contrast, the two *C. parapsilosis* isolates retained comparatively higher metabolic activity, suggesting species-dependent adaptation to the vaginal environment. These findings indicate that vaginal yeast isolates display species-dependent phenotypic adaptation to lactate-conditioned vaginal-like acidic conditions, highlighting adaptive responses that may contribute to persistence within the healthy vaginal niche and influence antifungal susceptibility.

## PO17 - The long pentraxin PTX3 shapes macrophage immunometabolic reprogramming during *Aspergillus fumigatus* infection

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Fungal infections are a major global health burden, affecting more than 1 billion people worldwide and causing an estimated 3 million deaths each year. Immunocompromised individuals are especially vulnerable to invasive pulmonary aspergillosis (IPA), most commonly driven by *Aspergillus fumigatus*. The long pentraxin PTX3 is a soluble, fluid-phase pattern-recognition molecule that is critical for efficient antifungal immune sensing. Produced mainly by macrophages and neutrophils, PTX3 supports host defense by binding fungal structures, promoting opsonization, and facilitating downstream effector responses. Because metabolic rewiring is increasingly viewed as a key determinant of macrophage activation during A. fumigatus challenge, we investigated how PTX3 intersects with these immunometabolic programs in IPA. We show that Ptx3-deficient (Ptx3−/−) bone marrow–derived macrophages exhibit reduced control of fungal germination, which parallels an inadequate shift of cellular metabolism toward glycolysis. In contrast, upon *A. fumigatus* exposure, Ptx3−/− macrophages accumulate pentose phosphate pathway (PPP) metabolites and upregulate the G6PD-SHPK axis, which controls PPP entry and downstream of sedoheptulose-phosphate metabolism, and shapes macrophage activation and redox homeostasis. Consistent with reports that *A. fumigatus* can influence PPP enzymes, our data support a model in which PTX3 deficiency drives SHPK activation, which redirects the glucose metabolism toward the PPP at the expense of glycolytic activation, thereby weakening macrophage antifungal effector functions and compromising protective immunity. Overall, these findings point to a previously unrecognized pathogenic mechanism in IPA linking PTX3 deficiency to disrupted macrophage metabolic programming and increased susceptibility to fungal infection.

**PO18 - Investigating the role of a LysR-type bacterial transcriptional regulator in *Starmerella bombicola***

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Yeasts belonging to the *Wickerhamiella/Starmerella* (W/S) clade are notable for having acquired multiple xenologous genes from bacteria and filamentous fungi (Pezizomycotina) through horizontal gene transfer (HGT). Although most of these acquired genes are associated with metabolic pathways, a few are involved in regulatory functions, including bacterial LysR-type transcriptional regulators (LTTRs). In bacteria, LTTRs regulate diverse genes involved in stress, virulence, metabolism, and motility. In a previous study, LTTR homologs were identified and found to be transcriptionally active in *Wickerhamiella domerqiae* and *Wickerhamiella versatilis*. A subsequent survey of more than one hundred genomes within the W/S clade revealed the presence of two distinct LTTR types across the lineage. To investigate the functional role of these regulatory genes in the W/S clade, a LTTR deletion mutant of *Starmerella bombicola* (LysRΔ::HygR) was constructed, and the phenotypic characteristics of the wild-type (WT) and mutant (MT) strains were compared. Growth performance was assessed using a microplate-based assay. Under oxidative stress induced by 25 mM $H_2O_2$ at 25°C, distinct growth profiles were observed between the WT and MT strains. This condition was therefore selected for subsequent transcriptomic analysis. RNA sequencing and comparative transcriptomic analyses revealed significant differences in gene expression profiles between the MT and WT strains, indicating that disruption of the LysR gene affects transcriptional regulation under oxidative stress. A total of 37 differentially expressed genes (DEGs) were identified. Notably, several of these DEGs corresponded to genes acquired from bacteria through HGT, suggesting that they may be regulated, either directly or indirectly, by the LTTR.

**PO19 - Dissecting eukaryotic horizontal gene transfer using yeast models**

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Horizontal gene transfer (HGT) is uncommon among eukaryotes, and the factors enabling the successful acquisition and retention of exogenous genes still remain poorly understood. The *Wickerhamiella/Starmerella* (W/S) yeast clade contains an exceptionally high number of horizontally acquired genes for an eukaryotic lineage, many of which have equipped these yeasts with relevant metabolic functions. We recently identified a *Wickerhamiella* species capable of growing on sucrose, a metabolic function that was seemingly absent in the W/S ancestor. Comparative genomics revealed that this strain harbours five maltase-like (ME) genes and eight maltose transporter-like (MT) genes of bacterial and fungal origins, organized either as enzyme-transporter clusters or as single genes. To assess their functional significance, heterologous expression in Saccharomyces cerevisiae demonstrated that one ME and two MT genes encode functional proteins involved in maltose/sucrose utilization. Although this illustrates how HGT can introduce metabolic capabilities that facilitate adaptation to the clade's ecological niche, more broadly it remains unclear which genetic and environmental factors enable HGT to become evolutionarily successful in eukaryotes. To address this question, ongoing research aims to establish a laboratory framework to simulate HGT and monitor hits dynamics in real time. The approach will be applied to both wild type W/S strains and knockout mutants lacking candidate genes predicted to influence HGT, allowing the identification of conditions that promote the acquisition, adaptation and stable maintenance of foreign DNA. This work will provide new insights into the mechanisms underlying eukaryotic HGT and help explain one of the most intriguing processes driving genome evolution.

## PO20 - Population-level dynamics of horizontally transferred genes in *Wickerhamiella versatilis*

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Horizontal Gene Transfer (HGT) is the non-sexual movement of genetic information between organisms. It is an important mechanism of adaptation in bacteria, but in eukaryotes it has long been considered rare. However, increasing evidence shows it is an important mechanism in some eukaryotic lineages. One of these lineages is the Wickerhamiella/Starmerella (W/S) yeast clade, which has much higher occurrence of HGT events than any other yeast clade, with bacteria and filamentous fungi as donors. Within this clade, Wickerhamiella versatilis is particularly interesting. Firstly, because it exhibits the highest rate of HGT in the clade, with hundreds of HGT-derived genes identified. Secondly, because it occupies two different environments: high salt, human-associated fermentations (e.g., soy sauce) and bee-associated environments (e.g., bees, honey, and flowers). To assess whether HGT-derived genes are differentially retained, lost, or expressed depending on the ecological context, 17 W. versatilis strains from contrasting environments were used (6 fermentation-associated and 11 bee-associated). First, high-quality genome assemblies were generated using Illumina and Nanopore technologies. Genetic relatedness among the isolates was assessed using both average nucleotide identity metrics and phylogenomic reconstruction. HGT-derived genes from bacteria and fungi were identified using a phylogenetic-based in house pipeline. Afterwards, the HGT-derived gene repertoire was compared between ecological and geographic groups, including analysis of presence/absence, chromosomal location and differential expression. This study provides a pioneering population-level investigation of HGT dynamics, offering new insights into the evolutionary and ecological factors shaping the acquisition, retention, and expression of horizontally transferred genes.

**PO21 - Comparative evolutionary genomics of *Candida albicans* clinical isolates spanning asymptomatic colonisation, mucosal infection, and invasive candidiasis**

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*Candida albicans* is a member of the human microbiota that can persist as an asymptomatic coloniser, cause mucosal infection, or produce life-threatening invasive disease. Its genome displays extensive variation, including high single nucleotide polymorphism (SNP) density, chromosomal rearrangements, copy number variation, and aneuploidy. Some alterations can arise under environmental and host-associated pressures and influence clinically relevant traits such as stress tolerance, biofilm formation, and yeast-to-hyphal transitions. However, the genomic features distinguishing colonising, mucosal infections, and invasive isolates remain poorly understood. We applied comparative and evolutionary genomics approaches to 124 clinical isolates from distinct anatomical niches and three clinical categories: asymptomatic colonisation, mucosal infection, and invasive candidiasis. Whole genome comparisons were integrated with reference-based SNP analysis and detection of copy-number variation and aneuploidy. This approach revealed clade-specific gene content, including variation in clade 13 (*Candida africana*), whose reduced virulence and vaginal niche restriction have been linked to lineage-specific gene loss. SNP analyses indicated that genomic diversity was structured primarily by phylogenetic clade rather than clinical status or host-site isolation, highlighting the need to account for population structure in clinical comparisons. Reference-based sequence mapping also revealed extensive additional variation, including insertions/deletions and other non-biallelic differences, that are not captured by standard SNP pipelines. Partial and whole-chromosome aneuploidies were detected in 20 genomes; complete aneuploidies predominantly involved chromosomes 4 and 5 and were observed mainly in isolates from mucosal infections or invasive candidiasis. Overall, integrating SNP-based, structural, and whole-genome analyses is essential to capture genomic diversity potentially relevant to host interaction and disease.

**PO22 - Leveraging nanopore sequencing for elucidation of high-resolution genomic analysis of *Hanseniaspora guilliermondii* strains**

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*Hanseniaspora guilliermondii* (Hg) is commonly found in the microbiome of grapes and its presence improves wine sensorial profiles by producing aromas not produced (or produced at low concentrations) by *Saccharomyces cerevisiae*, the yeast that leads the vinification process. The genome sequence of a Hg strain (UTAD222) was first disclosed by our laboratories in 2019, shedding light into the biology of this species. Among other factors, it stands out the lack of functional neoglucogenesis and glyoxylate cycles and what appears to be a new family of alcohol acetyl transferases, the enzymes responsible for the formation of ethyl esters. In this work we extended the genomic analysis to several other Hg strains combining results from Illumina and NanoPore sequencing that helped us to reconstruct, almost at the chromosome level, the genomic repertoire of these strains. Subsequent annotation of these genomes also allowed us to make in silico comparative proteomic analyses that led to the identification of a set of what appears to be species-specific proteins of this species. This work expands the genomic resources available for *Hanseniaspora*, providing chromosome-organized, annotated genomes for ten Hg strains and a solid foundation for exploring the genetic and proteomic diversity of this underexplored genus, supporting its future use as a microbial cell factory.

## PO23 - Gene losses and gains shape genome evolution in the bee-associated *Wickerhamiella/Starmerella* yeast clade

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The *Wickerhamiella/Starmerella* yeast clade comprises species with a close association with bees and flowers, making them a valuable model for studying the impact of host-associated lifestyles at a genomic level. Previous studies have suggested that this lineage exhibits an unusually high rate of horizontal gene transfer (HGT) combined with extensive gene loss compared to other yeast lineages. However, the extent, timing, and functional impact of these events remain largely unexplored. In this study, close to 100 proteomes from across the W/S clade were analysed using a comparative phylogenomics approach to reconstruct gene gain and loss dynamics throughout their evolutionary history. Orthogroups were identified across all species, a species tree was reconstructed from single-copy orthologs and gene gains and losses were mapped onto this tree to determine which genes were lost, when these losses occurred, and whether HGT events compensated for ancient gene loss events. This study will provide a comprehensive view of gene gain/loss dynamics in the W/S clade, identifying candidate genes and metabolic pathways that may underpin ecological adaptation to bee-associated lifestyles. More broadly, it will highlight the importance of gene loss as a fundamental evolutionary process that can drive adaptation and innovation, particularly when coupled with the acquisition of novel functions through HGT.

## PO24 - Exploring the phenotypic space leading to ecological diversification in a complex yeast lineage

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Understanding how phenotypes arise from the interaction of genes, environment and evolution remains a central challenge in biology, with important implications for biomedicine, biotechnology, and the development of novel fermentations. Recently, we documented several cases of intraspecific hybridization in Zygosaccharomyces, identifying the parental species and the genomic structure of hybrid genomes. Moreover, we hypothesize that hybrids have enhanced capacities that are relevant for the colonization of certain anthropic niches that pose physiological stresses, like for example food substrates rich in sodium chloride. Here we test our hypothesis by employing a systematic characterization of circa 40 hybrid and non-hybrid Zygosaccharomyces strains under multiple stress conditions using microplate-based growth assays, and measuring lag phase, growth rate and carrying capacity. We employed multivariate analyses to identify phenotypic patterns of stress adaptation across species. Finally, and taking advantage of the availability of whole-genome data for all cultures, we attempt to link phenotypic data with possible genetic signatures associated with stress resistance, by surveying presence/absence and copy number variation of an extended dataset of genes linked to stress tolerance.

Mariana Versiani acknowledges the financial support provided by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) through a PhD scholarship.

## PO25 - Urban waters: a hidden reservoir of antifungal-resistant *Candida* spp.

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Urban freshwater systems are increasingly recognized as reservoirs of opportunistic pathogenic yeasts, yet their role remains insufficiently understood. Within a One Health framework, this study investigated the occurrence, community dynamics, antifungal susceptibility, and biofilm-forming ability of *Candida* spp. recovered from an urban freshwater lake system in Aveiro, Portugal. Water samples were collected from five sites during four sampling campaigns, between June 2025 and March 2026. In total, 290 yeast-like isolates were recovered, of which 174 were identified as *Candida* spp. based on MSP-PCR fingerprinting and sequencing of the ITS region and partial 28S rRNA gene. The community included *C. parapsilosis* (n = 67), *C. lusitaniae* (n = 47), *C. palmioleophila* (n = 17), *C. albicans* (n = 15), *C. glabrata* (n = 11), *C. krusei* (n = 11), *C. bovina* (n = 4), *C. rugosa* (n = 1), and *C. tropicalis* (n = 1). Alpha diversity did not differ significantly among sampling sites or campaigns, whereas beta diversity analyses revealed significant temporal variation in community composition. Antifungal susceptibility testing of 53 representative isolates revealed substantial variability in MIC profiles. Nineteen isolates (35.8%) were classified as resistant according to available EUCAST clinical breakpoints, most frequently to echinocandins, particularly anidulafungin, although azole and amphotericin B resistance were also observed. All resistant isolates formed metabolically active biofilms. The recovery of antifungal-resistant, biofilm-forming, clinically relevant Candida spp. from an urban freshwater system supports the inclusion of urban freshwater ecosystems in One Health surveillance strategies to target antifungal resistance and the circulation of potential fungal pathogens.

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**PO26 - SF1 is a novel marine-derived compound with promising antifungal activity**

Margarida Gonçalves; Susana Ribeiro; Nuno Miguel Saraiva; Cláudia Malta-Luís; Teresa Pissarro; Américo Gonzalez Duarte; Oscar Lenis Rojas; Maria Rita Ventura; Catarina Pimentel

Instituto de Tecnologia Química e Biológica António Xavier

Invasive fungal infections 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. We have recently identified a potent antifungal compound derived from a marine sponge extract, designated SF1. SF1 exhibits strong efficacy against several *Candida* species responsible for IFI, including those inherently tolerant to azoles such as *C. glabrata* and *C. krusei*. It is fungicidal, capable of overcoming resistance to existing antifungals, and acts through a novel mechanism of action. Furthermore, SF1 addresses gaps in the current antifungal pipeline, showing activity against species such as *C. krusei* and *C. guilliermondii*. To ensure a scalable and sustainable supply, efforts are currently underway to establish a synthetic route for SF1.

## PO27 - Developing an interactive web application for promoter-region analysis in antifungal resistance studies

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Fungal infections represent a global health challenge, accounting for 3.5 million deaths annually, and the problem is aggravated by the emergence of resistance to antifungal agents in clinical isolates. After decades of research, it is widely accepted that antifungal resistance evolves through the accumulation of point mutations (e.g. PDR1 gain-of-function mutations in *Nakaseomyces glabratus*). However, it has not yet been possible to define a comprehensive drug-resistance signature based on mutations in coding regions. Therefore, our work proposes to extend the commonly used comparative genomic analysis pipelines towards non-coding promoter regions, and to develop tools to enable researchers to streamline this analysis. We will present our prototype interactive web application developed in Python using Streamlit, Pandas, and Plotly, designed to: 1) load and process GFF3, VCF, and reference FASTA files, as well as clinical metadata tables; 2) extract SNPs located in gene promoter regions and map them onto a physical scale of 1000 bp upstream of each gene; 3) test data homoscedasticity and apply statistical tests for genotype–phenotype association analyses; and 4) integrate Position Weight Matrices from the YEASTRACT database (https://yeastract.com/) to calculate regulatory impact. We demonstrate the utility of the new tool considering the CDR1 gene as a case study, by characterizing CDR1 promoter-region diversity across 84 clinical isolates and integrating these data with clinical metadata, thereby supporting the investigation of promoter-region mutations as potential determinants of antifungal resistance.

**PO28 - From environmental microbiome to antifungal leads**

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The increasing emergence of antifungal-resistant fungi, together with insufficient investment in the discovery and development of novel antifungal agents over the past decade, has made antifungal resistance an escalating global health threat. In MicroMundo@Oeiras, a citizen science project implemented at ITQB NOVA under the "Ciência + Cidadã" programme, with the support of the Municipality of Oeiras, students isolated soil bacteria in teaching laboratory settings and investigated their capacity to produce antibiotic compounds. In its most recent edition, the project's scope was expanded to include the search for antifungal-producing bacteria, with bacterial isolates also being tested against the baker's yeast Saccharomyces cerevisiae. A collection of 37 bacterial isolates was subsequently tested against *Candida* species, medically relevant opportunistic pathogenic yeasts for which antifungal resistance is an increasing concern. Five isolates exhibited antifungal activity and were effective against strains resistant to marketed antifungal drugs. Ongoing work aims to identify the bioactive compounds responsible for this activity.

## PO29 - Dissecting the role of the ABC transporter aus1 in azole resistance in the pathogenic yeast *Candida glabrata*

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Azole resistance among *Candida* yeasts is problematic as it increases therapeutic failure, resulting in prolonged hospital stays and worse clinical prognosis. Clinical strains of the *Nakaseomyces glabratus* species (formerly classified as *Candida glabrata*) acquire resistance to azoles mainly by acquiring gain-of-function (GOF) mutations in the regulator Pdr1. However, some strains (including ISTB218, the strain at the center of this work) exhibit a resistance phenotype but encode a “wild-type” (non-GOF) CgPDR1 allele. Recent OMICS analysis of the ISTB218 strain led to hypothesized mechanisms explaining the observed resistance phenotype, including the over-expression of the ATP-binding cassette (ABC) transporter Aus1. In line with this, deletion of CgAUS1 abolished the resistance phenotype to fluconazole of ISTB218 cells and led to prominent alterations in the fluidity of the plasma membrane. In this work we scrutinize in more detail the role of CgAus1 in azole tolerance in *C. glabrata* exploring for that a lipidomic analysis to clarify which molecules could serve as substrates for this transporter and impacting the biophysical properties of the plasma membrane.

**PO30 - Landscape of the frequency of isolation of *Candida* strains in clinical samples obtained from healthcare settings in the Lisbon area**

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The importance of *Candida* yeasts as human pathogens is being increasingly recognized as more attention is given to the burden posed by fungal infections. This issue has been boosted by the recent identification by the World Health Organization of various fungal species (including *C. albicans* and *C. auris*) as priority agents. To understand the frequencies of *Candida* isolation across clinical samples in healthcare settings in the Lisbon area, our laboratory began a survey around 2015. Since then, around 1333 isolates have been gathered, subsequently identified at the species level and then phenotyped for their phenotype of resistance to azoles, the most commonly used antifungals in clinical practice. In this work, we will show and discuss these results, putting in light the mechanisms underneath the azole-resistance phenotype exhibited by the identified resistant strains. It is expected that this work provides an updated overview of the local epidemiology of azole resistance in *Candida* spp. while also offering relevant insights for microbiological surveillance and the optimization of antifungal therapeutic strategies.

## PO31 - Toxicogenomic analysis of the cellular response to cymoxanil

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Cymoxanil is a fungicide widely used in agriculture; however, the molecular and cellular mechanisms underlying its toxicity remain poorly understood. Toxicogenomic approaches employing Saccharomyces cerevisiae have proven to be powerful tools for elucidating mechanisms of chemical toxicity due to the high conservation of fundamental eukaryotic cellular pathways and the availability of genome-wide functional genomic resources. In this work, complementary toxicogenomic approaches were used to investigate the cellular response to cymoxanil exposure and identify biological processes involved in its mechanism of action. Transcriptomic analyses revealed genome-wide alterations, particularly affecting membrane transport systems. In parallel, a functional screen of the yeast knockout collection identified deletion mutants displaying altered susceptibility to cymoxanil, providing additional insight into the cellular pathways involved in the response to the fungicide. Mechanistic analyses further demonstrated that cymoxanil impairs the activity of the essential $H^+$-ATPase Pma1p, leading to plasma membrane depolarization, intracellular acidification and loss of cell viability. Together, these findings indicate that plasma membrane dysfunction and disruption of ion homeostasis are central to cymoxanil toxicity and highlight the value of integrating complementary toxicogenomic approaches to elucidate agrochemical modes of action and support mechanism-based pesticide hazard assessment.

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**PO32 - When less is more: activity of *Pterospartum tridentatum* in combination with fluconazole against *Candida guilliermondii***

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Fluconazole-resistant *Candida* species in vulvovaginal candidosis highlights the need for innovative therapeutic solutions. Plant-derived compounds may offer a promising approach to overcome antifungal resistance. This study aimed to assess the antifungal activity of *Pterospartum tridentatum* against vulvovaginal *Candida* isolates and to investigate its potential interaction with fluconazole. The aqueous extract of *Pterospartum tridentatum* was prepared from its aerial parts. Antifungal activity was determined by measuring the minimum inhibitory concentration and minimum fungicidal concentration against four *Candida* species: fluconazole-resistant *Candida albicans*, fluconazole-resistant *Candida glabrata* (currently classified as *Nakaseomyces glabratus)*, *Candida guilliermondii* (currently classified as *Meyerozyma guilliermondii*), and *C. krusei* (currently classified as *Pichia kudriavzevii*). The interaction between this aqueous extract and fluconazole was evaluated using checkerboard microdilution method. The aqueous extract of *Pterospartum tridentatum* exhibited no antifungal activity when tested alone against any of the strains (>2500 µg/mL). In combination with fluconazole, this plant extract demonstrated a concentration-dependent synergistic interaction against fluconazole-resistant *C. albicans* and *C. glabrata*. No interaction was observed against *C. krusei*. Against *C. guilliermondii*, the combination exhibited synergism at lower plant extract concentrations and antagonism at higher concentrations. In conclusion, the combination of *Pterospartum tridentatum* with fluconazole appeared to be a promising candidate for improving the treatment of *Candida* infections. Nevertheless, its activity in combination with fluconazole against *C. guilliermondii* emphasizes the critical importance of dose control. Consequently, its use in combination therapy should be approached with caution due to the need for precise dose optimization, as lower concentrations may enhance efficacy, whereas higher concentrations may compromise therapeutic outcomes.

XXIII Jornadas de Biologia de Leveduras
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## Tribute to Professor Isabel Spencer Martins

## Bound to Iron: How Caspofungin Loses Antifungal Activity and Finds Its Way into the Cell

Andreia Pedras

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Invasive fungal infections cause a high number of deaths each year and remain a major global health threat. Among these, invasive candidiasis (IC), caused by *Candida* species, is of particular concern, especially in patients undergoing immunosuppressive or intensive antibacterial therapies. Echinocandins are a cornerstone of IC treatment; however, emerging evidence suggests that their activity against *Candida albicans* can be compromised by an unexpected factor: iron. Through a multidisciplinary approach, we uncovered a novel mechanism underlying iron-mediated resistance to caspofungin (CAS). We demonstrated that iron binds directly to CAS, compromising its ability to inhibit its molecular target, Fks1. We further identified the siderophore transporter Sit1 as a potential mediator of the intracellular uptake of iron-bound CAS, revealing an unexpected link between iron homeostasis and antifungal drug activity. Together, these findings provide new insights into how iron availability in the host may influence antifungal efficacy and identify molecular players that could potentially be exploited to enhance CAS activity.

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