JL

XXII Jornadas de Biologia de Leveduras

Professor **Nicolau van Uden**

ABSTRACTS BOOK

20th & 21st of September, 2024



Hotel Termas da Curia

SUMMARY

WELCOME MESSAGE 6
COMMITTEES 7
Honor Committee 8
Organizing Committee 9
Local Organizing Committee 10
Scientific Committee 11
PROGRAM 12
KEYNOTE LECTURES 21
Yeast-mediated production of carboxylic acids and of their "new-to-nature" derivatives 22
Unlocking the Potential of *Torulaspora delbrueckii*: Phenotypic and Genomic Insights for Enhanced Bread and Wine production 23
Killer toxins and evolution of the genetic code in fungi 25
Experimentally evolving cellular miniaturization 26
The lifestyle of *Cryptococcus neoformans* within mammalian hosts 27
Aging and rejuvenation – a consequence of cellular asymmetry? 28
Lipid Droplets: The Unexplored Connection Between Aging and Neurodegeneration 29
United we stand, divided we fall: The antifungal promise of copper-azole complexes 31
ABSTRACTS 32
Topic: YEAST BIOTECHNOLOGY AND SYNTHETIC BIOLOGY 32
ORAL COMMUNICATIONS 33
ID 251 | Lipid Irony: Impairment of Inositol Metabolism and Iron Sensing Yeast Model of N88S Human Seipinopathy 33
ID 262 | From wastes to worth: valorization of biowastes into added-value compounds by non-*Saccharomyces* yeasts 35
ID 265 | Microbial Biosynthesis of Resveratrol: Engineering *Kluyveromyces marxianus* for Sustainable Production 37
FLASH PRESENTATIONS 39
ID 259 | Aiming at the Automation of Genome-wide Regulatory Network Inference in *Saccharomyces cerevisiae* 39

ID 272 | Using CRISPR-Cas9 edited *Saccharomyces cerevisiae* to reduce *Listeria monocytogenes* 40
ID297 | Structural insights on the *Aspergillus niger dicarboxylate exporter Dct-02* 41
ABSTRACTS 42
Topic: FERMENTED FOODS AND BEVERAGES 42
ORAL COMMUNICATIONS 43
ID 280 | In search for a sustainable control approach for *Dekkera bruxellensis* in wine 43
ID 284 | Harnessing *Lachancea thermotolerans*: Genomic Insights for Advanced Wine Biocontrol and Bioflavouring 44
FLASH PRESENTATIONS 46
ID 273 | Advancing *Hanseniaspora guilliermondii* as a bioflavorant: functional analysis of genes involved in acetate ester production and in ethanol tolerance 46
ABSTRACTS 48
Topic: GENOMICS AND EVOLUTION 48
ORAL COMMUNICATIONS 49
ID 250 | Showcasing *Candida glabrata's* Genomic Plasticity and its Effect on the Evolutionof Biofilm Formation in Clinical Isolates 49
ID 278 | Interplay between gene loss and horizontal gene transfer extensively remodeled central metabolism in fructophilic yeasts 50
ID 279 | Tracking alternative versions of the galactose gene network in the genus *Saccharomyces* and their expansion after domestication 51
ID 294 | Compensatory Evolution to DNA Replication Stress is Robust to Glucose Availability 53
FLASH PRESENTATIONS 54
ID 267 | Non-*Saccharomyces* mobilome and its impact in yeasts biotechnological potential for winemaking 54
ID 277 | Yeast hybridization comes in different flavors – the case of *Torulaspora* and *Zygosaccharomyces* 55
ID 281 | Genetic diversity of commensal and clinical *Candida albicans* isolates 57
ID 288 | Uncovering the plasma membrane compartmentalization and dynamics of carboxylate transporter proteins in yeast 58
ID 295 | The (a)symmetrical physiological effects of aneuploidy 60

ABSTRACTS 61
Topic: YEASTS AND HEALTH 61
ORAL COMMUNICATIONS 62
ID 282 | *Candida* spp. extracellular metabolism of purines contribution to immune evasion 62
ID 287 | Unveiling the Crucial Role of Nutrient Transporters in *Candida albicans* Pathogenesis 64
FLASH PRESENTATIONS 66
ID 254 | Essential Oils: A Potent Strategy Against Drug-Resistant *Candida auris* 66
ID 255 | Lab-on-chip technology for diagnosis of yeast infection 68
ID 256 | Assessment of the pathogenic potential of yeasts isolated from urine 70
ID 266 | Evaluation of functional properties of peptides secreted by *Saccharomyces cerevisiae* with potential skin benefits 71
ID 268 | Delving into the Fungicidal Mechanisms of ZnO and Ag-ZnO Thin Films Against *Candida albicans* 73
ID 286 | Environmental Reservoirs of *Candida auris*: Insights from Natural and Hospital Settings 74
ID 289 | Characterizing Ato2 proteins: Expression profiles and Subcellular Dynamics in *Candida albicans* 75
ID 290 | Antifungal susceptibility profile and resistance mechanisms displayed by *Candida parapsilosis* species complex isolates 77
ABSTRACTS 78
Topic: YEAST ADAPTATION, CELL DEATH AND AGING 78
ORAL COMMUNICATIONS 79
ID 252 | Vps27 is essential for mitochondrial respiration and lifespan extension in Sit4 deficient cells 79
ID 283 | pH-dependent downregulation of a yeast nutrient transporter: mechanisms and metabolic implications 81
FLASH PRESENTATIONS 83
ID 258 | Bacteria isolated from the microbiome of two marine microalgae species display promising antifungal activity against *Candida* spp. 83
ID 263 | Iron antagonizes with fluconazole against *Candida glabrata* 84

ID 276 | Influence of different sweeteners on *Saccharomyces cerevisiae* during aging 85
ABSTRACTS 86
Topic: DRUG AND STRESS RESISTANCE 86
ORAL COMMUNICATIONS 87
ID 253 | Essential Oil Vapors: A Novel Approach to Preventing Antifungal-Resistant *Candida*Biofilms 87
ID 261 | A copper complex of a fluconazole derivative demonstrates outstanding antifungal properties 89
FLASH PRESENTATIONS 90
ID 257 | Caspofungin binding to iron reduces its antifungal activity against *Candida albicans* 90
ID 260 | New insights into *Saccharomyces cerevisiae* arsenic detoxification 92
ID 269 | Tackling molecular players underlying tolerance to SO_2 in *Saccharomyces cerevisiae* wine strains: beyond the sulphite efflux pump SSU1 93
ID 270 | Combining fluconazole with benzo[a]phenoxazine derivatives as a promising strategy against fluconazole-resistant *Candida* species 95
ID 271 | Unraveling new mechanisms governing azole resistance mechanisms in clinical *Candida glabrata* strains: beyond CgPdr1 97
ID 285 | Antimicrobial activity of rowan leaf and berries *(Sorbus aucuparia)* extracts against spoilage microorganisms 99
ID 291 | *Debaryomyces hansenii* Adaptation to NaCl: Compairing Physiological Traits in Exponential and Stationary Growth Phases 100
SPONSORS 101
SCIENTIFC SPONSORSHIP: 103
WITH THE SUPORT OF: 103

WELCOME MESSAGE

Dear colleagues,

After the challenging years of COVID-19, we are pleased to announce that the **XXII Jornadas de Biologia de Leveduras Professor Nicolau van Uden** will be held next **20th and 21st of September in Hotel Termas da Curia, Portugal**. This venue will be an opportunity to share the latest advances in the field of Yeast biology as well as to network with portuguese colleagues experts from diverse scientific areas, early-career scientists and students, while creating valuable networking opportunities, or to initiate collaborations in a friendly setting. The topics of the Program include:

- Genomics and Evolution
- Yeast Biotechnology and Synthetic Biology
- Yeasts and Health
- Fermented Foods and Beverages
- Drug and stress resistance
- Yeast Adaptation, Cell Death and Aging

We are also inviting you to share your work by submitting abstracts for 10 min-selected oral communications and 5 min-flash presentations. Those of you who obtained your PhD degree between June 2021 and June 2024 are also invited to apply for the Prize, "the *Young Researcher Award – Prof. Isabel Spencer Martins*".

The meeting will start on Friday, September 20th, 2024, during the morning and will end on the 21st September in the afternoon. Besides the oral presentations, the social programme includes Coffee breaks and lunches (covered by the registration fee) which will create opportunities of interaction between the attendees. The conference dinner will take place on the 20th of September.

The meeting will be entirely held at the **Hotel Termas da Curia**, 108 km away from Porto, 230 km from Lisbon and 28 km from Coimbra, a classical hot springs hotel in Portugal, surrounded by luxurious gardens.

We all look forward to meeting you soon again, and exchange inspiring discussions on the latest research!

See you all in Curia!

Organizing Committee

COMMITTEES

Honor Committee



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ULisboa

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IST-ULisboa, iBB, I4HB



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FCT NOVA, UCIBIO, i4hb



Paula Ludovico

Uminho, ICVS-Uminho



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Uminho, CBMA



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UC, FMUC, CIBB, CNC-UC

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UC, FMUC, CIBB, CNC-UC



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Rita Domingues

UC, FMUC, CIBB, CNC-UC



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Paula Sampaio
CBMA-UMinho

Sofia Costa Oliveira
FMUP

Vítor Costa
I3S, IBMC, ICBAS, UP

PROGRAM

Keynote Lectures – 15 min presentation + 5min discussion | **Oral Communications** - 10 min presentation + 5 min discussion | **Flash presentations** - 5 min presentation + 2 min discussion.

September 20th | Friday

09:00 am - Opening Registration

10:00 am - Welcome Coffee

10:30 am - Welcome Session Professor Nicolau van Uden

Opening Session
Teresa Gonçalves on behalf of the Organizing Committee

"A few words on Professor Nicolau van Uden"
José Paulo Sampaio – *FCT NOVA, ICIBIO, i4hb*

11:00 am - Session I - Yeast Biotechnology and Synthetic Biology

Chairs: Björn Johansson | Lucília Domingues

11:00 am – Keynote Lecture

Yeast-mediated production of carboxylic acids and of their "new-to-nature" derivatives

Nuno Mira – *IST-ULisboa, iBB, i4HB*

11:20 am – ORAL COMMUNICATIONS

11:20 am – **From wastes to worth: valorization of biowastes into added-value compounds by non-*Saccharomyces* yeasts**
Ticiana Fernandes

11:35 am – **Microbial Biosynthesis of Resveratrol: Engineering *Kluyveromyces marxianus* for Sustainable Production**
Marlene Baptista

11:50 am – **Lipid Irony: Impairment of Inositol Metabolism and Iron Sensing Yeast Model of N88S Human Seipinopathy**
Mariana Ribeiro

12:05 pm – FLASH PRESENTATIONS

12:05 pm – **Aiming at the Automation of Genome-wide Regulatory Network Inference in *Saccharomyces cerevisiae***
Diogo Couceiro

12:12 pm – **Using CRISPR-Cas9 edited *Saccharomyces cerevisiae* to reduce *Listeria monocytogenes***
David Moreno

12:19 pm - **Structural insights on the *Aspergillus niger dicarboxylate exporter Dct-02***
Isabel Soares-Silva

12:30 pm - Session II – Fermented Foods and Beverages

Chairs: Alexandra Mendes-Ferreira | César Fonseca

12:30 pm - Keynote Lecture

Unlocking the Potential of *Torulaspora delbrueckii*: Phenotypic and Genomic Insights for Enhanced Bread and Wine production

Maria João Sousa – *UMinho, CBMA, IB-S*

12:50 pm – ORAL COMMUNICATIONS

12:50 pm – **Harnessing *Lachancea thermotolerans*: Genomic Insights for Advanced Wine Biocontrol and Bioflavouring**
Marcos Esteves

01:05 pm – **In search for a sustainable control approach *for Dekkera bruxellensis* in wine**
João Sousa

01:20 pm - FLASH PRESENTATIONS

01:20 pm – **Advancing *Hanseniaspora guilliermondii* as a bioflavorant: functional analysis of genes involved in acetate ester production and in ethanol tolerance**
Diogo Santos

01:30 pm - Lunch

03:00 pm - Session III - Yeast Genomics and Evolution

Chairs: José Paulo Sampaio | Margarida Casal

03:00 pm - Genome PT Talk

Killer toxins and evolution of the genetic code in fungi

Keneth H. Wolfe - *University College Dublin, Ireland*

03:45 pm - Keynote Lecture

Experimentally evolving cellular miniaturization

Marco Fumasoni - *Instituto Gulbenkian de Ciência*

04:05 pm - ORAL COMMUNICATIONS

04:05 pm – **Interplay between gene loss and horizontal gene transfer extensively remodeled central metabolism in fructophilic yeasts**
Carla Gonçalves

04:20 pm – **Showcasing *Candida glabrata's* Genomic Plasticity and its Effect on the Evolution of Biofilm Formation in Clinical Isolates**
Inês Vieira Costa

04:35 pm – **Compensatory Evolution to DNA Replication Stress is Robust to Glucose Availability**
Mariana Natalino

05:05 pm – Coffee Break

05:35 pm - FLASH PRESENTATIONS

05:35 pm – **Genetic diversity of commensal and clinical *Candida albicans* isolates**
Maria João Carvalho

05:42 pm – ***Non-Saccharomyces* mobilome and its impact in yeasts biotechnological potential for winemaking**
Fábio Rafael Moutinho Pereira

05:49 pm – **Yeast hybridization comes in different flavors – the case of *Torulaspora* and *Zygosaccharomyces***
Margarida R. Silva

05:56 pm – **The (a)symmetrical physiological effects of aneuploidy**
Manuel Alberto Vásquez Ibarra

06:03 pm – **Uncovering the plasma membrane compartmentalization and dynamics of carboxylate transporter proteins in yeast**
Beatriz-Conceição Leite

08:00 pm - Yeast Dinner

Keynote Lectures – 15 min presentation + 5min discussion | **Oral Communications** - 10 min presentation + 5 min discussion | **Flash presentations** - 5 min presentation + 2 min discussion.

September 21st | Saturday

08:30 am - Opening Registration

09:00 am - Session IV – Yeasts and Health

Chairs: Isabel Miranda | Joana Rolo

09:00 am - Keynote Lecture

The lifestyle of *Cryptococcus neoformans* within mammalian hosts

Carolina Coelho - *University of Exeter, UK*

09:20 am - ORAL COMMUNICATIONS

09:20 am – **Unveiling the Crucial Role of Nutrient Transporters in *Candida albicans* Pathogenesis**
Faezeh Ghasemi

09:35 am – ***Candida* spp. extracellular metabolism of purines contribution to immune evasion**
Lisa Rodrigues

09:50 am - FLASH PRESENTATIONS

09:50 am – **Essential Oils: A Potent Strategy Against Drug-Resistant *Candida auris***
Liliana Fernandes

09:57 am – **Lab-on-chip technology for diagnosis of yeast infection**
Maria Zolotareva

10:04 am – **Assessment of the pathogenic potential of yeasts isolated from urine**
Filipa Oliveira Castro

10:11 am – **Evaluation of functional properties of peptides secreted by *Saccharomyces cerevisiae* with potential skin benefits**
Patrícia Branco

10:18 am – **Delving into the Fungicidal Mechanisms of ZnO and Ag-ZnO Thin Films Against *Candida albicans***
Patrícia Pereira-Silva

10:25 am – **Environmental Reservoirs of *Candida auris*: Insights from Natural and Hospital Settings**
Sofia Costa de Oliveira

10:32 am – **Characterizing Ato2 proteins: Expression profiles and Subcellular Dynamics in *Candida albicans***
Patrícia Ataíde

10:39 am – **Antifungal susceptibility profile and resistance mechanisms displayed by *Candida parapsilosis* species complex isolates**
Isabel M. Miranda

11:00 am - Coffee Break

11:30 am - Session V- Yeast Adaptation, Cell Death and Aging

Chairs: Manuel Santos - *Scientific Director of MIA-Portugal* | Vitor Costa

11:30 am - MIA-Portugal Lecture

Aging and rejuvenation – a consequence of cellular asymmetry?

Thomas Nyström – *University of Gothenburg, Sweden*

MIA Portugal
Multidisciplinary
Institute of Ageing

This project has received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 857524

12:15 pm - Keynote Lecture

Lipid Droplets: The Unexplored Connection Between Aging and Neurodegeneration

Vitor Teixeira – *FCUP, I3S, ICBAS*

12:35 pm - ORAL COMMUNICATIONS

12:35 pm **– Vps27 is essential for mitochondrial respiration and lifespan extension in Sit4 deficient cells**
Miguel Correia

12:50 pm – **pH-dependent downregulation of a yeast nutrient transporter: mechanisms and metabolic implications**
Cláudia-Barata Antunes

01:05 pm - FLASH COMMUNICATIONS

01:05 pm – **Influence of different sweeteners on *Saccharomyces cerevisiae* during aging**
Sara Dourado

01:12 pm – **Bacteria isolated from the microbiome of two marine microalgae species display promising antifungal activity against *Candida* spp.**
Teresa Pissarro

01:19 pm – **Iron antagonizes with fluconazole against *Candida glabrata***
Carolina Mariano

01:30 pm - Lunch

03:00 pm - Session VI – Drug and stress resistance

Chairs: Paula Sampaio | Sofia Costa Oliveira

03:00 pm - Keynote Lecture

United we stand, divided we fall: The antifungal promise of copper-azole complexes
Catarina Pimentel – *ITQB NOVA*

03:20 pm - ORAL COMMUNICATIONS

03:20 pm – **Essential Oil Vapors: A Novel Approach to Preventing Antifungal-Resistant *Candida* Biofilms**
Maria Elisa Rodrigues

03:35 pm – **A copper complex of a fluconazole derivative demonstrates outstanding antifungal properties**
Cláudia Malta-Luís

03:50 pm - FLASH PRESENTATIONS

03:50 pm – ***Debaryomyces hansenii* Adaptation to NaCl: Compairing Physiological Traits in Exponential and Stationary Growth Phases**
Tomás Gonçalves

03:57 pm - **Unraveling new mechanisms governing azole resistance mechanisms in clinical *Candida glabrata* strains: beyond CgPdr1**
Maria Joana Pinheiro

04:04 pm – **New insights into *Saccharomyces cerevisiae* arsenic detoxification**
Sofia M. da Silva

04:11 pm – **Antimicrobial activity of rowan leaf and berries (*Sorbus aucuparia*) extracts against spoilage microorganisms**
Patrícia Lage

04:18 pm – **Caspofungin binding to iron reduces its antifungal activity against *Candida albicans***
Andreia Pedras

04:25 pm – **Tackling molecular players underlying tolerance to SO2 in *Saccharomyces cerevisiae* wine strains: beyond the sulphite efflux pump *SSU1***
Bárbara Coelho

04:32 pm – **Combining fluconazole with benzo[a]phenoxazine derivatives as a promising strategy against fluconazole-resistant *Candida* species**
Maria Inês Pacheco

04:45 pm - Conference "Jovem Investigador" | Tribute to Professor Isabel Spencer Martins

Sponsored by *Sociedade Portuguesa de Microbiologia (SPM)*
Chairs: Professor Paula Ludovico | Professor Jorge Pedrosa – *President of tHE SPM*

05:30 pm - Closing Session

Farewell Coffee

KEYNOTE LECTURES

NUNO MIRA

IST-ULisboa, iBB, i4HB

Yeast-mediated production of carboxylic acids and of their "new-to-nature" derivatives

Many carboxylic acids (CAs) have a recognizably high economical interest due to their versatility to be used as catalysts in a multitude of chemical routes explored by industries to produce commodities of our daily life. Several of the more interesting CAs are produced through microbial fermentation as they are intermediates of known metabolic pathways; but in some cases (such as levulinic acid) a production pathway is not known. This complicates the engineering of suitable production strategies as there is no biochemical pathway to intervene on. In other cases, the production pathway to obtain the CA is known but it is unclear how to transform them into their most valuable derivatives. The strategies used by our lab leveraging computation-assisted approaches and design new synthetic pathways to obtain CAs and/or their valuable derivatives will be herein discussed using as platforms levulinic and itaconic acids. The results obtained in our favorite production hosts, the Yeasts *Saccharomyces cerevisiae* and *Pichia kudriavzevii*, will be shown. The approaches will encompass not only the designed synthetic pathways, but others implemented with the help of metabolic engineering supported by computational metabolic modelling.

MARIA JOÃO SOUSA

CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga Portugal; INRAE, Institut Agro Montpellier, Montpellier, France

Flávia Silva-Sousa1; Ticiana Fernandes1; Fábio Pereira1; Bruna Oliveira1; Carolina Osório1; Teresa Rito1; Pedro Soares1; Carole Camarasa,2; Ricardo Franco-Duarte,1; Maria João Sousa1,2

1 CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Braga, Portugal;
2 INRAE, Institut Agro Montpellier, Montpellier, France.

Unlocking the Potential of *Torulaspora delbrueckii*: Phenotypic and Genomic Insights for Enhanced Bread and Wine production

Background: *Torulaspora delbrueckii* (Td) is an ascomicetous yeast evolutionary close to *Saccharomyces cerevisiae* (Sc), whose particular traits have caught the attention of the bread and wine industries, due to several advantageous properties when compared to the traditional Sc yeast.

Methods: Aiming to get an in-depth understanding of the species phenotypic and genomic diversity, we gathered a set of 40 Td strains with varied geographical and technological origins, sequenced them and performed a phenotypic characterization regarding parameters of biotechnological interest. Additionally, we conducted a comprehensive metabolic characterization of 15 Td strains (including the determination of fermentation parameters, metabolite production and aroma compounds) which was then integrated with genomic data. As sulphite resistance was identified as a potential weakness for Td application in wines, we also performed ALE to improve its resistance and analysed the differences of the evolved strains at genome level.

Results: Our results suggest that the technological origin of the strains seems to prevail over the geographical origin in what regards influence on yeast properties. Data analysis also revealed that the majority of the strains shared an advantageous resistance to several stress environments. Metabolic characterization displayed inter-strain variability within Td. Results also showed that the overall aromatic profiles of Td strains differed significantly from Sc, exhibiting higher levels of monoterpenes and higher alcohols, while producing less acetate esters, fatty acids, and their corresponding ethyl esters, as well as lactones. Moreover, we identified potential genomic links for these variations. ALE allowed to improve Td resistance to sulphites and revealed genetic determinants linked to this phenotype.

Conclusions: This work highlights the remarkable potential of Td and underscores links between its genotype and phenotype. It also emphasizes the need to fully understand specificities Td's metabolism and adaptation during alcoholic fermentation.

Keywords: *Torulaspora delbrueckii*; wine and bread fermentation; metabolism; aromas.

Acknowledgments
The work was supported by national funds (FCT) via the institutional programs supporting CBMA (UIDB/04050/2020, DOI:10.54499/UIDB/04050/2020) and funding from FCT fellowships to FSS - UI/BD/150873/2021; TF - DOI: 10.54499/2021.04595.BD; FP - 10.54499/2023.00204.BD; BO - 10.54499/2023.00306.BD; CO - DOI:10.54499/2023.00660.BD; TR - 2023.06638.CEECIND; RFD DOI:10.54499/2022.00340.CEECIND/CP1718/CT0018.

KENNETH H. WOLFE

Conway Institute & School of Medicine, University College Dublin, Ireland

Killer toxins and evolution of the genetic code in fungi

For more than 30 years, we have known that *Candida albicans* and related yeast species use a non-standard genetic code in their nuclear genes: they translate CUG codons as Ser instead of Leu. More recently, a CUG-Ala clade and a second CUG-Ser clade were discovered. Remarkably, these clades are the only 3 examples known of a codon being reassigned from one amino acid to a different amino acid, in the nuclear genetic code of any eukaryotes. So, why did all 3 reassignments occur in the same part of the eukaryotic phylogenetic tree, and why did they all involve the same codon? We hypothesized that the driving force may have been a zymocin-like killer toxin. Zymocin (also called γ-toxin) is encoded by a natural plasmid found in some strains of *Kluyveromyces lactis*. It is a ribonuclease that specifically cleaves the anticodon of one tRNA type, tRNA-Glu (UUC). We hypothesized that an ancient killer toxin might have targeted the tRNA-Leu that read CUG codons, leading to evolutionary pressure to read CUG using a tRNA that is not cleaved, i.e., a tRNA for a different amino acid such as Ser or Ala. However, zymocin-like toxins are rare. They are present in only 1-2% of yeast strains. Their phylogenetic distribution is unknown, and only 4 of them have been studied. We searched for new zymocin-like toxins by using public genome sequence data and new bioinformatics methods. We identified 45 new toxin genes, and verified that many of them are toxic to S. cerevisiae when expressed. The toxins are extraordinarily diverse in sequence and show evidence of positive selection. Toxin genes were found in five taxonomic orders of budding yeasts, including two of the three orders that reassigned CUG codons, indicating that killer plasmids coding for tRNA-cleaving toxins have been parasites of yeasts for at least 300 million years, and that their existence pre-dates the genetic code changes.

MARCO FUMASONI

Instituto Gulbenkian de Ciência

Experimentally evolving cellular miniaturization

Cell volume is a fundamental determinant of cellular physiology, maintained through a process known as cell size homeostasis. Studies have shown that large deviations from the typical cell size often produce harmful effects. Nonetheless, cell sizes have dramatically diverged during evolution, ranging from micrometers to millimeters. How does cell size homeostasis evolve to produce such diversity without impacting cell physiology? To address this paradox, we designed an experimental evolution strategy to select progressively smaller cells from *Saccharomyces cerevisiae* populations. Over 1500 generations, we achieved a five-fold reduction in cell volume compared to wild-type cells, marking the largest decrease observed. We show that the new size homeostasis is stably maintained. Strikingly, evolved cells maintain robust fitness, suggesting that the dramatic change in cell volume did not severely impact physiology. We investigated the genetic basis of cellular miniaturization through whole-genome sequencing of the evolving populations. The roles of known and novel players involved in cell size regulation, as well as putative suppressors of defects associated with cell miniaturization will be discussed. Our results demonstrate the evolutionary plasticity of cell size homeostasis and offer novel insights into how variability in a major cellular feature can be evolutionarily achieved.

CAROLINA COELHO

University of Exeter, UK

The lifestyle of *Cryptococcus neoformans* within mammalian hosts

Cryptococcus neoformans is a pathogenic fungus that results in ~180, 000 deaths per year and is responsible for 15% of HIV-associated deaths. Following inhalation, *C. neoformans* rapidly crosses a series of tissue barriers – from the lungs, into the blood and finally across the blood-brain barrier where it causes meningoencephalitis. The mechanisms by which *C. neoformans* can disseminate from the lungs to the brain are poorly understood. Concomitantly, in the lungs, *C. neoformans* can either be engulfed and reside within macrophages or avoid phagocytosis by enlarging to form Titan cells. Deciphering these host-pathogen interactions requires information at multiple scales. We will be discussing latest discoveries in how this lethal fungal pathogen interacts and overcomes host defenses.

THOMAS NYSTRÖM

University of Gothenburg, Sweden

Aging and rejuvenation – a consequence of cellular asymmetry?

The 'Evolutionary Theory of Aging' regards aging as an evolved characteristic of the soma but it is clear that the life history of some vegetatively reproducing, unicellular, microorganisms – such as budding yeast - encompasses mandatory replicative aging. In the process of yeast budding, an aged and deteriorated mother cell gives rise to a youthful and pristine daughter cell. This remarkable event offers an interesting and tractable model system for identifying factors affecting life expectancy and our data has demonstrated that aged and damaged proteins are specifically retained in the aged mother cell during cytokinesis; an asymmetrical, non-Mendelian, inheritance of damage. The data supports the hypothesis that asymmetric division in vegetatively reproducing organisms can give rise to epigenetic stratification and age-structured populations as is also observed in adult stem cells. In line with such experimental and theoretical considerations, it has been proposed that the evolution of aging (as a byproduct) was first contingent on the development of polarization and a division of labor between mother-type and daughter-type cells in clonal populations of unicellular microorganisms rather than the separation of soma from germ line. In this talk, I will summarize some data on how the yeast mother cell recognizes and retain protein damage during cytokinesis and discuss how virus-like particles may have an effect on damage segregation, protein quality control and cellular aging.

MIA Portugal
Multidisciplinary Institute of Ageing

This project has received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 857524

VITOR TEIXEIRA

Yeast Signalling Networks (YSN), i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Lipid Droplets: The Unexplored Connection Between Aging and Neurodegeneration

The aging process is marked by a decline in cellular function and increased vulnerability to neurodegenerative diseases. Emerging evidence underscores the pivotal role of lipid droplets (LDs) in these processes, influencing cellular signaling pathways, lipid metabolism, and inflammation. Intriguingly, LD accumulation is directly linked to age-related neurodegeneration, positing LDs as potential therapeutic targets to tackle this issue.

Genetic studies have recently unveiled mutations in genes associated with LD biology, leading to neuropathological conditions with significant clinical variability and distinct presentations, such as seipinopathy. Seipin *(BSCL2)* is an evolutionarily conserved membrane Endoplasmic Reticulum (ER) protein that forms a homo-oligomer specifically targeted to ER-LD contact sites to facilitate triacylglycerol delivery from the ER to nascent LDs. The protein is highly expressed in motor neurons and key brain regions including the frontal lobe, hypothalamus, and brainstem, and is essential for excitatory synaptic transmission and neurotransmitter release. Gain-of-function mutations identified in seipin, specifically N88S and S90L, are implicated in autosomal dominant motor neuron diseases like hereditary spastic paraplegias (Silver syndrome), Charcot-Marie-Tooth disease type 2, and distal hereditary motor neuropathy type V.

Our research has pioneered in the development of a yeast model of N88S seipinopathy, which recapitulates the main human seipinopathy cellular features: induction of the ER stress response and formation of inclusion bodies (IBs) containing WT-N88S heteromers or N88S homo-oligomers. Notably, cells expressing N88S homo-oligomers exhibit cell death and reduced viability due to increased oxidative damage, elevated reactive oxygen species (ROS) accumulation, lipid peroxidation, and impaired antioxidant activity. Using a multi-omics

approach, we now propose that human seipinopathy is not only a proteinopathy, but also a lipidopathy associated with oxidative damage and loss of iron homeostasis.

In this lecture, the use of yeast systems biology in understanding principles of physiology underlying human neurodegeneration and defining innovative therapeutic strategies targeting LD biology, will be discussed.

CATARINA PIMENTEL

Yeast Molecular Biology, ITQB NOVA

United we stand, divided we fall: The antifungal promise of copper-azole complexes

We have recently shed light on the molecular mechanisms by which copper enhances the efficacy of fluconazole, the most widely used antifungal drug. These findings inspired the design of dual-function compounds that combine azoles with metal-binding groups. In this talk, I will share our journey towards the development these compounds, which culminated in the synthesis of a novel copper-binding azole derivative with remarkable antifungal activity against *Candida* spp., capable of overcoming fluconazole resistance.

ABSTRACTS

Topic: YEAST BIOTECHNOLOGY AND SYNTHETIC BIOLOGY

ORAL COMMUNICATIONS

ID 251 | Lipid Irony: Impairment of Inositol Metabolism and Iron Sensing Yeast Model of N88S Human Seipinopathy

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Background: Lipid droplets (LDs) are organelles dedicated to lipid storage, with a core containingtriacylglycerols (TAG) and sterol esters [1, 2]. Seipin, encoded by human BSCL2 and yeast SEI1, is amembrane protein in the endoplasmic reticulum (ER) that forms homo-oligomers at ER-LD contact sites forTAG delivery to nascent LDs [3-5]. Gain-of-function mutations in BSCL2 (N88S/S90L) lead to motor neurondiseases named seipinopathies [6-9]. Our yeast model of N88S seipinopathy [10] exhibits inclusion bodies(IBs) composed of WT-N88S and N88S-N88S seipin oligomers, and reduced viability due to increasedoxidative damage, lipid peroxidation and diminished antioxidant activity [10]. In this project, we performed a proteomic and lipidomic profilling of this model, aiming at defining changes in protein and lipid composition.

Methods: Comparative quantitative untargeted mass spectrometric proteomic and lipidomic analyses wereconducted to examine changes in protein and lipid abundance in wild-type (WT) and N88S seipin-expressing mutant cells. Changes in lipid levels were quantified, and differentially expressed proteins were categorized into functional networks to highlight altered protein function sand signaling pathways.

Results: We observed alterations in phosphatidic acid (PA) levels, associated with disrupted TAG and inositol metabolism and decreased flux towards phospholipid biosynthesis. These changes contribute to ERstress beyond IB formation. Additionally, N88S seipin-expressing cells exhibit iron accumulation and impaired ability to cope with iron deficiency. This was linked to changes in the expression of Aft1-controlled iron regulon genes, including the mRNA-binding protein CTH2 and the high-affinity iron transport system member FET3, in a p38/Hog1- and Msn2-dependent manner. Transcriptomics analysis supported that genes transcriptionally activated by Aft1 in response to iron deficiency are downregulated in N88S-expressing cells.

Conclusions: This model highlights iron dyshomeostasis and disruption of lipid metabolism as potential hallmarks of human seipinopathy. The ultimate goal is to uncover new diagnostic markers and therapeutic targets through translational studies.

Keywords: Lipid droplet; neurodegeneration; seipin, seipinopathy

Acknowledgements

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ID 262 | From wastes to worth: valorization of biowastes into added-value compounds by non-*Saccharomyces* yeasts

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Background: Volatile fatty acids (VFAs), primarily sourced from biowastes anaerobic digestion, are emerging as promising substrates for the synthesis of added-value compounds. Non-*Saccharomyces* yeasts display phenotypic robustness in the challenging environments typically found in waste streams, positioning them as attractive biotechnological hosts. Production of succinic acid (SA), lipids and other added-value compounds, are key platform chemicals with wide-ranging applications, that have traditionally relied on petroleum-based approaches. Leveraging biowastes use as substrates and the metabolic plasticity of unconventional yeasts, offers a reduced environmental footprint and potentially cost-effective productions.

Methods: Forty-eight isolates were assessed for their capacity to grow on seven synthetic VFAs, tested individually and in mixtures, at concentrations found in real effluents: acetic(5.1g/L), propionic(2.7g/L), butyric(3.5g/L), isobutyric(0.2g/L), valeric(0.9g/L), isovaleric(0.3g/L), caproic(1.0g/L) acids.

Results: Data analysis unveiled heterogeneous responses to each carbon source, with ten strains consistently outperforming the other isolates of the collection. These exceptional performers were identified as *Apiotrichum brassicae, Cyberlindnera jadinii, Meyerozyma guilliermondii, Pichia kudriavzevii, Saccharomyces cerevisiae, Starmera stellimalicola, Wickerhamiella vandeerwaltii, Yarrowia lipolytica* and *Zygossacharomyces rouxii*. Different VFAs combinations allowed for the assessment of potential synergisticand antagonistic effects on strains' performance. Results suggest a possible redirection towards metabolic pathways other than TCA cycle, as lipids productivity was higher compared to organic acids, ethanol, and glycerol. FTIR spectra profiles reinforce the lipids production under these conditions by the strains able to grow in the mixtures.

Conclusions: Future plans include adaptive evolutionary approaches to improve the use of VFAs assubstrates and increase metabolites production. This will allow for a wider exploitation of natural isolates as alternative platforms to metabolic engineering approaches. These findings reinforce the attractiveness of non-conventional yeasts for biotechnological

applications and help overcome the not yet full acceptance of genetically modified organisms in the industry.

Keywords:on*Saccharomyces*;VolatileFattyAcids(VFAs);biowastes;AdaptiveLaboratoryEvoluti on.

ID 265 | Microbial Biosynthesis of Resveratrol: Engineering *Kluyveromyces marxianus* for Sustainable Production

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Resveratrol is a phenolic compound renowned for its potent antioxidant properties and shows great potential across various industrial sectors. Traditionally extracted from plants or synthesized chemically, these methods often involve complex and unsustainable processes. However, microbial biosynthesis offers a viable solution, mitigating such limitations and contributing to a more environmentally sustainable production. The non-conventional yeast *Kluyveromyces marxianus* has been emerging as an alternative cellfactory to produce high-value chemicals with a wide range of applications. Beyond its thermotolerance and resistance to different inhibitors, it can metabolise many substrates, including glucose, arabinose, and xylose, which can be obtained from lignocellulosic materials. Using these globally available and renewable feedstocks allows the establishment of sustainable processes that contribute to the development of the so aimed circular economy. Here, for the first time, *Kluyveromyces marxianus* strains were engineered by the integration into the genomic lac4 locus of a tyrosine ammonia lyase gene from *Flavobacterium johnsoniaeu* (FjTAL), under the control the native PDC1 promoter, which resulted in the production of p-coumaric acid, a key precursor of resveratrol. Further engineering of those strains by the introduction of the genes coding for a p-coumaroyl-CoA ligase (At4CL2) from *Arabidopsis thaliana*, and a resveratrol synthase (VvVST1) from *Vitis vinifera* under the control of the native strong promoters PDC1 and TEF1, respectively, led to resveratrol production from glucose, xylose, and/or ethanol. Xylose was found to be a better carbon source for resveratrol production. Ethanol supplementation in xylose medium proved to increase resveratrol titersby limiting the accumulation of xylitol by-product. Resveratrol production at 37 °C from a corn cob hydrolysate and whole slurry by simultaneous saccharification and fermentation greatly increased resveratrol yield on sugar, resulting in the production of 69.26 mg/L of resveratrol. This work establishes foundations for an integrated sustainable process for resveratrol production from lignocellulosic materials.

Keywords: Resveratrol; *Kluyveromyces marxianus*; corn cob;SSF

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project (PTDC/BII-BTI/1858/2021). Special thanks to Dr. Jean-Marc Daran from the Faculty of Applied Sciences of TU Delft who provided some parts from the YTK Kluyveromyces kit (plasmids containing the promoters and terminators used) and plasmids pGGKd015, pGGKd072 and pGGKd068.

FLASH PRESENTATIONS

ID 259 | Aiming at the Automation of Genome-wide Regulatory Network Inference in *Saccharomyces cerevisiae*

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Metabolism is dynamic and complex and much more than a network comprised of reagent-catalyst-product connections – an assumption too often made in metabolic modelling. Metabolism is in close association with external stimuli such as nutritional and general environmental conditions and must metamorphose accordingly via signaling and regulatory pathways. It is thus important to consider all these layers in order to faithfully represent cell behavior mathematically and so to apply the resulting models in cell factory optimization – for biofuel production for instance - or in the understanding and combating of pathogenesis.

In this work a tool for automated inferring Boolean regulatory networks from integrated regulatory associations present in Yeastract+ (containing around 200K TF-target associations for *Saccharomyces cerevisiae*) 1 [Teixeira et al, NAR, 51: D785-D791, 2023], in different environmental conditions is being developed. Currently only Direct Transcription Factor (TF) to metabolic genes associations have been modulated and higher level TF-TF regulation integration is still under development. For this a set of base Boolean rules has been defined and implemented and tested global yeast regulatory data, to assess the adequacy of the chosen Boolean forms to be used as general rules of TFs-TG interactions. *In silico* ethanol production optimizations have been performed and wet-lab validation has preliminarily shown promising predictive reliability.

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Keywords: Systems Biology; Mixed Metabolic-Regulatory Models.

Acknowledgements
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ID 272 | Using CRISPR-Cas9 edited *Saccharomyces cerevisiae* to reduce *Listeria monocytogenes*

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Background: In this study, we investigate the efficacy of *Saccharomyces cerevisiae* to deliver the endolysin Ply511 against *Listeria monocytogenes* as a proof of concept towards an engineered probiotic. Listeria monocytogenes is a Gram-positive pathogen causative of human infections, resulting in febrile gastroenteritis, perinatal infection, and central nervous system infections. *L. monocytogenes* is frequently acquired through the ingestion of contaminated food. Endolysins, bacteriophage-derived enzymes, have emerged as promising antimicrobial agents due to their ability to specifically target and degrade bacterial cell walls.

Methods: We employed CRISPR-Cas9 to genetically modify *S. cerevisiae* to display the listeria endolysin Ply511 on its surface or to secrete it. We used flow cytometer and flurescent microscopy to confirm the expression of the genetic construction integrated in the recombinant yeast. Also, the enzymatic activity of the engineered yeast was confirmed by using heat-killed *L. monocytogenes* cells. For killing assays, yeast and bacteria were incubated together at 30°C and Listeria counts were enumerated in OXFORD selective media agar.

Results: The recombinant yeast displaying Ply511 endolysin did reduce the CFUs of Listeria in comparison with the wild type, in contrast, the yeast secreting the endolysin could achieve an eradication in the number of *L. monocytogenes*. To enhance their effectiveness in vivo, this engineering strategy is currently being applied in the probiotic *Saccharomyces boulardii*, to ensure the achievement of effective delivery of the molecule to the gut.

Conclusions: This study demonstrates the potential of genetically engineered *S. cerevisiae* to secrete the endolysin Ply511 for reducing *Listeria monocytogenes*. Future efforts will focus on utilizing *Saccharomyces boulardii* as a probiotic delivery system to ensure efficient in vivo delivery of Ply511 to the gut, thus advancing towards a viable engineered probiotic that prevents *L. monocytogenes* infections.

Keywords: Surface display; endolysin; cytometer; *L.monocytogenes*.

Acknowledgements
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ID297 | Structural insights on the *Aspergillus niger dicarboxylate exporter Dct-02*

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Background: The *Aspergillus niger Dct-02* transporter is a malate and succinate transporter, that is expressed in microbial cell factories to increase the production of carboxylic acids. This transporter belongs to the Tellurite-resistance/Dicarboxylate Transporter (TDT) family, whose members are found in all domains of life.

Methods: To investigate deeper into the structural aspects of the Dct-02 transporter, we conducted a sitedirected mutagenesis approach targeting 20 amino acid residues, totalizing 31 mutant alleles. These residues were selected based on their conservation across the TDT family, 3D structural modelling, and molecular docking analyses. The phenotypic impact of these mutations was assessed by expression of mutant alleles in the *Saccharomyces cerevisiae* CEN.PK 113-5D strain and evaluation of cell growth on media containing carboxylic acids as sole carbon and energy source, as well as the ability to consume and secrete succinate. Further examination involved the determination of the subcellular localization of GFPtagged Dct-02 transporter and mutant alleles.

Results: We identified nine mutations that affected the proper localisation of Dct-02 at the plasma membrane. Among the properly localised transporters, 11 mutations enabled an improved cell growth on carboxylic acids, suggesting a decreased activity of Dct-02. Other mutations resulted in a growth profile behaviour intermediate between the empty vector and wild-type expressing strains. Three mutants promoted a growth phenotype corresponding to a potential increase of succinate export capacity of Dct-02. In liquid cultures, cells expressing these alleles presented different succinate secretion capacities and growth profiles.

Conclusions: This work provides a comprehensive overview of putative key amino acids that play crucial roles in the capacity of the Dct-02 transporter.

ABSTRACTS

Topic: FERMENTED FOODS AND BEVERAGES

ORAL COMMUNICATIONS

ID 280 | In search for a sustainable control approach for *Dekkera bruxellensis* in wine

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Background: *Dekkera (Brettanomyces) bruxellensis* is still one of the major microbiological contaminants of wine and a serious concern for the wine industry all over the world, giving its ability to produce off-flavour volatile phenolic compounds (4-ethylphenol and 4-ethylguaicol). SO2 remains the main preservative used toprevent and control their development. However, with the increase of chemical-resistant strains and a demand for greener alternatives, there has been a growing search for safer methodologies. Thus, this work aims to harness antagonist wine yeasts as biocontrol agents against *D. bruxellensis.*

Methods: Firstly, we evaluated the antagonistic potential of around 2,000 yeasts using a killer-assay againsta strain of *D. bruxellensis*, selected from previous studies based on its spoilage potential. The most promising antagonistic yeasts were tested against 15 strains of *D. bruxellensis*, representatives of the genomic diversity of a group of 57 isolates, as assessed by PCR-fingerprinting.

Results: Around 7% of the strains tested appeared to show inhibitory activity against the target, with *Metschinowia pulcherrima* being the most prevalent species. Among the different yeasts tested, one stood out as being the most effective against the 16 strains of *D. bruxellensis.*

Conclusions: This study demonstrate the biocontrol potential of wine yeasts. Undergoing work is focus on the mechanisms underlying this antagonistic activity and its application in winemaking conditions.

Keywords: *Dekkera bruxellensis*; Wine yeasts; Biocontrol

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ID 284 | Harnessing *Lachancea thermotolerans*: Genomic Insights for Advanced Wine Biocontrol and Bioflavouring

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Background: The species *Lachancea thermotolerans* is frequently found among the microbiota of grapes and is a current trend in wine technology research, mainly due to its ability to produce lactic acid from the fermentation of sugars, reducing wine acidity. Additionally, it is also relevant for this industry for its production of volatile compounds, which not only have bioflavouring potential, but biocontrol activity against phtytopathogenic fungi, as we have shown in a previous study. However, the genetics and physiology of this species, in general, are still elusive, as are the molecular mechanisms underlying oenology-relevant phenotypes.

Methods: Two *L.thermotolerans* strains (UTAD|731, UTAD|2092) with biocontrol potential were selected for 72-hour single-culture fermentations of synthetic grape juice, along with two reference strains (commercial strain and type strain CBS6340). The higher lactic acid production determined a strain selection for genomesequencing, using PacBio and Illumina. Assembly of the obtained reads was performed in a hybrid format, followed by manually validation of *ab initio* gene predictions. The predicted ORFeome was compared with that of relevant strains/species both oenologically and phylogenetically and used to reconstruct the metabolic network of this strain.

Results: Divergence in lactic production was found, with the native strains UTAD|731 and UTAD|2092 producing higher amounts of lactic acid than the reference strains, particularly UTAD|2092 that produced 21.56 g/L, compared with 4.13 and 6.28 g/L produced by commercial and type strains, respectively. The difference in production of lactic acid prompted us to sequence the genome of UTAD|2092 strain. Assembly of the UTAD|2092 genome yielded 20 contigs totaling 10.41 Mb of assembled DNA and encoding 5029 protein-encoding genes. Comparative proteome analysis between the ORFeome of UTAD|2092 and of CBS6340T highlighted genetic variations that may explain the variability detected in lactic acid production, besides helping us to identify species-exclusive proteins.

Conclusions: The results of the herein detailed comparative genomics analysis between *L.thermotolerans* strains is expected to shed light into relevant properties in oenology of this species, improving its rational use in this industry as a biocontrol, bioacidifier and bioflavouring agent.

Keywords: Keywords: Lachancea thermotolerans, winemaking, comparative genomics, lactic acid

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FLASH PRESENTATIONS

ID 273 | Advancing *Hanseniaspora guilliermondii* as a bioflavorant: functional analysis of genes involved in acetate ester production and in ethanol tolerance

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Background: *Hanseniaspora guilliermondii* is a non-conventional yeast with recognized potential as a bioflavorant (especially in wines) due to its enhanced capacity to produce volatile acetate esters, associated with fruity and floral aromas. The genomic sequence of a *H. guilliermondii* strain disclosed by our laboratories revealed a seemingly new family of alcohol acetyltransferases (AATs), the enzymes involved in acetate ester biosynthesis. Herein we will shed light into Hanseniaspora AAT genes, while also addressing the issue of low ethanol tolerance in this genus, one recognized “Achilles-heel” constraining its industrial exploration.

Methods: Functional analysis of AAT genes was studied through their heterologous expression in *S. cerevisiae*, along with studies focused on phylogenetic relatedness and gene expression regulation in several *Hanseniaspora* species. To study ethanol tolerance mechanisms in Hanseniaspora we relied on transcriptomics based on RNA sequencing and adaptive laboratory evolution.

Results: In striking contrast to *S. cerevisiae*, *H. guilliermondii* was found to overproduce acetate esters and higher alcohols at low carbon-to-assimilable-nitrogen ratios, using amino acids as precursors. The higher acetate ester production was accompanied by a higher expression of the HgAAT genes, confirming their anticipated involvement in this process. We will show results comparing this pattern of gene expression and amino acids preference for different *Hanseniaspora* species, revealing a clearer picture of how this phenotype is established across the genus. We will also discuss the massive alterations observed in the transcriptome of *H. guilliermondii* cells subjected to ethanol stress (6% for 1h), which include

305 up- and 392 down-regulated genes, compared to the control transcript levels. This is the first reported study so far to examine stress tolerance in this poorly characterized species.

Conclusions: Our work contributes to elucidate fundamental aspects of *H. guilliermondii's* biology and physiology, unlocking its potential as a bioflavorant and as a cell factory for production of add-value volatiles.

Keywords:*Hanseniaspora-guilliermondii*;non-*Saccharomyces*_yeasts; alcohol_acetyltransferases; acetate_esters_and_higher_alcohols; ethanol_stress

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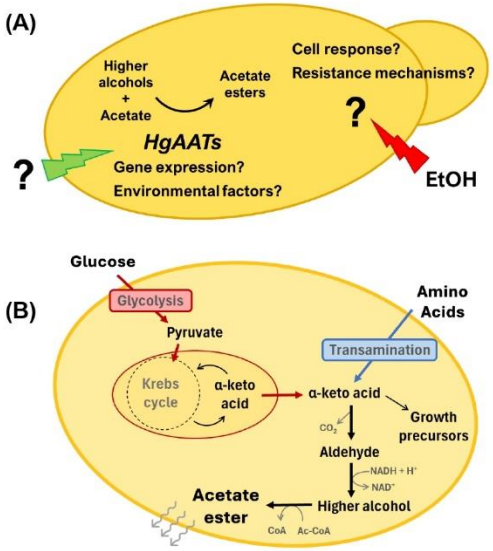


Figure 1. (A) Schematic detailing the work herein presented, focused on HgAAT genes expression regulation and *Hanseniaspora guilliermondii*'s ethanol stress response mechanisms. (B) Proposed model derived from the obtained results. *H. guilliermondii* seems to prefer low C:N ratios (in blue), contrarily to *S. cerevisiae* (in red), preferably producing esters from amino acid derivatives rather than from the central carbon metabolism.

ABSTRACTS

Topic: GENOMICS AND EVOLUTION

ORAL COMMUNICATIONS

ID 250 | Showcasing *Candida glabrata's* Genomic Plasticity and its Effect on the Evolutionof Biofilm Formation in Clinical Isolates

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Background: Fungal infections affect millions of people worldwide. *Candida* species are one the major causative agents of these infections. In particular, *Candida glabrata* is a pathogenic yeast of increasing concern, due to its rising prevalence associated with inherently reduced susceptibility to azole drugs. It can also form biofilms, on account of its versatile ability to adhere to a variety of different substrates, from host mammalian cells, other microorganisms, and abiotic surfaces like those of catheters, further aggravating its virulence. Despite the advantages biofilms confer to pathogenic yeasts, there has been limited research on the evolutionary aspects of biofilm formation among clinical isolates.

Methods: The phenotypic variability in biofilm formation across a collection of *C. glabrata* clinical isolates was examined. Combined comparative genomics with experimental microevolution of the clinical isolates was undertaken, leading weak biofilm producers towards a more robust biofilm producing phenotype. The aim was to pinpoint crucial regulators and effectors involved in this process.

Results: We observed a rapid specialization towards enhanced biofilm formation, characterized by genomic alterations, changes in adhesin encoding genes, and variations in effectors across multiple regulatory levels, from epigenetic to post-translational mechanisms. Through comprehensive virulence and adhesion assays we also found that there was no consistent correlation between biofilm formation and virulence/adhesion across all clinical isolates.

Conclusions: Genes with predictive roles in biofilm formation were identified as exhibiting consistent mutations in high biofilm producing strains, including those encoding uncharacterized adhesins from the EPA and PWP gene families, as well as transcription factors, telomeric silencing proteins, and proteins involved in cell-wall biogenesis. These genes hold promise for future exploration as targets for disrupting biofilms and as markers of biofilm evolution

Keywords: *Candida glabrata*; Biofilms; Genomics; Evolution

ID 278 | Interplay between gene loss and horizontal gene transfer extensively remodeled central metabolism in fructophilic yeasts

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In yeasts belonging to the subphylum Saccharomycotina, genes encoding components of the main metabolic pathways are generally conserved. However, in fructophilic species belonging to the floral *Wickerhamiella* and *Starmerella* genera (W/S clade), we found that many central metabolic pathways were unprecedently impacted by events of gene loss and horizontal gene transfer (HGT), the latter being an uncommon mechanism of genetic exchange among eukaryotic organisms. Species belonging to the W/S clade currently encode the higher number of bacterial genes ever detected in yeast genomes, raising several questions: i) what is the impact of HGT-derived genes in the metabolism of these yeasts? ii) howdid foreign genes adapt to the eukaryotic setting? iii) why was HGT rampant in this clade?

We found that both loss and HGT events deeply shaped the metabolic landscape of extant species belonging to the W/S clade, which display an incredible phenotypic diversity concerning sugar preference, sugar consumption rates or fermentation profiles.

Here we will discuss the possible links between gene loss and acquisition events and evolution of metabolism within an ecological framework, in the light of recent genomic and phenotypic data obtained for more than 50 species belonging to the W/S clade. Moreover, we will put forward hypotheses that attempt to explain why HGT was so extensive in this clade.

Keywords: Horizontal gene transfer; sugar metabolism

ID 279 | Tracking alternative versions of the galactose gene network in the genus *Saccharomyces* and their expansion after domestication

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Background: In nature microorganisms frequently face fluctuating environmental conditions, which can favoradaptive phenotypic plasticity. When Saccharomyces cerevisiae grows on mixtures of glucose and galactose, galactose utilization is repressed by glucose and induction of the GAL gene network only occurs when glucose is exhausted. Contrary to the reference GAL alleles, recently detected alternative alleles support faster growth on galactose, thus enabling distinct galactose utilization strategies maintained by balancing selection.

Methods: GAL genes were searched across the dataset and the sequences were used to constructphylogenies to assess the evolutionary history of these genes in the species/strains under study. Topology tests were also conducted to confirm phylogenetic relationships. Phenotypic tests, including growth rates in glucose, galactose, and a mixture of the two sugars, were performed. For the mixed condition glucose and galactose consumption was measured to assess glucose repression.

Results: We report on the discovery, using phylogenetic inference, of new wild populations of S. cerevisiae harboring the alternative GAL version and, for the first time, of S. paradoxus alternative alleles. In addition, we show that the non-functional GAL version found earlier in S. kudriavzevii is phylogenetically related tothe alternative versions, which constitutes a case of trans-specific maintenance of highly divergent alleles that encompasses the entire genus.

We characterized glucose and galactose utilization in strains harboring the different GAL network variants and exposed different levels of alleviation of glucose repression and growth proficiency on galactose.

Conclusions: We propose that domestication involved specialization toward thriving in milk from a generalistancestor partially adapted to galactose consumption in the plant niche.

Keywords: GAL network; *Saccharomyces*; microbial ecology;

ID 294 | Compensatory Evolution to DNA Replication Stress is Robust to Glucose Availability

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Evolutionary repair refers to the compensatory evolution that follows a perturbation of a cellular process. While the resulting evolutionary trajectories have often been deemed reproducible, other studies have proposed that they are shaped by the interaction of the genotype with the environment (GxE). Here, we testthe predictability of evolutionary repair in response to DNA replication stress, a cellular perturbation present in cancers with widely different tissue environments. We conducted high-throughput experimental evolution on budding yeast, Saccharomyces cerevisiae, experiencing constitutive replication stress under widely different glucose availability. Despite cell cycle and physiology being significantly affected by glucose availability, we found a high level of robustness in the evolutionary trajectories of cells experiencing DNA replication stress under different glucose conditions. Cells recover fitness with similar dynamics, andmutations in cellular modules were recurrently selected independently of the environment in which they evolved. We identified a novel role for the mediator complex of RNA polymerase II in the adaptation to replication stress. Our results highlight the robustness of evolutionary repair to DNA replication stress and provide new insights into the evolutionary aspects of genome stability, with potential implications for understanding cancer development.

Keywords: Evolutionary Repair; Replication Stress; Nutrients

FLASH PRESENTATIONS

ID 267 | Non-*Saccharomyces* mobilome and its impact in yeasts biotechnological potential for winemaking

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Saccharomyces cerevisiae is the most important yeast used in wine production worldwide. In recent years, its activity has been affected by climate change which influences both grape must composition andfermentation environmental conditions. This has had an impact on its role as a wine fermentation starterand increases the importance of non-conventional yeasts. These yeasts are resistant to higher fermentationtemperatures, higher grape sugar content and have lower ethanol yields. Therefore, recent research has been conducted to evaluate the potential of non-Saccharomyces species in wine production, showing their role in improving wine fermentation, and in diversifying and introducing new wines to the market.

Our previous work showed the genetic background underlying fermentation in non-Saccharomyces yeasts. Although the well-established phylogenetic relationship of the fermentation-related genes, it is unknown how their regulation has evolved in *non-Saccharomyces* species. Transposable elements (TEs) are mobile genetic elements able to change their location in the genome, and therefore produce genetic diversity. Theyare well known for changing the gene regulation of nearby genes when inserted in the promoters. In this work, we identified whether key genes for the fermentation process have TEs in their regulatory regions in 293 *non-Saccharomyces* species, demonstrating the molecular mechanism by which these genes might be regulated, and highlighting their potential for biotechnological applications. Our preliminary analysis revealed already at least 15 yeast species with TEs on the promoter regions of 3 fermentation-related genes, which are now being explored to understand their impact in wine fermentation.

Keywords: Genomics; Fermentation; *Non-Saccharomyces*; Transposable Elements.

ID 277 | Yeast hybridization comes in different flavors – the case of *Torulaspora* and *Zygosaccharomyces*

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A better comprehension of the causes and consequences of domestication of non-conventional yeasts may foster their exploitation in novel fermentations or in other economically-relevant processes. *Torulaspora,* along with its closest relatives *Zygotorulaspora* and *Zygosaccharomyces*, exemplify such cases with potential for modern bioeconomy. For example, T. delbrueckii can enhance wine and bread fermentations and Z. bailli, despite being a spoilage agent, is seen as a promising cell-factory for high-valued organic acids.

We are conducting a phylogenomic analysis of these genera to define species circumscriptions, ecological associations, identify cases of domestication, and to detect and evaluate hybridization events. We also wantto understand the hybrids' genomic architecture and ancestry and possible associations with domestication events.

We conducted an extensive phylogenomic study across the three genera, including multiple representativesof all known species, encompassing nearly 300 genomes. Our phylogenetic reconstruction was based on single copy orthogroups (SCO) from the predicted proteomes. These SCO were independently aligned, andthe concatenated alignment was then used to construct a Maximum Likelihood tree. Hybridization events were detected using SpplDer and were analysed further with diverse approaches.

We detected various cases of inter-species hybridization, especially in *Zygosaccharomyces,* besides those already reported in the literature. A few cases were uncovered in *Torulaspora* and none in *Zygotorulaspora*.In *Zygosaccharomyces*, a novel species was found to be the progenitor of at least three hybrid "species"and, in one case, a hybrid had three parental genomes.

Our study revealed, not only various hybridization events, but also that they were markedly distinct with respect to the degree of genetic divergence of parental species and of genetic composition of the hybrids. In some cases, an association between hybridization and domestication could be made, thus providing valuable insights into the evolutionary transitions that occurred in this complex yeast lineage.

Keywords: domestication; hybridization; genomics; *Torulaspora; Zygosaccharomyces*

Acknowledgements

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ID 281 | Genetic diversity of commensal and clinical *Candida albicans* isolates

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Background: *Candida* species are common human commensals and major causes of human fungal infections, with *C. albicans* leading life-threatening invasive infections in developed countries. Mortality ratescan reach 40% despite treatment, emphasising the need for ongoing monitoring of various *C. albicans* features, such as antifungal resistance, epidemiology, evolutionary trends, and virulence/commensalism.

Methods: From the iBiMED biobank collection of *Candida spp*. isolates (n=238), 165 *C. albicans* isolates were whole genome sequenced (Illumina) and distinct genomic traits were investigated. Phenotypic features were also explored, including isolates' antifungal susceptibility to amphotericin B, anidulafungin, micafungin, itraconazole and fluconazole according to the EUCAST guidelines.

Results: Genomic variation was evident in the form of polymorphisms (SNPs), copy number variations (CNVs), and loss of heterozygosity (LOH). Multilocus sequence typing revealed 80 sequence types (ST), from which 44 were novel, indicating shared ST with worldwide distributed *C. albicans* isolates and ST only found in Portugal. Most isolates were susceptible to the antifungals tested.

Conclusions: The *Candida albicans* isolates collection is phylogenetically diverse and most isolates are susceptible to the antifungal drugs commonly used for treatment of fungal infections. The ongoing study includes the establishment of associations between genotypic and phenotypic features. The iBiMED biobank collection of *Candida spp*. isolates continues to expand warranting the genomic surveillance of these organisms not only in Portugal but also in a worldwide context.

Keywords: Surveillance; genomic variants; resistance markers

Acknowledgements

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ID 288 | Uncovering the plasma membrane compartmentalization and dynamics of carboxylate transporter proteins in yeast

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Background: Jen1 and Ato1 proteins are reported to mediate the transport of carboxylic acids through the plasma membrane (PM). These proteins can be internalized under environmental changes or stressconditions to enable rapid adaptation. In the presence of non-fermentable carbon sources, Jen1 and Ato proteins are localized at the PM. However, in the case of Jen1, certain conditions, such as the addition of a preferable carbon source (i.e glucose) or the prolonged growth in lactate, trigger its internalization and degradation in the vacuole. It is known that certain PM proteins are recruited to specific PM domains, called Eisosome Membrane Compartments (EMCs), where they are protected from endocytosis. Therefore, investigating the potential localization of these nutrient transport proteins in those specific PM domains, under endocytic and non-endocytic grown conditions, is crucial to uncover the cellular response to nutrient fluctuations or stress.

Methods: The goal of this work was to check whether carboxylate transport proteins localize at PM domains, such as EMCs, under specific environmental conditions. For that purpose, we generated *S. cerevisiae* strains harboring the genes of interest (JEN1, ATO1, ATO2 or ATO3) with a GFP fusion at the C-terminal and also a Sur7-RFP fusion, to label the eisosomes. TIRF microscopy was performed in order to observe and compare the distribution pattern of those proteins.

Results: Our results show that, in the conditions tested, Jen1 localization at the plasma membrane is mostly excluded from eisosomal regions. Although we have observed some punctual spots of overlap. Additionally, we observed Jen1 co-localization with MCP (membrane compartment occupied by Pma1). Also, Ato1 displayed an interesting dot-like-pattern that differs from EMCs and requires further investigation.

Conclusions: Jen1 and Ato1 PM proteins do not seem to strongly co-localize with the eisosomes under the conditions tested. Further analyses are essential to understand the role of Jen1 localization

Keywords: Keywords: transporters; endocytosis; alkali stress,

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ID 295 | The (a)symmetrical physiological effects of aneuploidy

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Background: Aneuploidy, the presence of an abnormal number of chromosomes, is a hallmark of cancer and is associated with several medical conditions. In S. cerevisiae, aneuploidy has been shown to cause fitness defects that scale with chromosome size. Previous studies have primarily focused on the effects of additional chromosome copies, leaving a gap in understanding how the excess or scarcity of gene products affects cell physiology.

Methods: To address this point, we have engineered a system to parallelly produce strains with specific chromosome gains and losses in S. cerevisiae. We will measure the strains' fitness and chromosome missegregation rates and follow the population dynamics of aneuploid strains using experimental evolution.

Conclusions: Our findings will contribute to a deeper understanding of aneuploidy by decoupling the underlying factors responsible for its physiological impact.

Keywords: Aneuploidy; Chromosome segregation; Evolution

ABSTRACTS

Topic: YEASTS AND HEALTH

ORAL COMMUNICATIONS

ID 282 | *Candida* spp. extracellular metabolism of purines contribution to immune evasion

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Background: *Candida spp*., particularly *C. albicans* (Ca), colonize and are life threatening opportunistic agents of human infection. These yeasts biological traits strongly contribute for their ability to cross barriers and evade human immune defenses. Phagocytic cells like neutrophils, constitute the first line of host defenses, and neutrophil extracellular traps (NETs), web-like structures with nucleic acids and proteins, immobilize and kill invading microorganisms, preventing the infection establishment. The purinergic system, through ectophosphatases and ectonucleotidases activities, is critical in immunity and inflammation homeostasis, influencing several microbes-associated diseases severity. Moreover, the unique activity of a 3'-nucleotidase/nuclease (3'NT/NU), able to hydrolyze AMP and nucleic acids, has been associated to othermicroorganisms' mechanisms of escape from NETs, as a survival strategy. Thus, this study global aim was to evaluate *Candida spp*. NET-mediated killing escape, through its 3'NT/NU enzymatic activity. We also aimed to characterize Ca clinical isolates enzymatic profiles obtained from different human infection sites.

Methods: 5'&3'NT/NU enzymatic activity was quantified as Pi released at different pHs (4-8), using Fiske- Subbarow colorimetric methodology. Fifty Ca clinical isolates were selected from different infection sites (oral, bloodstream, vaginal, medical devices, and gut-associated infections). Neutrophils-*Candida* interaction phenotypes were assessed using immunofluorescence and microscopy approaches.

Results: During neutrophils-yeasts interaction, when NETs are formed and release, NETs digestion occurs and Ca survival is promoted. These effects are prevented by a nucleotidase inhibitor, ammonium tetrathiomolybdate. All selected *Candida spp*. isolates are endowed with 5'&3'NT/NU activity, with highest enzymatic activity obtained at pH 4. Vaginal and oral isolates had the highest and lowest enzymatic activity,respectively.

Conclusions: Although yeasts ectonucleotidases exact nature is not known, these enzymes are important inthe context of infection, helping Ca to overcome host neutrophils attack and NET-mediate killing. Moreover, these yeasts enzymatic properties seem to be niche-associated, with a contribution to Ca success as a pathogen.

Keywords: *Candida;* human infection; ectonucleotidases; NETs

ID 287 | Unveiling the Crucial Role of Nutrient Transporters in *Candida albicans* Pathogenesis

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Background: In recent years, *Candida albicans* has emerged as one of the significant global health threats, leading to systemic candidiasis and nosocomial bloodstream infections. This commensal opportunistic microorganism exhibits the capacity to colonize various parts of the host under abnormal conditions. *C. albicans* displays high adaptability to environmental changes and utilizes alternative carbon sources like acetate and lactate in glucose-poor niches of the host body. Jen and Atos are plasma membrane (PM) transport proteins that seem to play a crucial role in carboxylates utilization in *C. albicans*. This study was focused on the characterization of the 10 Ato (acetate-transporter-ortholog)-like proteins.

Methods: Initially an in-silico analysis of Ato proteins was carried out to predict and characterize their three- dimensional structure, specificities, and docking with different substrates. Furthermore, the CRISPR-Cas9 system was used to generate single and multiple *C. albicans* mutants on ATO genes as well as *C. albicans* strains harboring ATOS fused with GFP. The growth phenotypes of the generated mutants have been evaluated both in vitro and in vivo. Simultaneously, the expression and localization of Ato-GFP variants have been analyzed by fluorescence microscopy in different metabolic and stress conditions.

Results: According to our findings, at the plasma membrane, the expressions of Ato proteins are induced, and the deletion of Ato1 affects the expression of other Ato proteins in the presence of specific carboxylates. Our data showed that Ato1 appears to play a fundamental role within this family as the uptake and utilization of specific carboxylates are largely dependent on a functional Ato1.

Conclusions: This study advances our knowledge on how the pathogenicity of *C. albicans* is directly influenced by the metabolism of specific nutrients (carboxylates) and by the regulation of PM carboxylate transporters, particularly Atos.

Keywords: Alternative carbon sources; *Candida albicans*; plasma membrane transporters

Acknowledgments
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FLASH PRESENTATIONS

ID 254 | Essential Oils: A Potent Strategy Against Drug-Resistant *Candida auris*

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Background: *Candida auris*, a drug-resistant species, has recently emerged as a significant global health threat due to its high prevalence in hospitals and associated mortality rates. Its transmissibility and resistance to antifungal agents, coupled with its adaptability, are a challenge in the treatment of infections caused by *C. auris*. The intensified virulence, attributed to its multidrug resistance and biofilm infection, underscores the urgent need for an understanding of its biology. Such insights are essential for the development of effective strategies in the treatment, prevention, and management of *C. auris* infections.

Methods: The study evaluated the efficacy of three essential oils (EOs) – tea tree, niaouli and cajeput – against *C. auris* NCPF8971, assessing planktonic growth using minimum inhibitory concentration determination and agar disc diffusion methods. The antifungal activity of four Thymus EOs (Thymusvulgaris, Thymus zygis, Thymus satureioides, and Thymus mastichina) against planktonic cells of *C. auris* was determined using the agar disc diffusion method. The impact of these EOs, in direct contact or in vapor-phase, on biofilms was evaluated by enumeration of colony-forming units.

Results: The results revealed significant inhibition of *C. auris* planktonic growth by the tested EOs, with MIC50 values between 0.78-1.56%. Tea tree EOs exhibited halos of 20-21mm, while cajeput and niaouli showed 13-14 mm. The EOs effectively prevented biofilm formation, with cajeput completely eradicating pre-formed biofilms. The efficacy of Thymus EOs varied substantially, with T. vulgaris demonstrating the highest antifungal effect and T. mastichina showing lower activity. Chemical composition analysis revealed distinct profiles among Thymus EOs, explaining variations in antifungal actions.

Conclusions: This research underscores theefficacy of EOs in inhibiting *C. auris* growth and biofilm formation. The varying effectiveness among Thymusspecies highlights the importance of specifying species and chemotypes for accurate evaluation. Overall, EOs present a promising therapeutic alternative in combating *C. auris.*

Keywords*: C.auris*; Virulence; Biofilm; Phytotherapeutic; Thymus

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ID 255 | Lab-on-chip technology for diagnosis of yeast infection

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The worldwide burden of yeast infection has been increasing due to the expansion of the “endemic borders” and host range of certain mycosis and the emergence of novel opportunistic, drug and multi-drug resistant pathogenic yeast. Many deaths are to blame on slow, inaccurate, or absent diagnostic testing and lack of timely administration of effective antifungal treatment. While fungal disease diagnostics have improved greatly in the last decade, both access to and actual usage of these tests are limited.

In this study, we evaluate the state-of-art lab-on-chip technology, envisioning its application in the development of ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment- free, and Deliverable to end-users) diagnostic tools for taxonomic identification of pathogenic yeast. We designed a prototype divided into four modules: cell capture, DNA extraction, DNA amplification, and DNA detection. We started by building the DNA extraction device while also working on the detection and signal readout strategy. Our preliminary results include a microfluidic device for thermal and chemical lysis ofyeast cells – capable of obtaining quantifiable DNA from diluted yeast suspensions – and a set of DNA barcoding probes for 3 Candida species, with specificity and sensitivity evaluated in silico against adatabase of 57 species (1258 DNA sequences) and microarray assays against each other.

We will present our perspective on the future of diagnostics, and share our most recent efforts in developingan innovative diagnostic strategy.

Keywords: yeast infection; molecular diagnosis; microfluidics;

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ID 256 | Assessment of the pathogenic potential of yeasts isolated from urine

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Background: Urinary tract infections (UTIs) caused by yeast are uncommon, but still comprise around 10% of all UTIs. However, yeast isolated in urine samples are not routinely characterized and therefore its epidemiology and clinical relevance are illusive. In this study, the aim was to compare the species distribution, antifungal susceptibility profiles and virulence factors of yeast isolated from the urinary tract.

Methods: We isolated 37 yeasts from urine samples (17 from males and 20 from female), mostly from inpatients (64%), but also from outpatients (36%). Broth microdilution method was used for determination ofminimal inhibitory concentration for two antifungal drugs. Biofilm biomass of yeast isolates was quantified bystaining with crystal violet and the formation of germ tube was monitored microscopically. In addition, the ability to adhere to cervical HeLa cells was performed in-vitro for representative isolates.

Results: Overall, we found that *C. albicans* was the most frequent yeast isolated. Nakaseomyces glabratus was found exclusively in female urinary samples. Noteworthy, all yeasts isolated from the urinary tract were susceptible to both antifungals, regardless of its origin. Urinary *Candida* isolated from females were greater biofilm producers and had a greater ability to adhere to HeLa cells, while germ tube formation was more prevalent among male urinary Candida.

Conclusions: Although yeast species distribution in the female and male urinary tract were different, the antifungal susceptibility profiles were the same. Nonetheless, *Candida* isolates from the urinary tractexpressed relevant virulence factors, evidencing the need for more studies to unravel the pathogenic potential of urinary yeast.

Keywords: Urinary infections; *Candida*; Virulence factors

ID 266 | Evaluation of functional properties of peptides secreted by *Saccharomyces cerevisiae* with potential skin benefits

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Background: Yeast peptides have gained significant interest in the cosmetics industry since they may exhibit functional properties with skin benefits, such as antimicrobial, anti-inflammatory, antioxidant and anti- collagenase effect (ACE), as well as wound healing properties, rendering them excellent candidates for inclusion in cosmetic formulations.

Methods: Antimicrobial activity of the *S. cerevisiae* 2-10 kDa peptide fraction (SPF) (obtained as describedin [1]) was assessed against several dermal pathogens, using the broth microdilution method, according to [2]. ACE of the SPF was assessed In Vitro using the Collagenase Activity Colorimetric Assay Kit (Merck KGaA). Antioxidant activity was determined spectrophotometrically monitoring the reduction of nitroblue tetrazodium (NBT) to the blue chromogen formazan by superoxide anion, as described in [3]. Anti- inflammatory effect of the SPF was measured by quantification of tumor necrosis factor alpha (TNF-***α***) mRNA via RT-qPCR on dermal fibroblasts after exposure to lipopolysaccharides (LPS), as described in [1]. Wound healing assays were performed in human fibroblasts, endothelia cells and dermal keratinocytes by assessing the in vitro formation of capillary-like tubes by endothelial cells [4] and by the Scratch test [5]upon exposure to SPF.

Results: The SPF showed antimicrobial activity against 10 dermal pathogen species, exhibiting MICs ranging from 125 µg/ml for *Staphylococcus aureus* to 1000 µg/ml for *Candida albican*s and *Streptococcus pyogenes*. *In Vitro* collagenase activity was inhibited by the SPF, with a reduction of 41.8%, and 94.5% for SPF at 250 and 1000 µg/mL, respectively. Pre-incubation of dermal fibroblasts with the SPF resulted in a 0.15 ± 0.02 decrease of TNF-***α*** expression levels, revealing its protective effect against inflammation. No wound healing effect was found in the tests performed on dermal cells with the SPF.

Conclusions: The SPF analyzed showed functional properties (antimicrobial, anti-oxidant, ACE and anti- inflammatory) with potential skin benefits, revealing to be promising candidate for inclusion in cosmetic formulations.

Keywords: Yeast peptides; antimicrobials; antioxidant, anti-collagenase; anti-collagenase;anti-inflammatory

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ID 268 | Delving into the Fungicidal Mechanisms of ZnO and Ag-ZnO Thin Films Against *Candida albicans*

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Background: Cross-contamination of the near-patient surfaces contributes to the spread of Hospital- acquired infections (HAIs), with *Candida spp*. emerging as the predominant fungal species. Developing effective antimicrobial coatings holds promise for mitigating HAI spread. This study explores the molecular mechanisms underlying the antifungal action of Zinc Oxide (ZnO) and Silver (Ag)-ZnO thin films against *Candida albicans*.

Methods: *Candida albicans* cells were directly incubated on the thin films surface and their antifungal efficacy was assessed by colony forming units (CFUs) counting. Fungicidal mechanisms were investigated through morphological changes, ROS production, cell membrane damage, and leachate activity.

Results: ZnO and Ag-ZnO thin films exhibited remarkable antifungal efficacy. Ag-ZnO thin films demonstrated complete inhibition (100%) of *Candida albicans* growth, while ZnO thin films achieved 80% inhibition. Analysis of the fungicidal mechanisms revealed intriguing differences between the two materials. Ag-ZnO thin films induced robust ROS production (99%), whereas ZnO films induced modest oxidative stress (20%) and cell membrane damage (10%). The addition of the antioxidant Ascorbic Acid (AA)effectively reversed Ag-ZnO films' antifungal activity, underscoring the antifungal activity of Ag by ROS production. Conversely, AA exacerbated the pro-oxidative effect of ZnO films, reducing cell survival due to increased ROS production and membrane damage. Interestingly, the ZnO thin films leachate exhibited substantial antifungal activity (70%), though without significant ROS production or membrane damage, suggesting the presence of soluble components contributing to their effectiveness.

Conclusions: These findings provide insights into ZnO and Ag-ZnO thin films' antifungal mechanisms. WhileAg induces oxidative stress, ZnO operates via an alternative mechanism. This study deepens understanding of antimicrobial coatings and offers potential for novel strategies in combating fungal infections.

Keywords: *C.albicans*; Antifungal Coatings; Fungicidal Mechanisms

ID 286 | Environmental Reservoirs of *Candida auris*: Insights from Natural and Hospital Settings

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Candida auris, a multidrug-resistant yeast, poses significant challenges in healthcare settings worldwide. Understanding its environmental reservoirs is crucial for effective control strategies. Here, we aim to systematically review the literature regarding the natural and hospital environmental reservoirs of *C. auris*.

Published studies until October 2023 were searched in three databases: Pubmed, Web of Science, and Scopus. Information regarding the origin, sampling procedure, methods for laboratory identification, and antifungal susceptibility were collected and analyzed.

Twenty-five papers focused on hospital environmental sources of *C. auris*, mostly during outbreaks of this fungal infection. *C. auris* (mainly Clade I and III) was frequently detected on surfaces near the patient's bed area, in high-touch objects, and on mobile medical devices, such as thermometers and stethoscopes. Additionally, 13 studies have detected the presence of this yeast in areas outside the patient's rooms, such as keyboards, sinks, and air vents. Regarding natural reservoirs, *C. auris* (Clade I) was detected in diverse environmental substrates, including ears and skin from dogs, apples, soil from a salt marsh, beach sand, and seawater in India, as well as water from estuaries in Colombia. Detection methods varied, with molecular techniques often used alongside culture. Susceptibility profiles revealed resistance patterns tothe main antifungal drugs. Phylogenetic studies highlight the potential of environmental strains to influence clinical infections.

Recognizing the environmental reservoirs of *C. auris* is crucial to comprehending pathogen transmission. It will contribute to understanding the dynamics of the disease, leading to the development of more effective control strategies.

Keywords: *Candida auris*; environmental reservoirs; hospital

ID 289 | Characterizing Ato2 proteins: Expression profiles and Subcellular Dynamics in *Candida albicans*

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Background: While typically harmless as a commensal organism, *Candida albicans* assumes pathogenic potential particularly in immunocompromised individuals. The World Health Organization (WHO) has highlighted concerns about the growing prevalence of this fungus, the rising incidence of antifungal resistance and the significant morbidity and mortality associated with it.

In specific areas of the host, especially where glucose is scarce, carboxylic acids are produced (e.g.,acetate or lactate) and can be utilized by *C. albicans*, leading to a metabolic adaptation that significantly contributes to its pathogenicity.

Jen transporters and possibly Acetate Transporter Ortholog (Ato) proteins facilitate the uptake of carboxylates across the plasma membrane (PM). The Ato proteins belong to the acetate uptake transporter (AceTr) family, which includes 10 proteins (Ato1 – Ato8), whose roles and full functions are not yet fully understood.

Methods: In this study, we investigated the subcellular localization and aimed to characterize the expressionprofiles of CaAto proteins in distinct metabolic and physiological conditions, with more focus on CaAto2. To achieve this, the CRISPR-Cas9 system was used to generate a *C. albicans* strain harbouring a ATO2-GFP fusion at the chromosomal level. The expression of Ato2-GFP was assessed using fluorescence microscopy in living *C. albicans* cells grown in conditions mimicking human host environments, such as the gut, which typically contain relevant short chain fatty acids.

Results: The results indicate that the expression of Ato2-GFP is induced in the presence of acetic and lacticacids, predominantly at the plasma membrane, with subsequent localization to the vacuole observed after 24 hours. A weak Ato2-GFP internal signal is also noted in the presence of propionic, formic and butyric acids.

Conclusions: These observations suggest that Ato2 likely plays pivotal roles in the utilization and uptake of lactate and acetate in *C. albicans*, pointing to Ato proteins as potential targets for therapeutic intervention.

Keywords: Fungal infections; Yeast plasma membrane

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ID 290 | Antifungal susceptibility profile and resistance mechanisms displayed by *Candida parapsilosis* species complex isolates

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The increased prevalence of the healthcare-associated pathogen Candida parapsilosis urges their continuous monitorisation. Here we aimed to characterize the azole susceptibility of a *C. parapsilosis* complex collection and elucidate the molecular mechanisms involved in *C. parapsilosis* fluconazole (FLC) resistance.

Among a total of 281 clinical strains, 88.97% were identified as *C. parapsilosis*, 6.05% as C. metapsilosis, and 4.98% as *C. orthopsilosis*. According to CLSI protocol, the susceptible/wild-type phenotypes to FLC, voriconazole (VRC), and posaconazole (PSC) drugs were the most prevalent in the psilosis complexstrains. Resistant phenotype to FLC and VRC was displayed by 10.4% and 3.2%, respectively, of the *C. parapsilosis* isolates while only 1.2% displayed a non-wild type profile to PSC. In the subset of *C. parapsilosis* FLC-resistant strains (n=26), MDR1 was upregulated in 21 resistant strains, while ERG11 and CDR1 overexpression was detected in 7 isolates. The analysis of these encoding sequences and their respective transcription factors revealed several amino acid substitutions.

Our data reveals *C. parapsilosis* as the main etiologic agent in this Portuguese psilosis complex collection and associates the MDR1 efflux pump overexpression as the major mechanism underlying fluconazole resistance.

Keywords: Azole resistance; multidrug efflux pumps;

ABSTRACTS

Topic: YEAST ADAPTATION, CELL DEATH AND AGING

ORAL COMMUNICATIONS

ID 252 | Vps27 is essential for mitochondrial respiration and lifespan extension in Sit4 deficient cells

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Background: *Saccharomyces cerevisiae* Sit4 is a PP2A-like ser/Thr protein phosphatase implicated in a wide landscape of cellular processes, namely regulation of cell cycle and budding, carbohydrate and lipid metabolism, and organelle function. Previous work demonstrated that yeast cells lacking Sit4 exhibit an extended chronological lifespan associated with mitochondrial derepression and alterations in vacuolar morphology and function. A proteomic analysis of vacuolar membranes in Sit4-deficient cells showed an increase in the levels of the endosomal sorting complex required for transport-0 complex (Vps27-Hse1). Notably, Vps27 is critical for the sit4Δ increased lifespan. This work aimed to decipher the impact of Vps27 on vacuolar trafficking pathways and mitochondrial function and its implications in sit4Δ longevity.

Methods: Vacuolar trafficking pathways and mitochondrial protein levels were analysed by Western blotting. Mitochondrial fitness was assessed by analysing cell growth in fermentative (glucose) vs. respiratory (glycerol) media and oxygen consumption rate using a Clark's electrode. Iron levels were quantified using a colorimetric assay. All experiments were conducted in triplicates. Statistical analyses were performed using One-Way ANOVA.

Results: The analysis of protein trafficking and vacuolar sorting pathways showed that Vps27 did not increase sit4Δ lifespan by improving autophagy or the Cvt and CPY pathways. However, Vps27 was required for iron homeostasis and mitochondrial derepression in *SIT4* deleted cells, as sit4Δvps27Δ mutants exhibited high iron levels, low Atp2 and Cox2 protein levels and very low oxygen consumption rate.

Conclusions: Vps27-dependent longevity in Sit4-deficient yeast cells was found to be unrelated to alterations in some of the major vacuolar trafficking pathways. Our work unveiled a negative genetic interaction between *SIT4* and *VPS27* affectingg mitochondrial respiration and chronological lifespan in yeast.

Keywords: Sit4; Vps27; Longevity; Vacuoles; Mitochondria

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ID 283 | pH-dependent downregulation of a yeast nutrient transporter: mechanisms and metabolic implications

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Background: *Saccharomyces cerevisiae* cells alkalinize their surroundings when cultivated with lactate as a carbon source, which leads to the turnover of the Jen1 lactate transporter. Endocytosis was previously reported to depend on a functional Jen1 transporter and to involve Bul1 α-arrestin and an active TORC1 complex [1]. However, it remained unclear whether internalization of Jen1 during the prolonged growth on this monocarboxylate was exclusively due to increase in external pH.

Methods: To deeply explore the regulatory mechanisms behind the alkali stress-induced endocytosis of Jen1, *S. cerevisiae* cells stably expressing a *JEN1-GFP* fusion were studied in aerobic carbon-limited continuous cultures subjected to a linear increase of the culture pH. Jen1 internalization in these bioreactor cultures occurred at a specific pH transition value.

Results: This pH threshold coincided with wash-out of the chemostat cultures and with release of pyruvate and acetate (substrates of Jen1). Transcriptome analysis showed upregulation of genes involved in oxidative phosphorylation, suggesting that the energy status of the cells is being affected around this pH range.

Conclusions: These data support the hypothesis that Jen1 internalization prevents loss of crucial metabolites when extracellular pH exceeds the intracellular pH and, consequently, the driving force for Jen1-mediated carboxylate-proton symport changes from an inward- to an outward-direction. Jen1 endocytosis seemed to have evolved as a mechanism to increase cellular robustness in a pH changing environment.

Keywords: transporters; lactate; endocytosis; alkali stress

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FLASH PRESENTATIONS

ID 258 | Bacteria isolated from the microbiome of two marine microalgae species display promising antifungal activity against *Candida* spp.

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Background: Invasive fungal infections (IFIs) caused by opportunistic yeast *Candida spp*. are generally associated with high rates of mortality and morbidity among hospitalized patients, and impose significant socioeconomic burdens. The progress in life-saving medical treatments, the limited repertoire of effective drugs, and the increasing resistance to available antifungals have worsened this scenario and created a huge demand for new antifungal drugs.

The marine environment remains an untapped source of valuable medicines, and, in particular, the microbiome of marine organisms has triggered great interest because, if cultivable, it represents a sustainable and low-cost approach to developing effective drugs.

Methods: We screened a library of bacteria isolated from the microbiome of marine microalgae *Phaeodactylum tricornutum* and *Nannochloropsis oceanica* for antifungal activity against *Candida albicans* through agar diffusion assays. Bacteria exhibiting activity were further characterized for lipidase, chitinase and protease activity, and their genomes were inspected for the presence of relevant biosynthetic gene clusters (BGCs).

Results: We have identified several bacterial species with antifungal activity against *Candida spp.* At least one of them requires contact with yeast in order to secret the active compound, as confirmed by growth curve analyses using spent growth medium from mono and co-cultures. Analysis of the genome of bacteria displaying antifungal activity revealed promising BGCs.

Conclusions: Marine bacteria secrete compounds with antifungal potential against *Candida spp.* Some of these compounds are only secreted upon contact of the bacterium with the yeast. These findings underscore the marine microbiome as a valuable source for novel antifungals. We are currently investigating the nature of the active compounds.

Keywords: *Candida albicans*; antifungals; marine bacteria

ID 263 | Iron antagonizes with fluconazole against *Candida glabrata*

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Invasive fungal infections (IFIs) represent a significant health concern worldwide, as recently acknowledged by the World Health Organization upon releasing the first priority list of fungal pathogens. Fluconazole is the most frequently prescribed antifungal for treating IFIs, due to its affordability, oral bioavailability, broad spectrum of activity, and safety profile. This drug targets lanosterol 14-α-demethylase, an enzyme that catalyzes the conversion of lanosterol to ergosterol, whose inhibition arrests cell growth, due to the accumulation of toxic sterol species and disruption of membrane integrity. Recently, we found that iron loading conditions decrease fluconazole activity against *Candida glabrata*, the second most prevalent cause of nosocomial IFIs. In this work, we explored the mechanisms underlying the antagonistic interaction between iron and fluconazole.

Checkerboard assays were used to investigate the antagonism between iron and fluconazole. We performed qRT-PCR to measure mRNA levels of genes related to fluconazole resistance. We also quantified ergosterol and fluconazole levels using HPLC, to infer their amount on the cell membrane and inside the cell, respectively.

We have confirmed the antagonistic effect between iron and fluconazole across a wide range of concentrations. The presence of both iron and fluconazole does not decrease ergosterol levels as much as the drug alone. Co-treatment of iron and fluconazole increases the expression of genes encoding proteins crucial for fluconazole detoxification, such as Cdr1 and Cdr2, and their transcription factor Pdr1, compared to fluconazole alone.

The fact that the combination of iron and fluconazole prevents the accentuated reduction of ergosterol, along with the upregulation of CDR1, CDR2, and PDR1 by iron, suggests that co-treatment conditions may hyperactivate Pdr1. However, further studies are needed to strengthen our hypothesis. As iron excess is associated with many medical conditions or treatments, this finding can have important clinical implications regarding treating IFIs caused by *Candida glabrata* in such contexts.

Keywords: *Candida glabrata*; fluconazole; iron; Pdr1

ID 276 | Influence of different sweeteners on *Saccharomyces cerevisiae* during aging

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Background: With the increasing prevalence of obesity, Diabetes *mellitus* and other metabolic diseases, sweeteners have emerged as appealing substitutes of glucose in food, as well as in oral hygiene and pharmaceutical products. They give sweetness without adding calories or glycemic effects. Although many sweeteners are approved for use by International Health Agencies, controversies still exist regarding their impact on body weight, metabolic diseases, neurotoxicity and carcinogenicity. Additionally, there is a lack of studies exploring the influence of sweeteners on cellular aging, a complex and multifactorial biological process determined by a combination of genetic and environmental factors. The main objective of this work was to investigate the influence of usual sweeteners might have on the yeast Saccharomyces cerevisiae during chronological lifespan (CLS).

Methods: To access the impact of sweeteners, the CLS of the wild type *S. cerevisiae* BY4741 cells and its mutants hog1Δ, sch9Δ, and ras2Δ was evaluated. Cell cultures were supplemented with either glucose or sweeteners, and the osmolarity of each sweetener in the growth medium was measured. Then, the cellular mechanisms associated with aging were assessed (autophagy flux by GFP-ATG8 processing; cell cycle and accumulation of the reactive oxygen species by flow cytometry analysis).

Results: The findings revealed that cyclamate prolonged CLS, concomitant with reduced autophagy flux and ROS accumulation in wild type (WT) cells. Conversely, saccharin did not have a major impact in CLS or ROS formation, although the WT cells exhibited an increased autophagic flux.

Conclusions: The sweeteners might show divergent effects on different intracellular signaling pathways associated with aging and these outcomes indicate that additional studies are needed to elucidate the long- term effects of sweeteners on cellular processes.

Keywords: aging; sweeteners; yeast.

Acknowledgements

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ABSTRACTS

Topic: DRUG AND STRESS RESISTANCE

ORAL COMMUNICATIONS

ID 253 | Essential Oil Vapors: A Novel Approach to Preventing Antifungal-Resistant *Candida*Biofilms

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Background: Human fungal infections, especially by *Candida* species, are on the rise. Current antifungal drugs have significant limitations, including side effects and reduced effectiveness, leading to drug-resistantstrains. Therefore, there is a growing need for new, more effective, and less toxic treatments. This study explored the antifungal properties of essential oils (EOs) on drug-resistant *Candida* species.

Methods: For this study, *C. albicans* (n=3), *C. glabrata* (n=8), *C. krusei* (n=1) and *C. guilliermondii* (n=1) vaginal isolates belonging to a collection of yeasts created by the FBH group of Centre of Biological Engineering of University of Minho, in the scope of a large-scale recovery of vaginal specimens of *Candida*, carried out in Health Centers of North of Portugal, were used. All the isolates are at least resistant to one of the antifungalagents (fluconazole, ketoconazole or caspofungin). The antimicrobial effect of tea tree, niaouli, white thyme,and cajeput EOs on the planktonic growth of *Candida* isolates was initially evaluated using an agar disc diffusion method. The vapor-phase of tea tree EO (VP-TTEO) effect on biofilm formation and pre-formed biofilms was evaluated by crystal violet staining, XTT reduction assay, colony forming units' enumeration, and scanning electron microscopy.

Results: The results revealed high antifungal activity of tea tree, cajeput,niaouli, and white thyme EOs against strains with extreme resistance to common antifungals, such as *C. albicans, C. glabrata, C. guilliermondii*, and *C. krusei*. The VP-TTEO showed a significant inhibitory effecton biofilm formation (up to 4log reduction) of all tested isolates and caused a substantial reduction in mature *C. albicans* biofilms (3log reduction). Tea tree, cajeput, and

niaouli EOs demonstrated significant antifungal activity, while VP-TTEO exhibited notable preventive effects against biofilm of antifungal-resistant strains.

Conclusions: Overall, this study suggests that VP-EOs may be a promising solution to prevent biofilm infections caused by antifungal-resistant strains.

Keywords: Drug-resistance; Biofilm; *Candida;* Phytotherapy; Essential-oils

ID 261 | A copper complex of a fluconazole derivative demonstrates outstanding antifungal properties

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Background: *Candida* species are the leading cause of Invasive Fungal Infections (IFIs) in hospitals. Existing antifungal drugs are not entirely effective due to undesirable side effects and the emergence of resistance. We have recently shown that combining fluconazole, the most widely prescribed antifungal worldwide, with copper enhances its antifungal activity against *Candida glabrata*. This species, intrinsically tolerant to fluconazole, is a frequent causative agent of nosocomial IFIs. Analyses of the transcriptomic profile of *C. glabrata* following the combination of copper and fluconazole revealed a decrease in the expression of the multidrug transporter gene *CDR1,* suggesting that fluconazole efflux could be affected. Consistent with this observation, we found that copper inhibits the transactivation of Pdr1, the transcription regulator of several multidrug transporter genes, leading to the intracellular accumulation of fluconazole. These findings prompted us to synthetize a copper complex of a fluconazole derivative and evaluate its antifungal potential.

Methods: ICP-AES (copper measurements), HPLC (ergosterol measurements), antifungal susceptibility assays (CLSI), cytotoxicity assays and RT-PCR (gene expression).

Results: The novel compound is effective against all *Candida* species tested, with MICs substantially lower than those of fluconazole. Like fluconazole, it shows no *in vivo* toxicity against HeLa cells. We detected intracellular copper accumulation in yeast treated with the fluconazole-copper complex, suggesting that it can enter the cells. The complex is more effective than fluconazole in reducing the ergosterol levels and it is active against a fluconazole-resistant *Candida glabrata* strain. Interestingly, we found that the complex downregulates *CDR1* gene expression, indicating a possible inhibitory mechanism on Pdr1, which we are currently investigating.

Conclusions: This work demonstrates that the combination of metals with azoles can lead to more powerful bi-functional antifungal drugs. These are capable of disrupting fungal metal homeostasis while also inhibiting the mechanisms fungi use to eliminate toxic levels of antifungal drugs.

Keywords: *C.glabrata*; Fluconazole; Copper; Antifungal-Resistance; Pdr1.

FLASH PRESENTATIONS

ID 257 | Caspofungin binding to iron reduces its antifungal activity against *Candida albicans*

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Background: Invasive fungal infections caused by *Candida spp*. are the most common severe fungal diseases among hospitalized patients receiving immunosuppressive or intensive antibacterial therapies. Iron availability affects the progression of fungal infections and has been described to alter the susceptibility to therapeutic interventions. Here we reveal an additional mechanism by which iron overload interferes with the activity of caspofungin, an echinocandin drug that acts by inhibiting the synthesis of β-1,3-D-glucan, an important component of the fungal cell wall.

Methods: We studied the effect of iron on the activity of caspofungin against *Candida albicans* by flow cytometry and SEM. The interaction between caspofungin and iron was investigated using checkerboard microdilution assays. By UV-Visible spectroscopy, NMR and molecular dynamics, we tested caspofungin's iron-chelating properties. Enzymatic assays were performed to quantify β-1,3-D-glucan synthase activity in the presence of caspofungin and iron. Caspofungin efficacy *in vivo* in the context of iron overload was assessed using a *Galleria mellonella* model mimicking invasive candidiasis.

Results: The effect of caspofungin on *Candida albicans* viability, cell wall integrity and biofilm formation is mitigated under iron overload conditions, indicating a clear antagonism between the metal and the drug. This effect is caspofungin-specific and is observed in other clinically relevant fungal species. We show for the first time that caspofungin is able to bind iron. The *in vitro* activity of caspofungin's target, β-1,3-D- glucan synthase, is restored in the presence of iron. Importantly, iron overload compromises caspofungin activity *in vivo* against *Candida albicans,* leading to a decrease in *Galleria mellonella* larvae survival and an increase in fungal burden.

Conclusions: Iron decreases caspofungin activity against *Candida albicans* by binding to the drug, which ultimately renders caspofungin unable to inhibit its target. Our findings may have

significant implications for the treatment of invasive candidiasis in medical settings associated with iron overload.

Keywords: *Candida*, iron, echinocandins

ID 260 | New insights into *Saccharomyces cerevisiae* arsenic detoxification

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Background: Arsenic (As) is a naturally occurring and highly toxic metalloid, widely distributed in the environment, which contaminates water and food resources. In the yeast Saccharomyces cerevisiae the arsenate (As^{V}) reductase Acr2 and the arsenite (As^{III}) permease Acr3 are the key players in arsenic detoxification, and their mode of action is well documented. However, our recent data indicate that arsenatedetoxification is considerably more complex than currently assumed.

Results: We found that the deletion of both ACR2 and ACR3 genes increased *S. cerevisiae* sensitivity to As^{V} and led to a massive cellular accumulation of arsenic, which does not occur in the Δacr2 single mutant.These results challenge the accepted model for As^{V} detoxification in yeast, and suggest the direct involvement of the Acr3 transporter in As^{V} detoxification or the presence of an additional arsenate reductase. Our data show that both alternative pathways should coexist. We found that the Acr3 permease is able to transport not only As^{III} but also As^{V}, albeit with lower affinity, and an as yet unidentified reductasecan reduce As^{V} in the absence of Acr2.

Methods: Intracellular As was quantified by ICP-AES and As^{V}/As^{III} speciation was determined by mass spectrometry. To investigate other possible pathways of arsenate detoxification, we used RNA sequencing to search for differential expressed genes in Δacr2 cells stressed with As^{V}. In order to determine if As^{V} is a substrate of the Acr3 permease we expressed a recombinant form of the protein on yeast Δacr2Δacr3 cells and prepared plasma membrane vesicles by two-phase partitioning. We then evaluated the ability of these vesicles to transport As^{V} as well as other metals, and determined the parameters of the translocations.

Conclusions: Our findings bring to light additional pathways for arsenate detoxification in yeast.

Keywords: Arsenic; metalloid detoxification; arsenate reductase.

ID 269 | Tackling molecular players underlying tolerance to SO_2 in *Saccharomyces cerevisiae* wine strains: beyond the sulphite efflux pump SSU1

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Background: In the stressful environment of winemaking *Saccharomyces cerevisiae* is the leading species but the performance of the strains used is prominently affected by their tolerance to sulphite (SO_2), themost commonly preservative in vinification. Previous studies have identified the *SSU1* gene as the key player mediating the different degrees of tolerance of S. cerevisiae strains to SO_2. *SSU1* encodes asulphite efflux pump and in more tolerant *S. cerevisiae* strains a chromosomal rearrangement resulting in increased strength of its promoter is observed, resulting in higher tolerance.

Methods: In this work, we screened a cohort of approximately 50 *S. cerevisiae* strains (including commercial and “natural isolates” recovered from grape musts) for their tolerance to SO2. We performed extensive genotype regarding *SSU1* gene locus, along with comparative genomic analyses over the strains identified.

Results: Considerable phenotypic heterogeneity was found across the tested *S. cerevisiae* strains to SO_2 with some being highly tolerant while others were highly susceptible. Surprisingly, the SO_2-phenotype could not be clearly correlated with the occurrence of modifications in the *SSU1* promoter region which is a significant change to what has been described in the literature until this far. We could, for example, identify strains harbouring *SSU1-R1* rearrangement sensitive to SO_2, while others harbouring the wild-type *SSU1* allele were highly tolerant. Besides presenting these results (undertaken under different experimental settings), we will also show comparative genomic and transcriptomic analyses of these strains as a mean toclarify the full set of genes that determine tolerance to SO_2 in *S. cerevisiae.*

Conclusions: The results of our work enable a more efficient selection of *S. cerevisiae* strains to be used in wine production, while at the same time disclose important aspects by which

the physiology of this species is impacted by its domestication (in this case, concerning SO_2 tolerance).

Keywords: *Saccharomyces cerevisiae*; sulphite tolerance; *SSU1*

Acknowledgements
This research was supported by Fundação para a Ciência e a Tecnologia through SMARTWINE 2.0 project (2022.06777.PTDC).

ID 270 | Combining fluconazole with benzo[a]phenoxazine derivatives as a promising strategy against fluconazole-resistant *Candida* species

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Background: *Candida* infections remain a significant health threat despite medical advances, with non-*albicans* species increasingly showing resistance to common antifungals. Benzo[a]phenoxazines offerpromising treatment options. Manipulating their polycyclic system with different substituents can yield more effective antifungal agents. C34, with propyl and dipropyl groups at the 5- and 9-positions, showed strong antifungal activity against *S.cerevisiae* and in preliminary tests against *Candida spp*, including the multi- drug resistant *C.auris.* In the present work we evaluated three derivatives of C34: C35, with a chloropropyl group on the 5-position; A44 and A42, both incorporating a julolidine moiety at the 9-position of C34 and C35, respectively.

Methods: Antifungal activity of these compounds against different *Candida* species, was assessed by determining their Minimum Inhibitory Concentration (MIC), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocols for yeasts. Their *in vitro* interaction with fluconazole against resistant strains, using an EUCAST-based checkerboard method, was also determined by analysing the results through fractional inhibitory concentration index (FICI) calculation and response surface analysis, based on the Bliss model.

Results: The overall range of MIC values was very similar among the four compounds, except for A42, which exhibited the highest MIC values, followed by A44. Compound C34 showed the best antifungal activity with the lowest MIC geometric mean, closely followed by C35. Combining C34 with fluconazole resulted in indifferent interactions across the species tested. However, the presence of the chloropropyl group - C35 – showed a synergistic effect in *C.krusei*. A44, with the julolidine moiety, also exhibited synergy in *C.auris.* Notably, A42, which combines both alterations, was the only compound to show synergistic effects with fluconazole across all tested species (*C.glabrata,C.krusei* and *C.auris*).

Conclusions: Our findings demonstrate that combining fluconazole with benzo[a]phenoxazine derivatives may be effective against fluconazole-resistant *Candida*

species, suggesting that these compounds could be a promising strategy for overcoming antifungal resistance.

Keywords: *Candida*; benzo[a]phenoxazines; fluconazole; synergy.

ID 271 | Unraveling new mechanisms governing azole resistance mechanisms in clinical *Candida glabrata* strains: beyond CgPdr1

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The pathogenic yeast *Candida glabrata* has a prominently high ability to rapidly acquire resistance to azoles, a trait that reduces the efficacy of antifungal treatments and, consequently, increases mortality ratesin infected patients. Years of studies focused on this species provided us with a landscape of the molecular mechanisms implicated in azole tolerance in this yeast species1, however, most studies were performed using lab strains and not clinical strains. Consequently, it remains to be studied what drives the phenotypeof resistance in clinical strains that are exposed to a multi-stress environment while colonizing the host. Our lab has been working to elucidating resistance mechanisms in clinical strains. To disclose azole resistance mechanisms in clinical *C. glabrata* strains we have been exploring comparative genomic and transcriptomic analyses (based on high-throughput DNA and RNA sequencing) supported by confocal microscopy and membrane lipid analyses. Screening for resistance to fluconazole and voriconazole in a cohort of ~600 *Candida* isolates led us to identify 8 *C. glabrata* strains resistant to voriconazole and fluconazole. 5 of these strains were found to encode hyperactive variants of the CgPdr1 transcriptional regulator, a central mediator of azole resistance in *C. glabrata*. Consistently, a high predominance of CgPdr1 hyper-active mutants among azole-resistant strains is commonly described, although three mutants were only described in our strains: I392M, I803T and K274Q. Notably, we found three resistant strains (isolates ISTB218, ISTD190 e ISTF58) that encoded a wild-type CgPDR1 allele. Herein we will discuss results from comparative genomic and transcriptomic analyses undertaken in these azole-resistant strains that resulted in the identification of new resistance mechanisms, independent of CgPdr1.The results of our work pave the way for the understanding of the acquisition of resistance to azoles *in vivo* while also foster the development of new diagnostic tools for a rapid detection of resistant strains.

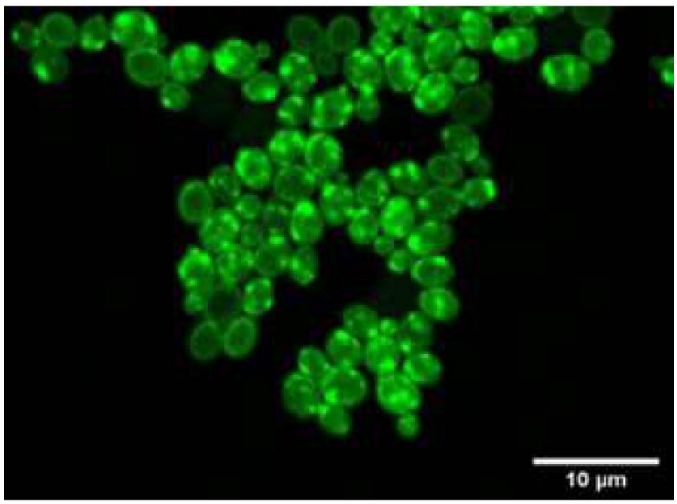
Keywords: CgPdr1-dependent and independent azole-resistance; *C.glabrata*

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ID 285 | Antimicrobial activity of rowan leaf and berries *(Sorbus aucuparia)* extracts against spoilage microorganisms

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Background: The growing need for safe and sustainable food preservation methods has led researchers to explore alternative antimicrobial agents as substitutes for chemical preservatives. One approach has been to harness plant-derived products as antimicrobial agents for use in food production chains to preserve and control microbial spoilage.

Methods: The antimicrobial activity of 14 extracts of leaves and berries of *Sorbus aucuparia* against three spoilage yeasts (*Zygosaccharomyces bailii, Saccharomycodes ludwigii* and *Dekkera bruxellensis*), and four filamentous fungi (*Aspergillus niger, Botrytis cinerea, Mucor sp. and Penicillium sp.*) was assessed using broth micro-dilution method, and the minimum inhibitory concentrations (MIC) values were determined according to EUCAST guidelines.

Results: The rowan leaf and berries extracts showed variable antimicrobial activity against the three tested yeast species, with MICs ranging from 19.54 mg/L to 633.4 mg/L and 180.8 mg/L to 1138 mg/mL against *Z. bailii* and *S. ludwigii*, respectively. On the other hand, all extracts were less effective against *D. bruxellensis* and the targeted molds.

Conclusions: The extracts that showed the highest antimicrobial activity warrant further investigation,including the identification and isolation of the active compounds, for the development of new bio-based food preservation strategies.

Keywords: antimicrobial activity;plant extracts;spoilage yeasts;molds;biobased preservatives

ID 291 | *Debaryomyces hansenii* Adaptation to NaCl: Compairing Physiological Traits in Exponential and Stationary Growth Phases

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Debaryomyces hansenii is an unconventional yeast species with attributes that make it highly promising for various biotechnological applications. Its notable ability to thrive in NaCl-rich environments, even with low potassium concentrations, is particularly intriguing.

The primary objective of this work was to compare several physiological parameters of cells cultivated in mineral media containing 1 M NaCl and those grown in 1 M KCl. Special emphasis was placed on comparing cells in the exponential and stationary growth phases, focusing on growth kinetics, glucose utilization, and intracellular concentrations of trehalose, Na+, K+, and Pi. Additionally, we assessed intracellular pH, proton efflux, and proton membrane permeability.

Cells cultivated with both 1 M NaCl and 1 M KCl reached the stationary phase without depleting their carbon and energy sources. The responses were similar for both cations, although cells in NaCl exhibited a longer latency period and slightly lower final biomass. Regarding other cellular parameters, the behavior of cells grown in 1 M NaCl and 1 M KCl varied depending on the cation and growth phase. These discrepancies were more pronounced in intracellular contents, proton fluxes, intracellular pH, and glucose transport. Notably, exponential phase cells displayed more differences than stationary phase cells when grown in either cation. Exponential phase cells grown in 1 M NaCl exhibited higher proton efflux, reduced proton influx rates, similar K+ content, and higher intracellular pH compared to those grown in 1 M KCl.

The findings suggest that distinct resistance mechanisms may be present in cells at different growth stages.Additionally, they confirm that despite significant intracellular accumulation, sodium does not exert toxic effects and does not impede cellular metabolism, affirming the adaptability of *Debaryomyces hansenii's* cellular physiology to the presence of sodium in the growth medium.

Keywords: *D.hansenii*; salt-tolerance; Physiological traits; NaCl

Acknowledgments: This work was financially supported by the Portuguese Foundation for Science and Technology (FCT) (LEAF Research Unit UIDB/AGR/04129/2020).

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