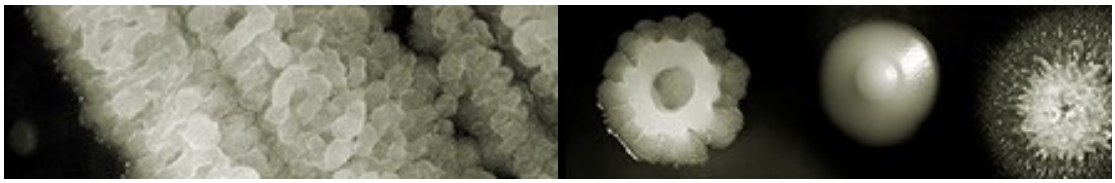
Livro de Resumos

XXI Jornadas de Biologia de Leveduras

"Professor Nicolau van Uden"



8 e 9 de Junho | 2018
Universidade do Minho, Braga

XXI Jornadas de Biologia de Leveduras

"Professor Nicolau van Uden"

As XXI Jornadas de Biologia de Leveduras "Professor Nicolau van Uden" decorrem na Universidade do Minho numa organização conjunta dos:

. Centro de Biologia Molecular e Ambiental (CBMA) | Departamento de Biologia da Escola de Ciências,

. Instituto de Investigação em Ciências da Vida e Saúde (ICVS) | Escola de Medicina

. IB-S - Instituto de Ciência e Inovação para a Bio-Sustentabilidade da Universidade do Minho.

Comissão organizadora

Maria João Sousa (CBMA/DB- EC) (*Chair*)
Cecília Leão (ICVS-EM)
Cândida Lucas (IB-S, CBMA/DB- EC)
Célia Pais (CBMA/DB- EC)
Fernando Rodrigues (ICVS-EM)
Manuela Côrte-Real (CBMA/DB- EC)
Margarida Casal (CBMA/DB- EC)
Maria Judite Almeida (CBMA/DB- EC)
Paula Ludovico (ICVS-EM)
Sandra Paiva (CBMA/DB- EC)
Susana Chaves (CBMA/DB- EC)

Programa

Sexta-feira, 8 de junho

10:00 **Receção e Entrega de Documentação**

11:00 - 11:20 **Sessão de Abertura**

Maria João Sousa (Presidente da Comissão de Organização)
Cândida Lucas (Diretora do IB-S)
Jorge Pedrosa (Diretor do ICVS)
Fernanda Cássio (Diretora do CBMA)

11:20 – 12:20 **Conferência "Professor Nicolau van Uden"**

Moderadores: Mª da Conceição Loureiro Dias e Cecília Leão

Dos fungos filamentosos aos unicelulares e das vinhas aos hospitais: espectro de um fungicida de última geração

Virgílio Loureiro

Sessão I - Exploring Yeast Diversity for Biotechnology

Moderadores: Arlete Mendes-Ferreira e Madalena Oom

12:20-12:35 **A genomic and phenotypic characterization of a lineage of *Saccharomyces cerevisiae* associated with olive brine**

Ana Pontes, Paula Gonçalves and José Paulo Sampaio

12:35-12:50 **Development of potential yeast protein extracts for wine clarification and stabilization**

Leonor Gaspar, Amadeu Machado, Rute Coutinho, Adriana Xavier, Manuel Figueiredo, Victor Freitas, Maria de Fátima Teixeira, Filipe Centeno and João Simões

13:00-14:30 **Almoço**

Sessão II - Exploring Yeast Diversity for Biotechnology (cont.)

Moderadores: José Paulo Sampaio e Catarina Prista

14:30-14:45 **Genome sequence of the non-conventional wine yeast *Hanseniaspora guilliermondii* UTAD222 unveils relevant traits of this species and of the *Hanseniaspora* genus in the context of wine fermentation**

Isabel Seixas, C. Barbosa, A. Mendes-Faia, U. Güldener, R. Tenreiro, A. Ferreira* and N. P. Mira*

14:45-15:00 **Exploring organic acid producer microorganisms – Identification, morpho- and physiological characterization of wild yeast strains**

Maria Sousa-Silva, David Ribas, Ricardo Franco-Duarte, Célia Pais, Margarida Casal and Isabel Soares-Silva

15:00-15:15 **Yeasts as biocontrol agents of fungal phytopathogenic diseases**

Pedro Ferraz, Mariana Rodrigues, Fernanda Cássio and Cândida Lucas

15:15-15:30 **Port and Douro Wines Institute, IP (IVDP) Yeast Culture Collection**

Margarida Roseira, Natália Ribeiro, Vitória Maciel and Maria João Sousa

Sessão III – Yeast Physiology and Genetics

Moderadores: Paula Gonçalves e Isabel Soares-Silva

15:30-15:45 **Impact of horizontal gene transfer on sugar metabolism in a fructophilic yeast lineage**

Carla Gonçalves, Madalena Oom, Maria José Leandro and Paula Gonçalves

15:45-16:00 **Dephosphorylation of the ATP synthase subunit Atp2 by the Ser/Thr protein phosphatase Sit4p contributes to the regulation of mitochondrial function**

Clara Pereira, Andreia T. Pereira, Hugo Osório, Pedro Moradas-Ferreira and Vítor Costa

16:00-16:15 **Copper detoxification in yeast – a new link with iron homeostasis and Fe-S biogenesis**

Ana Gaspar-Cordeiro*, Soraia Marques Caetano*, Catarina Amaral*, Claudina Rodrigues-Pousada*, Catarina Pimentel

16:15-16:30 **Pkh1-dependent activation of Ypk1 is triggered by membrane stress in a yeast model of Niemann-Pick type C1 disease**

Rúben Gonçalves, Vasco Fontes, Vítor Costa and Rita Vilaça

16:30 -17:00 **Café**

Sessão IV – Yeast Cell Death and Aging

Moderadores: Pedro Moradas-Ferreira e Vítor Costa

17:00-17:15 **Pkh1p-Ypk1p and Pkh1p-Sch9p pathway activation underlies mitochondrial-dependent regulated cell death induced by acetic acid**

António Rego, Katrina F. Cooper, Justin Snider, Yusuf A. Hannun, Vítor Costa, Manuela Côrte-Real, and Susana Rodrigues Chaves

17:15-17:30 **Characterization of the yeast cell death induced by a benzo[a]phenoxazine derivative**

João C. Ferreira, C. Lopes, A. Almeida, A. Preto, M. S. T. Gonçalves and Maria J. Sousa

17:30-17:45 **Deciphering the mechanisms underlying bovine milk lactoferrin anticancer activity using yeast and cancer cell lines as complementary models**

Cátia S. Pereira, Joana P. Guedes, Susana R. Chaves, María T. Andrés, José F. Fierro, Hernâni Gerós, Lígia R. Rodrigues and Manuela Côrte-Real

17:45-18:00 **Caloric restriction rescues aged yeast cells from α-synuclein toxicity through regulation of proteolytic systems**

Belém Sampaio-Marques, Hélder R. Pereira and Paula Ludovico

18:00-18:15 **Impact of nutrients in longevity regulation of the yeast *Saccharomyces cerevisiae***

Júlia Santos, Maria João Sousa, Cecília Leão and George van der Merwe

18:15-18:45 **Apresentações *FLASH***

Moderadores: Sandra Paiva e Paula Ludovico

Understanding the molecular mechanisms of homothallism in the order Cystofilobasidiales

Alexandra Cabrita, Márcia David-Palma, Marco A. Coelho, José Paulo Sampaio and Paula Gonçalves

High-throughput screening of oleaginous yeasts by using innovative fluorimetric and spectroscopic approaches

Catarina Miranda, Sara Bettencourt, Tatiana Pozdniakova, Raul Machado, Paula Sampaio, Ricardo Franco-Duarte and Célia Pais

Volatile fatty acids as carbon source for single cell oil production by oleaginous yeasts

Sara Bettencourt, Catarina Miranda, Tatiana Pozdniakova, Paula Sampaio, Ricardo Franco-Duarte and Célia Pais

***Candida albicans* Cht3 as a novel recombinant subunit antigen against fungal infections: characterisation and liposomal encapsulation**

Augusto Costa-Barbosa, M. Dias, M.E.C.D.R. Oliveira, T. Collins and P. Sampaio

Microbiome shift in the context of recurrent vulvovaginal candidosis (RVVC): a new biomarker for recurrence?

Joana Rolo, C. Gaspar, P. Gonçalves-Faria, T. Barata, J. Martinez-de-Oliveira and A. Palmeira-de-Oliveira

Quantitative assessment of DNA damage in the industrial ethanol production strain *Saccharomyces cerevisiae* PE-2

Paulo César Silva, Lucilia Domingues, Tony Collins, Rui Oliveira and Björn Johansson

20:00 **Jantar das Leveduras**
Restaurante CENTURIUM

Sábado, 9 de junho

Sessão V - Yeast and Human Health I

Moderadores: Nuno Mira e Paula Sampaio

9:00-9:15 ***Candida albicans* overcolonization of the elderly gut: Involvement of adenosine A2A receptors**
Lisa Rodrigues, Isabel Miranda, Geanne Andrade, Marta Mota, Luísa Cortes, Acácio Rodrigues, Rodrigo Cunha and Teresa Gonçalves

9:15-9:30 **New multiplex PCR based methodology for the diagnosis of *Candida* and *Aspergillus* species**
Joana Carvalho-Pereira, Jan Springer, Maria José Buítrago, Jurgen Löffler, Célia Pais and Paula Sampaio

9:30-9:45 **Beyond MDR transporter-mediated azole resistance in *Candida glabrata*: functional characterization of the transcription factors CgRpn4 and CgMrr1**
Pedro Pais, Mónica Galocha, Raquel Califórnia, Geraldine Butler and Miguel Cacho Teixeira

9:45-10:00 **Alternative carbon sources modulate *Candida glabrata* biofilm development on medical devices**
Alexandra Gomes-Gonçalves, Rosana Alves, M. Casal, Patrick Van Dijck and Sandra Paiva

10:00-11:30 **Café**

Posters

Molecular mechanisms of chronological lifespan modulation
Hélder Pereira, Alexandra Teixeira, Belém Sampaio-Marques and Paula Ludovico

The versatile role of mannitol in central metabolism of the fructophilic yeast *Starmerella bombicola*
Carolina Ferreira, Carla Gonçalves and Paula Gonçalves

Semen supports growth of *Candida albicans*: a possible role of this pathogen in infertility?
Caixeirinho P., J.Rolo, P.Ruivo-Gomes, C.Gaspar, R.Palmeira-de-Oliveira , J.Martinez-de-Oliveira and A. Palmeira-de-Oliveira

Exposure of recurrent vulvovaginal *Candida spp* isolates to azole compounds prevents biofilm formation?
Faria-Gonçalves P., J. Rolo, C. Gaspar, A. S. Oliveira, T. Barata, J. Martinez-de-Oliveira, and A. Palmeira-de-Oliveira

Functional characterization of *Cyberlindnera jadinii* carboxylate transporters in *Saccharomyces cerevisiae*
Maria Sousa-Silva, David Ribas, Margarida Casal and Isabel Soares-Silva

Towards a genetic toolkit for *Sporothrix brasiliensis* analysis
Beatriz H Ferreira, Gabriela Neves, Jorge H Ramírez-Prado, Paula Sampaio, Célia Pais, Maria SS Felipe, Gustavo Goldman, Agostinho Carvalho, Leila Bezerra, Cristina Cunha and Fernando Rodrigues

The Ady2 "NPAPLGL(M/S)" motif is critical for acetate uptake and binding
David Ribas, Isabel Soares-Silva, Daniel Vieira, Maria Sousa-Silva, Joana Sá-Pessoa, João Azevedo-Silva, Sandra Cristina Viegas, Cecília Maria Arraiano, Sandra Paiva, Pedro Soares and Margarida Casal

Assessing yeast diversity in Portuguese honey samples
Cláudia Carvalho, Céline Freitas & José Paulo Sampaio

Fostering the utilization of Non-*Saccharomyces* yeasts as co-adjuvants of wine and beer fermentations through the exploration of comparative genomics data
Maria J. Tavares, Ulrich Guldener, Arlete Mendes Ferreira, Alexandra M. Ferreira and Nuno P. Mira

***Saccharomyces cerevisiae* as a model system to uncover target and off-target effects of fungicides of environmental concern**
Carla Carvalho, Maria João Sousa, Bruno B. Castro and Susana R. Chaves

Exploring yeast as a tool to study the human pro-apoptotic protein Bax
Vitória Baptista, Cátia S. Pereira, Joana P. Guedes, Maria João Sousa, Susana Chaves and Manuela Côrte-Real

Evaluation of decolourisation of textile dyes by selected yeasts
Marta Mendes, Patrícia Moreira, Paula Castro and Manuela Pintado

Posters (cont.)

Polyphasic identification of a yeast isolate with dye decolourisation ability
C. Neto, M. M. Pintado and P. Moreira

Role of vacuolar membrane proteins in acetic acid-induced cell death
Joana Terra-Matos, Cátia S. Pereira, Susana R. Chaves, Maria João Sousa, Hernâni Gerós, Manuela Côrte-Real

Unveilling the *Candida cylindracea* genome to study the role of the translational machinery on gene evolution
Rita Coimbra, Miguel Pinheiro, Andreia Reis, Ana R Bezerra, Gabriela Moura, Manuel

The role of ER-mitochondria contact sites in acetic acid-induced cell death
Vítor Martins, Tânia Fernandes, Catarina Afonso, Diana Lopes, Rosário Domingues, Manuela Côrte-Real and Maria João Sousa

Sessão VI - Yeast and Human Health II

Moderadores: Fernando Rodrigues e Célia Pais

11:30-11:45 **The role of *Candida albicans* transcription factor RLM1 in response to carbon adaptation**
João Oliveira-Pacheco*, Rosana Alves*, Augusto Costa Barbosa, B. Cerqueira-Rodrigues, P. Pereira-Silva, Sandra Paiva, Sónia Silva, Mariana Henriques, Célia Pais and Paula Sampaio

11:45-12:00 **Genomic adaptative mechanisms mediating azole resistance and adaptation to the human host in *Candida glabrata*, with emphasis on the role of CgPDR1 regulon**
Sara B. Salazar, Tiago Pedreira, Hiroji Chibana, Maria M. Lopes, Ulrich Güldener and Nuno P. Mira

12:00-12:15 **Study of the interaction between vaginal lactobacilli, *Candida albicans* and *Candida glabrata*: from physiological aspects to transcriptomic analyses**
Nuno A. Pedro and Nuno P. Mira

12:15-12:45 **Microbial Resource Research Infrastructure (MIRRI) and the Portuguese microBiological Resource Center Network (Pt-mBRCN): the current situation and future perspectives**
Nelson Lima and José Paulo Sampaio

12:45-14:15 **Almoço**

Sessão VII - Study of Transporters in Yeast

Moderadores: Margarida Casal e Isabel Sá Correia

14:15-14:35 ***in memorium* of André Goffeau**

14:35-14:50 **Understanding the role of grapevine NIPs aquaporins in transport of water, glycerol and atypical substrates in aqy-null *Saccharomyces cerevisiae***
Farzana Sabir, Maria Loureiro-Dias, Graça Soveral and Catarina Prista

14:50-15:05 **Functional characterization of the Human Copper Transporters hCTR1 and hCTR2 in the yeast *Saccharomyces cerevisiae***
Cláudia Barata-Antunes, A. B. Figueiredo, G. Talaia, R. Alves, A. M. Fernandes, V. Martins, P. A. De Beule, H. Gerós and S. Paiva

15:05-15:20 **The relationship between structure and function in the Acetate transporter family**
João Azevedo-Silva*, Davide Castagnoli, David Ribas, Isabel Soares-Silva, Diogo Athayde, Margarida Archer and Margarida Casal

15:20-15:35 **Yeast as model organism to study plant solute transporters**
Richard Breia, Artur Conde, Henrique Noronha, Viviana Martins, Hernâni Gerós

15:35-16:25 **Conferência "Jovem Investigador"**

Tributo à Professora Isabel Spencer-Martins

Moderador: Manuela Côrte-Real

Deciphering genomic and pheno-metabolomic determinants of yeasts towards adaptation to different ecological niches and potential use in biotechnology

Ricardo Franco-Duarte

16:25-16:45 **Sessão de Encerramento**

Lanche | Partida

Resumos

Conferência

"Professor Nicolau van Uden"

Dos fungos filamentosos aos unicelulares e das vinhas aos hospitais: espectro de um fungicida de última geração

V. Loureiro*
virgiliolourenco@gmail.com

Os fungos assumem uma enorme importância social e económica, tanto positiva como negativa. Os filamentosos são geralmente conotados negativamente, nomeadamente na agricultura, onde causam perdas da ordem de 20% da produção total, acrescidas de mais 10% de perdas pós-colheita. As leveduras têm, em regra, uma imagem mais favorável junto do grande público, graças ao seu papel na produção das bebidas alcoólicas e do pão, não obstante o enorme impacte negativo que assumem na saúde humana e animal. Nesta comunicação começaremos por apresentar um fungicida de características únicas descoberto em Portugal e patenteado em quase todo o mundo, que se tem revelado um sucesso na luta contra muito dos fungos filamentosos da agricultura e já comercializado em vários países, nomeadamente Estados Unidos, China, Coreia do Sul, Austrália e Canadá.

Em seguida serão abordadas as fragilidades e preocupações actuais na luta contra os fungos patogénicos das plantas, que têm conduzido à emergência de estirpes de fungos e leveduras cada vez mais resistentes a fungicidas, com graves efeitos colaterais na difícil luta contra as micoses do Homem e animais.

Após breve descrição dos modos de acção do fungicida de última geração já conhecidos serão discutidas as promissoras perspectivas de terapia tópica contra as leveduras patogénicas para o Homem e animais e enaltecida a enorme vantagem deste fungicida ser um polipéptido, que permite a construção de proteínas quiméricas com efeito microbiostático e/ ou microbicida contra bactérias Gram (+) e Gram (-), fungos filamentosos e leveduras. Serão apresentados resultados de um "super-fungicida", que foi sintetizado *in vivo* para controlo de fungos filamentosos e bactérias patogénicos de plantas.

Finalmente, a exemplo do que a Merck Sharp & Dohme Limited fez para a caspofungina, convida-se a comunidade científica das leveduras a estudar este fungicida, tanto em termos de mecanismos de acção como de abordagens de luta contra as leveduras patogénicas para o Homem e animais, dado o enorme potencial científico e de aplicação que demonstra.

*Com a colaboração de Ricardo Ferreira, Sara Monteiro, Alexandra Carreira, André Barata e Margarida Pinheiro

Conferência “Jovem Investigador”

Tributo à Professora Isabel Spencer-Martins

Deciphering genomic and pheno-metabolomic determinants of yeasts towards adaptation to different ecological niches and potential use in biotechnology

Ricardo Franco-Duarte

Molecular and Environmental Biology Centre (CBMA)/Department of Biology, University of Minho, 4710-057 Braga, Portugal

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Yeast genomics and pheno-metabolomics, combined with integrative data mining methods, have contributed to understand the vast genetic diversity of *Saccharomyces cerevisiae* strains that are adapted to different ecological niches and are used for most distinct biotechnological applications, in particular winemaking. During must fermentation thousands of volatile aroma compounds are formed. Our group showed the application of innovative computational approaches to analyse the pheno-metabolomic diversity of a strain collection with different origins. The presented method revealed as a breakthrough, to combine genetic, phenotypic and metabolomic data, which had not been possible before due to difficulties in comparing different types of data. This computational approach was successful to shed light into the holistic characterization of *S. cerevisiae* pheno-metabolome, which allowed the identification of combined relevant features with application in selection of good winemaking strains.In parallel, yeast genomics was used to understand genome variations that yeasts undergo during adaptation to diverse environments. We showed the occurrence of microevolutionary changes within the descendants of a winemaking commercial yeast – Zymaflore VL1 – that were re-isolated from vineyards surrounding wineries where this strain was applied during several years, in comparison with the commercial reference strain. The occurrence of microevolutionary changes was shown by comparative genome hybridization arrays, and supported by DNA whole-genome sequencing that revealed variations, when compared with the reference strain. Phenotypic screening and metabolic profiles also distinguished the recovered isolates from the reference strain. We showed that the transition from nutrient-rich musts to nutritionally scarce natural environments induces adaptive responses and microevolutionary changes promoted by Ty elements and by nucleotide polymorphisms that were not detected in the reference strain.

Additionally, ongoing work is dealing with the detailed characterization of our yeast collections in order to select promising and robust yeast isolates to be applied in several biotechnological contexts. One example is the yeast production of succinic acid. Genome sequencing revealed some key factors associated with an increased production of succinic acid using natural yeast isolates, overcoming in this way the limitations of using bacteria, and giving clues for future strain improvement.

Sessões I e II

Exploring Yeast Diversity for Biotechnology

O1

A genomic and phenotypic characterization of a lineage of *Saccharomyces cerevisiae* associated with olive brine

Ana Pontes, Paula Gonçalves and José Paulo Sampaio

UCIBIO, Departamento de Ciências da Vida, FCT-UNL

Microbe domestication has led to profound phenotypic changes in the lineages that underwent artificial selection. These changes are essential for the production of fermented foodstuffs and beverages. Here, the yeast *Saccharomyces cerevisiae*, the workhorse for the wine, beer and other industries, is used to better understand the genetic underpinnings of domesticated phenotypes. In this species, several populations have been uncovered using population genomics, and it has been demonstrated that *S. cerevisiae* comprises both wild and domesticated populations [1]. Among the latter, the wine population is probably the best studied one and several genomic signatures of domestication have been uncovered, together with domestication-related phenotypic traits [1]. This study investigates the genetic characteristics of a group of strains recently detected by us that share a common ecological origin, olive brine and related substrates. Eighteen *S. cerevisiae* strains obtained from olive related environments were investigated. For these strains, DNA from monosporic derivatives was extracted and paired-end whole-genome data were obtained. Reads for each isolate were mapped to the *S. cerevisiae* reference genome. Single nucleotide polymorphisms were extracted from multiple sequence alignments and the evolutionary history was inferred from a rooted phylogenetic tree constructed by Neighbor-Joining. Evidence of introgressions from other *Saccharomyces* species was investigated by mapping the reads to a combined reference of six *Saccharomyces* species. Preliminary phylogenetic analysis revealed that the eighteen strains formed a monophyletic group distinct from the already known lineages of *S. cerevisiae*. Subsequent analyses indicated that the olive-related strains had a considerable number of introgressed genes from the sister species *S. paradoxus*, which could suggest a hybridization event between these two species. Growth/survival experiments in sterile olive brine revealed that representatives of the olive population outperformed those of the wine population, attaining higher cell numbers by consuming more glucose.

Funding from Fundação para a Ciência e a Tecnologia, grant UID/Multi/04378/2013 is acknowledged.

1.Marsit S et al. 2017. Nat Rev Gen 18:581-598.

O2

Development of potential yeast protein extracts for wine clarification and stabilization

Leonor Gaspar[1], Amadeu Machado[1], Rute Coutinho[1], Adriana Xavier[2], Manuel Figueiredo[2], Victor Freitas[3], Maria de Fátima Teixeira[2], Filipe Centeno[2] and João Simões[1]

[1]BIOCANT - Centro de Inovação em Biotecnologia, BIOCANT PARK Cantanhede, Portugal; [2]PROENOL – Indústria Biotecnológica, Lda, Portugal; [3]Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto

The wine industry is one of the most competitive sectors all over the world. Recently, new technologies have been combined to improve quality and sensorial diversity of the wine. Several stabilization agents were developed to induce flocculation and sedimentation of particulate matter in wine, making its clarification and stabilization possible. The most commonly used fining agents are animal proteins, such as milk casein. However, its use is being related to food intolerance and to neurodegenerative diseases. To overcome this, suitable microorganisms must be selected for use in industrial processes. In previous studies performed by our consortium, the potential of yeast protein extracts (YPE) in white wine clarification, stabilization and curative processes was identified. Therefore, the main objective of the present work is to select YPE with the potential to develop stabilization agents for red wines, without risk to the consumer. As a result, seven YPE were produced from a diversified collection of oenological yeasts. YPE selection was based on dry matter weight, protein content and protein molecular weight profile of each fining agent by SDS-PAGE electrophoresis. The effectiveness of these YPE was evaluated by different oenological parameters such as turbidity, chromatic characteristics, lees volume and conductivity. SDS-PAGE electrophoresis showed that 50% of the total yeast protein is above 15 kDa of molecular weight, which is in accordance with the OIV demand. The denatured protein was also determined by Differential Scanning Fluorimetry. Hence, we believe this work will be an important contribution for the development of a new biological product which will improve the final quality of wines. In conclusion, the selected YPE revealed promisor results as fining agents with significant brilliance increase, along with a turbidity reduction and final color improvement.

Acknowledgments

This work is supported by the project P2020 BioClarVinoII (Ref. POCI-01-0247-FEDER-017687)

O3

Genome sequence of the non-conventional wine yeast *Hanseniaspora guilliermondii* UTAD222 unveils relevant traits of this species and of the *Hanseniaspora* genus in the context of wine fermentation

Seixas [1,2], I, Barbosa[1,2], C., Mendes-Faia[1,2], A., Güldener[3] , U., Tenreiro [2] , R., Mendes-Ferreira*[1,2], A., and Mira* [1], N. P

[1]WM&B—Laboratory of Wine Microbiology & Biotechnology, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal. [2]BioISI-Biosystems and Integrative Sciences Institute, Campo Grande, Lisbon, Portugal; [3]Department of Genome-oriented Bioinformatics, Wissenschaftszentrum Weihenstephan, Technische Universität München, Maximus von-Imhof-Forum 3, 85354 Freising, Germany; [4]IBB, Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.

Species of the *Hanseniaspora* genus are largely abundant among wine must microbiota and are among those found to be more interesting for the production of wines with stylistic properties. Despite their wide utilization, the genetics and physiology of *Hanseniaspora* species is still poorly understood. In this work, we have disclosed and annotated, for the first time, the genome of a *H. guilliermondii* strain (UTAD222) [1]. Herein, we present the results from the metabolic reconstruction as well as a comparative proteome analysis involving *H. guilliermondii* and other wine strains. This analysis revealed that *H. guilliermondii* is not equipped with a functional neoglucogenesis, nor it encodes enzymes required for catabolism of glycerol, galactose or acetate, or for biosynthesis of polyamines, biotin or thiamine. Moreover, we were able to identify 14 *H. guilliermondii*-specific genes, as well as a total of 870 proteins only found in the *Hanseniaspora* species. The release of *H. guilliermondii* genomic sequence and the comparative genomics and proteomics analysis herein performed, will accelerate research focused on these species, contributing for their more rational utilization by the wine industry or by other bio-industries where they could be explored as cell factories.

References

[1] Seixas I et al. Genome Announc 5:e01515-16

Acknowledgements

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O4

Exploring organic acid producer microorganisms – Identification, morpho- and physiological characterization of wild yeast strains

Maria Sousa-Silva, David Ribas, Ricardo Franco-Duarte, Célia Pais, Margarida Casal and Isabel Soares-Silva

Molecular and Environmental Biology Centre (CBMA)/Department of Biology, University of Minho, 4710-057 Braga, Portugal

A broad diversity of yeast species can be found in nature, including in wastes from food industries. The exploration of this biodiversity has captured great interest from food, pharmaceutical and even fuel companies due to the interesting features of these microorganisms [1]. Yeasts can convert sugars present in raw materials into different chemical building-blocks, as well as biofuels, a process more sustainable than those based on fossil fuels and refineries [2]. Among yeasts, *Saccharomyces cerevisiae* is considered the model organism. Characteristics that range from its simple cultivation, short replication period, sporulation efficiency, easy genetic manipulation and rare pathogenicity have turned it in an ideal organism for various biotechnological processes [3]. In this work, a group of isolates from the TransBio collection (Project FP7 KBBE–Nº289603) were selected, based on their ability to grow in organic acids. The microorganisms, were identified by molecular typing (DNA sequencing of the ITS regions) and characterized morpho- and physiologically. Morphological traits and sporulation patterns were evaluated for cell cycle determination. Phenotypically, evaluated yeasts revealed interesting physiological features regarding growth profiles using carboxylic acids as sole carbon and energy source. The full characterization of these yeast strains is currently ongoing.

References

1. Barnett, J. A., & Barnett, L. (2011). Yeast research: a historical overview. American Society for Microbiology Press.

2. Hong, K. K., & Nielsen, J. (2012). Metabolic engineering of *Saccharomyces cerevisiae*: a key cell factory platform for future biorefineries. Cellular and Molecular Life Sciences, 69(16), 2671-2690.

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Acknowledgments

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O5

Yeasts as biocontrol agents of fungal phytopathogenic diseases

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Phytopathogenic fungi without viable treatment options affect economically valuable plant crops with high socio-economic impact. One case is *Moniliophtora perniciosa*, causing the Witches' Broom Disease of cacao plant and fruit, a major economic sustainability hurdle for tropical countries. Another case are *Colletotrichum gloeosporioides* and *C. acutatum* species complex, causing the anthracnose of olive trees and fruits in the Mediterranean countries, which became a threat to the North of Portugal regional economy. New efficient and eco-friendly strategies of disease control are required, such as using biological control agents. Yeasts, which secrete peptides that are lethal to specific strains of other yeasts, bacteria or fungi, can provide this kind of solution. The present work explores yeasts as biocidal agents for the control of the cacao plant and olive tree fungal diseases. Biotechnology yeast strains originating from Brazilian bioethanol and cachaça fermentation industries and well-known killer yeasts were used to test biocidal activity against several strains of both phytopathogens, originating from infected fields on several producing countries. Antagonism was evaluated at different pH values, in different media, in solid and liquid cultures, and in several yeast and fungus life cycle phases. *M. perniciosa* and *C. gloeosporioides* growth were strongly inhibited by different industrial yeast strains, according to a specific antagonistic response. Strategies to enhance the antagonism simultaneously simplifying the biocidal/fungus contact process are under development. Eventually, economically useable biocontrol protocols for in field application on contaminated plants and/or prevention of either disease will be generated.

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O6

Port and Douro Wines Institute, IP (IVDP) Yeast Culture Collection

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The IVDP Yeast Culture Collection is housed in IVDP Porto, at the Laboratory of Microbiology. It currently holds approximately 600 yeast strains, mostly indigenous yeast from the Douro Demarcated Region (DDR), the cultural landscape of the Alto Douro Wine Region (UNESCO World Heritage Site). This Yeast Collection started in 1949 with a few isolates. The harvest of new isolates occurred again in 1960. From 1991 to 1997, a high number of wild wine yeasts were isolated from grape musts and wines collected, in accordance with a diversified sampling programme which took into account: the three Sub Regions of the DDR – "Baixo Corgo", "Cima Corgo" e "Douro Superior" (with different microclimates); way of vinification [lagar (stone treading tank)/ stainless steel)]; fermentation phase (harvested at the beginning of fermentation, just prior to the addition of grape spirit or fortified wine); grape variety (blend of varieties / monovarietal), among other characteristics. Since then, the IVDP Yeast Culture Collection has been supplemented by sampling and isolation associated with different research activities. At present, all yeasts strains are preserved in pure culture, are stored as live frozen cultures and are maintained by cryopreservation at -80 ºC, facilitating their characterization, classification and maintenance. A part of this collection (103 isolates) has been characterized regarding its fermentative behaviour in grape must, and the best performing yeasts were then further characterized, both phenotypically and genetically. All isolates studied displayed excellent fermentative capacity under stress conditions and an exceptional ethanol resistance. With the IVDP yeast database, the specific characteristics of a given strain in the Collection (for example, its origin and characterization) will allow quick access to all this data for later consultation as well as a perpetuation of the transmission of this information, with less risk of loss. The database of the IVDP collection already gives us information on taxonomy, physiology and ecology for a high number of yeasts strains. The Port and Douro Wines Institute Yeast Culture Collection is an important asset of strains, unique in the world.

Sessão III

Yeast Physiology and Genetics

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O7

Impact of horizontal gene transfer on sugar metabolism in a fructophilic yeast lineage

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Fructophily is a rare trait that consists on the preference for fructose over other carbon sources, including glucose. It has been so far identified in two ascomycetous yeast lineages (*Zygosaccharomyces* and the *Wickerhamiella*/*Starmerella* clades) and in a particular group of bacteria. All these organisms are associated with fructose-rich environments, which may account for an efficient fructose assimilation as an adaptive advantage. Despite the ecological overlap between fructophilic yeast and bacteria, the molecular bases of fructophily appear to be distinct. While for *Zygosaccharomyces* it was shown that fructophily strongly relies on the presence of a high capacity fructose facilitator (Ffz1), the loss of alcoholic fermentation is a hallmark in fructophilic bacteria. In this work we showed that *FFZ1* was introduced in *Saccharomycotina* through an horizontal gene transfer (HGT) event and that it is also crucial for fructophily in the *Wickerhamiella*/*Starmerella* (W/S) clade. The acquisition of *FFZ1* was concurrent with other genomic remodelling events that occurred in the most recent common ancestor (MRCA) of the W/S clade, the most striking being the acquisition of many genes of bacterial origin. One bacterial gene (Adh1) was probably involved in the reacquisition of alcoholic fermentation, as the entire alcoholic fermentation pathway (PDC1 and ADH1) appeared to have been lost in an ancestor lineage. The reinstatement of alcoholic fermentation also included the modification of a pre-existing enzyme, Aro10, which is now fulfilling the role of Pdc1. The elimination of the *ARO10* gene in *Starmerella bombicola* impacted glucose metabolism more than fructose metabolism, suggesting that the loss of alcoholic fermentation might have set the stage for the evolution of fructophily in this lineage, similarly to what happened in fructophilic bacteria. Other genes related with sugar metabolism were also acquired in this lineage by HGT, namely a gene encoding an invertase which was shown to be essential for sucrose assimilation in *St. bombicola*, strongly suggesting that HGT was probably pivotal in the adaptation of these yeasts to sugar rich environments.

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O8

Dephosphorylation of the ATP synthase subunit Atp2 by the Ser/Thr protein phosphatase Sit4p contributes to the regulation of mitochondrial function

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Sit4p is a yeast type 2A-related protein phosphatase involved in the glucose repression of mitochondrial genes, among other cellular processes. In this study, we show that Sit4p is also involved in post-translational regulation of mitochondrial proteins, including the ATP synthase (FoF1 complex) β subunit Atp2p that was hyperphosphorylated in SIT4 deleted cells. Moreover, Sit4p and Atp2p interacted both physically and genetically. Two Atp2p phosphorylation sites, T124 and T317, were identified by MS/MS. Notably, the expression of Atp2-T124D or -T317D phosphomimetics increased Atp2p levels, ATP synthase activity and ATP levels, promoting mitochondrial respiration and extending yeast lifespan. Our results reveal a role for Sit4p on the post-translational regulation of Atp2p which may play a role in the metabolic adaptation to distinct energetic demands.

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O9

Copper detoxification in yeast – a new link with iron homeostasis and Fe-S biogenesis

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In the yeast *Saccharomyces cerevisiae* Aft1, the low iron-sensing transcription factor is known to regulate the expression of the *FET3* gene. However, we found that a strain-lacking *FET3* is more sensitive to copper excess than a strain-lacking *AFT1*, and accordingly, *FET3* expression is not fully compromised in the latter. These findings suggest that, under such conditions, another regulator comes into play and controls *FET3* expression. In this work, we identify Ace1, the regulator of copper detoxification genes, as a regulator of *FET3*. We suggest that the activation of *FET3* by Ace1 prevents the hyper activation of Aft1, possibly by assuring the adequate functioning of mitochondrial iron–sulfur cluster biogenesis. In fact, we also show that in the ace1 mutant the activity of aconitase is compromised, which is in agreement with the fact that Fe-S clusters are targets of copper toxicity. While reinforcing the link between iron and copper homeostasis, this work unveils a novel protection mechanism against copper toxicity mediated by Ace1, which relies in the activation of *FET3* and results in the restriction of Aft1 activity as a means to prevent excessive copper accumulation.

O10

Pkh1-dependent activation of Ypk1 is triggered by membrane stress in a yeast model of Niemann-Pick type C1 disease

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The lysosomal Niemann-Pick type C1 (NPC1) disease is caused by recessive inherited mutations in NPC1, a lysosomal transmembrane protein that mediates the cholesterol export out of lysosomes. A progressive entrapment of cholesterol and sphingolipids inside the endosomal/lysosomal system compromises organelle function leading to neurodegeneration and cell death. NPC1 is highly conserved and deletion of the orthologue Ncr1 in yeast has been used to decipher molecular mechanisms that are compromised in this disease. We have previously shown that sphingosine and ceramide levels are increased in yeast cells modelling NPC1 (*ncr1*Δ). The activation of downstream signalling pathways like Pkh1/Sch9 and Sit4/CAPP, compromises mitochondrial function and integrity and shortens the lifespan of *ncr1*Δ cells. The levels of ceramide synthase are increased suggesting that sphingolipid synthesis is upregulated. In yeast, the sphingolipid biosynthesis is regulated by Ypk1, a downstream target of Pkh1 and the TORC2 complex that are present in distinct membrane compartments - eisosomes. In this study, we showed that Ypk1 is hyperactivated by TORC2 and Pkh1 in *ncr1*Δ. In accordance, the deletion of *YPK1* restored mitochondrial respiratory function and lifespan in these cells. Disruption of eisosome organization by deletion of a structural subunit (Pil1) decreased Pkh1-dependent phosphorylation of Ypk1 and suppressed cell death phenotypes of *ncr1*Δ. Also, we showed an abnormal distribution of phosphatidylinositol 4,5-bisphosphate (PIP2) in *ncr1*Δ, which may contribute to changes in the organization of eisosomes. The results suggest that Ypk1 is activated by TORC2 and Pkh1 due to eisosome organization instability possibly associated with changes in sphingolipids and PIP2 distribution in the plasma membrane of *ncr1*Δ cells.

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Sessão IV

Yeast Cell Death and Aging

O11

Pkh1p-Ypk1p and Pkh1p-Sch9p pathway activation underlies mitochondrial-dependent regulated cell death induced by acetic acid

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The yeast *Saccharomyces cerevisiae* undergoes a mitochondrial-dependent regulated cell death (RCD) exhibiting typical markers of mammalian apoptosis. Changes in sphingolipid metabolism have been linked to modulation of cell fate in both yeast and mammalian cells. We have previously shown that ceramide production contributes to RCD induced by acetic acid and is involved in mitochondrial outer membrane permeabilization and cytochrome *c* release, especially through hydrolysis of complex sphingolipids catalyzed by inositol phosphosphingolipid phospholipase C, Isc1p. The role of several signaling pathways in acetic acid-induced RCD was assessed. Our results show that Pkh1p, Ypk1p and Sch9p regulate acetic acid-induced RCD, since single mutants are resistant to acetic acid. We also found that acetic acid exposure leads a Pkh1p dependent-phosphorylation of both Sch9p and Ypk1p and that Isc1p is regulated by Sch9p under acetic acid stress. Both single and double mutants lacking Isc1p or/and Sch9p have the same resistant phenotype, and *SCH9* deletion impairs the translocation of Isc1p to mitochondria upon acetic acid exposure. We also found that the higher resistance of all mutants correlates with higher levels of endogenous mitochondrial phosphorylated long chain bases (LCBPs), suggesting that changing the sphingolipid balance in favour of LCBPs in mitochondria results in increased survival to acetic acid. In addition, our results suggest that Pkh1p-Ypk1p is necessary for *isc1*Δ resistance to acetic acid-induced RCD. Indeed, double deletion of *ISC1* and *PKH1* has a drastic effect on cell survival, which is associated with hyperactivation of the cAMP/PKA pathway and consequently increased ROS accumulation and release of cytochrome *c*. Overall, our results suggest that Pkh1p-Ypk1p and Pkh1p-Sch9p pathways contribute to RCD induced by acetic acid.

O12

Characterization of the yeast cell death induced by a benzo[a]phenoxazine derivative

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Phenoxazine derivatives have assumed an increasing importance in life sciences since they present antiproliferative proprieties that potentiated their study as possible antitumor and antimicrobial agents. We have recently found that one new benzo[a]phenoxazine derivative (MSG-111-cd3), induces cell death in *Saccharomyces cerevisiae*. In the present study, we used *Saccharomyces cerevisiae* as a model of a eukaryotic cell to determine the molecular pathways involved in the cell death process induced by MSG-111-cd3. To achieve this goal, cell viability and cytometric assays were performed in wild-type and mutant yeast strains when exposed to the compound. Several mutants deficient in proteins previously reported to be involved in cell death were tested, and necrotic, apoptotic and autophagic markers were assessed. We found that MSG-111-cd3 accumulates at the vacuolar membrane, endoplasmic reticulum and lipid droplets, and that it is inducing a regulated cell death process mediated by vacuolar membrane permeabilization. Also, our results showed that the cell death process is dependent on the vacuolar protease Pep4p and that the vacuole permeabilization resulted in its translocation from the vacuole to the cytosol. We observed that autophagy is not involved in the cell death process, and although MSG-111-cd3 leads to mitochondrial network fragmentation, apparently, the cell death process is independent of the mitochondrial pathway, since no other alterations were significantly induced in this organelle. Furthermore, we tested the compound cell toxicity in two colorectal cancer (CRC) cell lines and in normal colon cells. Our results showed that, as was observed in yeast, MSG-111-cd3 decreased cell viability in the cell lines, however, it was considerably more efficiently in CRC cells than in the normal colon cells, suggesting it as a promising candidate for CRC therapy.

Acknowledgments

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O13

Deciphering the mechanisms underlying bovine milk lactoferrin anticancer activity using yeast and cancer cell lines as complementary models

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Lactoferrin (Lf) is a milk derived iron-binding protein that exhibits a broad range of interesting biological activities, from which its anticancer and antifungal activities stand out. Our group has been elucidating the mechanisms and identifying the molecular targets underlying Lf anticancer/antifungal activities in order to improve its therapeutic efficacy and rational application. Indeed, we previously demonstrated that Lf triggers a mitochondrial and caspase-dependent regulated cell death in *Saccharomyces cerevisiae* (1). Moreover, we found that Lf selectively induces apoptosis in highly metastatic cell lines displaying the proton pump V-ATPase at the plasma membrane (2). However, much work is needed to further characterize Lf mechanisms of action. In the present work, we show how functional genomic approaches using yeast deletion mutants provided new insights on the activity of Lf against yeast that were then validated in human cancer cell lines. Results will be discussed in an integrated manner regarding their contribution towards understanding the molecular basis of Lf anticancer activity. In addition, this study highlights the great potential of yeast as a model to uncover mechanisms of action occurring in the more complex human cells.

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O14

Caloric restriction rescues aged yeast cells from α-synuclein toxicity through regulation of proteolytic systems

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Aging is a complex and multi-factorial process that results in the progressive accumulation of cellular damage and disruption of cellular homeostasis. Several hallmarks of aging that represent age-common denominators in different model organisms have been proposed, including deregulated nutrient-sensing and loss of proteostasis. The ubiquitin-proteasome system (UPS) and autophagy are crucial proteolytic systems responsible for the maintenance of proteostasis, however these processes are, themselves, affected by aging. Caloric restriction (CR) is still one of the most effective non-genetic intervention that promotes lifespan extension, across several aging model organisms. In the present work, we used the yeast *Saccharomyces cerevisiae* to understand the contribution of the proteolytic systems for the beneficial effects promoted by CR intervention, during aging. An aging scenario of proteotoxic stress is also promoted by the heterologous expression of human α-synuclein (aSyn), a protein associated with Parkinson's disease. The data gathered showed that CR increases chronological lifespan (CLS) of aSyn-expressing cells accompanied by enhanced UPS activity and maintenance of autophagy at homeostatic levels. Overall, data suggests that CR balances the UPS and autophagy activities during aging. Nevertheless, maintenance of autophagy at homeostatic levels appears to be central on cells´ rescue from aSyn-mediated toxicity and extension of longevity promoted by CR.

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O15

Impact of nutrients in longevity regulation of the yeast *Saccharomyces cerevisiae*

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The harsh environment that yeast encounter during wine fermentation makes yeast survival crucial for a good fermentation performance. Several compounds present in must, including the fermentation products ethanol and acetic acid are known to negatively influence yeast survival and performance. Ammonium toxicity and its effects on the chronological life span (CLS) of *Saccharomyces cerevisiae* have been previously described with ammonium being capable of affecting both auxotrophic and prototrophic strains CLS and emerging as a novel key modulator of yeast longevity. More recently, we demonstrated that CLS extension is dependent on a proper cell cycle arrest obtained due to either glucose or ammonium depletion. For higher concentrations of glucose, other nutrients present in the culture media influence the yeast CLS maximum extension by allowing the total consumption of glucose and/or ammonium before aging. In media supplemented with 10% glucose and 0.1% ammonium sulphate, increasing Yeast Nitrogen Base (YNB) from 1x to 2.5x significantly increased CLS extension in these conditions, demonstrating that the right nutritional equilibrium between carbon, nitrogen and micronutrients sources, regulates yeast longevity. In the present work, we used the *S. cerevisiae* haploid strain EC1118-59A, derived from the commercial wine strain EC1118, to further investigate the nature of the micronutrients capable of influencing yeast longevity in high glucose media conditions.

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Sessões V e VI

Yeast and Human Health

O16

***Candida albicans* overcolonization of the elderly gut: Involvement of adenosine A2A receptors**

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With the development and aging of our modern societies, immunosenescence and inflammaging of the elderly is becoming a public health issue. There is a strong association between this and increased morbidity and mortality rates due to infections and chronic inflammatory gut diseases. Though *Candida* spp. belong to human microbiota, they can turn into aggressive agents of opportunistic infections [1,2]. Purines operate an important role in immunity and inflammation homeostasis; adenosine A2A receptor (A2AR) contributes to fine-tuning inflammatory and immune responses, prompting an efficient elimination of threats while minimizing tissue damage [3]. This work aimed to explore and compare, in aged, adult and young mice, the relative intestinal overcolonization by *Candida albicans*. We studied the distribution of A2AR in the gut and the correlation between the density of A2AR and tissue damage. We showed that elderly mice are more prone to over-colonization by *C. albicans*, than adults and young. This seems to be related with higher growth rate in the intestinal lumen, independent of invasion of gut tissues, but resulting in higher inflammation of the elderly gut. In these mice there is a higher stomach colonization and increased yeast-to-hypha transition. When compared with young and adults, aged mice have a lower A2AR density in the gut and *C. albicans* failed to increase the density of this receptor [4]. In conclusion, these in vivo results indicate that aged mice have a lower ability to cope with inflammation due to *C. albicans* overcolonization, which is likely related to the inability to adaptively adjust the density of A2AR. This paves the way to exploit the impact of the purinergic system, in particular the A2AR, in the control of the gut over colonization/infectious process of *C. albicans* in the elderly, together with relevant purinergic *C. albicans* metabolic issues.

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O17

New multiplex PCR based methodology for the diagnosis of *Candida* and *Aspergillus* species

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Fungal pathogens are the major agents of invasive infections, in which *Candida* and *Aspergillus* species are the most frequent. The rapid and correct identification of the fungal species is essential since the susceptibility to antifungal drugs is different [1,2]. In clinical microbiology laboratories several methodologies are available, however all techniques present low specificity and sensibility. In this work, we developed a new methodology based in a multiplex PCR analysis for the specific identification of nine of the most clinically important *Candida* and *Aspergillus* species. This methodology is based on the design of a panel for species identification, combining fluorescence with molecular weight of specific PCR fragments. Our methodology was optimized using DNA extracted from strains previously identified, serum from healthy donors spiked with DNA and cDNA extracted from blood of patients. The optimization of the method by using DNA from known strains showed 100% of specificity. The calculated yield from DNA extracted from serum spiked with fungal DNA was of around 80%. This DNA was used to determine the sensitivity of the technique and results showed that we were able to detect samples with 20pg of fungal DNA. Results obtained with clinical samples showed that we were able to detect specific amplification in 75% of samples. This is a promising, fast, accurate and reproducible methodology and due to the design of the identification panel it is able to identify species involved in mixed infections.

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O18

Beyond MDR transporter-mediated azole resistance in *Candida glabrata*: functional characterization of the transcription factors CgRpn4 and CgMrr1

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Candida glabrata presents high levels of intrinsic and acquired resistance to antifungals, which limits their clinical effectiveness. The study of regulatory networks that coordinate resistance phenotypes is of the utmost importance.

In this study, the transcription factors (TFs) Rpn4 and Mrr1 were identified as fluconazole resistance regulators in *C. glabrata*. Transcriptomics analysis based on RNA-seq was carried out in order to determine their role in fluconazole resistance. Rpn4 regulates the expression of 212 genes (80 activated) upon fluconazole stress. The Rpn4-activated regulon shows a significant enrichment for ergosterol biosynthesis genes, which is consistent with fluconazole mode of action. Moreover, Rpn4 was found to affect the intracellular accumulation of fluconazole in *C. glabrata* cells, which is consistent with a role in plasma membrane structure via ergosterol biosynthesis regulation. On the other hand, Mrr1 regulates the expression of 337 genes (134 activated) in response to fluconazole. The Mrr1-activated regulon comprises fatty acid, phospholipid metabolism and sterol transfer genes; suggesting a possible role in plasma membrane organization. Accordingly, Mrr1 was found to decrease the intracellular accumulation of fluconazole. Consistent with the mode of action of fluconazole, both Rpn4 and Mrr1 are here proposed to be regulators of plasma membrane organization and structure. This is mediated through transcriptional control of plasma membrane lipid metabolism, which in turn is likely to affect membrane permeability and decreased drug accumulation. Moreover, both TFs were found to be integrated a network with Pdr1, the master regulator of azole resistance.

O19

Alternative carbon sources modulate *Candida glabrata* biofilm development on medical devices

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Candida glabrata is considered a major human fungal pathogen. The capacity to cause disease lies in the ability to grow within the human host environment and to adapt its metabolism to the carbon sources available. Previous transcriptional studies on this fungus demonstrated that the presence of alternative carbon sources, such as acetate and lactate, influence its behaviour, suggesting that the expression of carboxylic acid transporters has a crucial role in biofilm formation and resistance to antifungal drugs (Mota et al, 2015; Alves et al, 2017). Moreover, *C. glabrata* is able to grow outside the human body and colonize abiotic surfaces, being one of the most prevalent causes of fungal infections in medical devices. Medical device-associated biofilms are clinically important due to their intrinsic and prevalent resistance to conventional antifungal drugs and immune system, being associated with morbidity, mortality and cost. Here we investigated the effect of alternative carbon sources in biofilm formation on central venous catheters and the impact of carboxylic acid transporters in this process. Our results demonstrate that the amount of carbon source, as well as the extracellular pH, influence biofilm formation. The absence of glucose in the medium also impacts biofilms formation in some conditions. These results support the view that the adaptation of *Candida* cells to the alternative carbon sources present in the host environment influences their pathogenicity.

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O20

The role of *Candida albicans* transcription factor RLM1 in response to carbon adaptation

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Candida albicans is the main causative agent of candidiasis and one of the most frequent causes of nosocomial infections worldwide. In order to establish an infection, this pathogen supports effective stress responses to counter host defenses and adapts to changes in the availability of important nutrients, such as alternative carbon sources. These stress responses have clear implications on the composition and structure of *Candida* cell wall. Therefore, we studied the impact of lactate, a physiologically relevant carbon source, on the activity of *C. albicans* RLM1 transcriptional factor. RLM1 is involved in the cell wall integrity pathway and plays an important role in regulating the flow of carbohydrates into cell wall biosynthesis pathways. The role of *C. albicans* RLM1 in response to lactate adaptation was assessed in respect to several virulence factors, such as the ability to grow under cell wall damaging agents, filament, adhere or form biofilm, as well as to immune recognition. The data showed that growth of *C. albicans* cells in the presence of lactate induces the secretion of tartaric acid, which has the potential to modulate the TCA cycle on both the yeast and host cells. In addition, we found that adaptation of *C. albicans* cells to lactate reduces their internalization by immune cells and consequent % of killing, which could be correlated with a lower exposure of the cell wall ß-glucans. In addition, absence of RLM1 has a minor impact on internalization, compared with the wild-type and complemented strains, but it reduces the higher efficiency of lactate grown cells at damaging phagocytic cells and induces a high amount of IL-10, rendering these cells more tolerable to the immune system. The data suggests that RLM1 mediates cell wall remodeling during carbon adaptation, impacting their interaction with immune cells.

O21

Genomic adaptative mechanisms mediating azole resistance and adaptation to the human host in C*andida glabrata*, with emphasis on the role of CgPDR1 regulon

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The emergence of azole resistance among *Candida glabrata* strains is frequent and contributes to increase the incidence of infections caused by this species. In this work we aimed at elucidating the molecular mechanisms underlying resistance to fluconazole and voriconazole in a resistant clinical isolate (FFUL887). Whole-genome sequencing of FFUL887 and subsequent comparison with the genome of the susceptible reference strain CBS138 revealed the existence of prominent differences in several genes documented to promote azole resistance in *C. glabrata*. Among these was the transcriptional regulator CgPdr1 with a K274Q modification not documented in other azole-resistant strains. The significant increase in susceptibility to azoles of the FFUL887 strain upon deletion of the CgPDR1K274Q allele, along with results from transcriptomic profiling rendering evident the upregulation of 80 documented targets of CgPdr1 in the FFUL887 strain, support the idea that K274Q is a novel CgPdr1 gain-of-function mutation. Analysis of the non-coding genome of the FFUL887 and of CBS138 support the idea that in the FFUL887 strain alterations of the CgPdr1-controlled regulatory network may have changed its architecture to improve the expression of azole-resistance genes. Comparison of the genome of the FFUL887 and CBS138 also showed prominent differences in the sequence of adhesin-encoding genes, while comparison of the transcriptome of the two strains showed a significant remodelling of the expression of genes involved in metabolism of carbohydrates, nitrogen and sulphur in the FFUL887 strain; these responses probably reflecting adaptive responses evolved by the clinical strain during colonization of the host.

O22

Study of the interaction between vaginal lactobacilli, *Candida albicans* and *Candida glabrata*: from physiological aspects to transcriptomic analyses

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To successfully colonize the vaginal niche *C. glabrata* and *C. albicans* have to face multiple environmental insults, which include, among others, the presence of a commensal bacterial microflora. Metagenomic analyses revealed that lactobacilli are predominant in the vaginal microflora and a reduction in the abundance of these bacteria is associated with a higher risk of developing infections. In this work was examined the effect of *L. gasseri* and *L. jensenii*, two of the most abundant species in the vaginal tract, on the physiology and virulence of *C. albicans* and *C. glabrata* strains. The results obtained showed that exposure of *Candida* cells to the two bacteria in a co-culture setting reduces growth rate and cell viability of the yeast cells also attenuating relevant virulence traits. A similar effect was observed when the two yeasts were cultivated in the presence of a supernatant of the two lactobacilli. Notably, vaginal *C. glabrata* isolates were much more resilient to the presence of the two lactobacilli used indicating that prior adaptation to the vaginal environment influences the yeast-bacteria interaction. To gain further insights into the *C. glabrata-L. gasseri* interaction transcriptomic analysis (RNA-seq) was performed in a co-culture setting. Around 638 *C. glabrata* and 204 *L. gasseri* genes were found to be differently expressed in the co-culture setting. Interestingly, the *C. glabrata* genes found to be more expressed in the presence of *L. gasseri* encode proteins of poorly characterized function. The relevance of the differently expressed genes in the *C. glabrata-L. gasseri* interaction will be further discussed with particular emphasis on the role played by the recently described *C. glabrata* transcription factor CgHaa1. On the overall, the results presented in this study open the door to a better understanding of the interference between lactobacilli and *Candida*.

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Microbial Resource Research Infrastructure (MIRRI) and the Portuguese microBiological Resource Center Network (Pt-mBRCN): the current situation and future perspectives

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Since the OECD initiative, started with the workshop held in Tokyo 1999, for the development of microbial culture collections (CC) into the more advanced public service microbial domain Biological Resource Centres (mBRCs) that CC are facing 1) a multitude of regulations and international protocols, 2) aspects of biosafety and biosecurity, 3) recognition the differences in CC development, 4) the fragmentation of data with difficulties of biological material access, 5) a tiny percentage of all microorganisms studied and published are well preserved, and 6) poor long-term security and funding. This scenario contrast with the intensifying demands of the scientific user communities for better microbial resources to underpinning the research and development in the field of life sciences and biotechnology. By provision of high quality microorganisms, associated data and the broad expertise of the CC partners, MIRRI (www.mirri.org) aims to support research and development changing the current situation and accelerating the innovation in the field of life sciences and biotechnology.

After the preparatory phase (2012-2016), funded by the European Union's Seventh Framework Programme under grant agreement nº. 312251, MIRRI is now entering in a construction phase. The 1st step submission for the MIRRI-European Research Infrastructure Consortium (ERIC) application is scheduled for September 2018, in view of having MIRRI legally established by the end of 2019. To accomplish this schedule, MIRRI will be established as a not-for-profit legal entity following a distributed model with a Central Coordination Unit (CCU) accommodating the operational headquarters and the national nodes bringing together the partners and stakeholders in each European member country. The MIRRI CCU will consist of two distributed sections: 1) the Statutory Seat (SS) located in Portugal (University of Minho, Braga) and 2) the Collaborative Working Environment (CWE) hub operated from Spain (University of Valencia, Paterna) and supported by LifeWatch-Spain, a closely related e-infraestructure.

At national level, the Portuguese microBiological Resource Centre Network (Pt-mBRCN, www.mbrcn.pt) is foresee as a key initiative to strength the public service Portuguese CC activities and, in near future, a potential embryo for the Portuguese MIRRI Node.

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Sessão VII

Study of Transporters in Yeast

in memorium of André Goffeau

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O23

Understanding the role of grapevine NIPs aquaporins in transport of water, glycerol and atypical substrates in aqy-null *Saccharomyces cerevisiae*

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The nodulin-26-like intrinsic proteins (NIPs) belong to the subfamily of aquaporins, which are crucial for water and ion homeostasis in plants. NIPs present diverse substrate selectivity, considered being dependent on the amino acid residues present at ar/R filter. In addition to water they have also been proposed as transporters of other small molecules, like glycerol, H_2O_2, arsenic and boric acid. However, their function in plants is poorly understood. Thus, unveiling the role of NIPs may provide a key step towards understanding the uptake and translocation of these substrates in plants. Four NIPs (NIP1;1/NIP2;1/NIP5;1/NIP6;1) from *Vitis vinifera* (cv. Touriga Nacional) were cloned and expressed in the membrane of *S. cerevisiae* aqy-null strain. Water and glycerol transport in strains expressing Vitis NIPs was assayed through stopped-flow-spectroscopy. To identify the key residues for substrate specificity of NIPs, site directed mutagenesis were performed in their ar/R filters. Expression of NIP1;1 and NIP6;1 increased the permeabilities and reduced the activation energies for both water and glycerol transport in yeast cells. Additionally, their permeabilities were found to be mercury sensitive. Strains expressing mutants of NIP1;1 (W86T and V206I) and NIP6;1 (T118W and I239S) showed significantly lower permeabilities for glycerol, while their water permeabilities were slightly reduced. Drop tests were performed to examine the role of *Vitis* NIPs for atypical substrates (H_2O_2, arsenic and boric acid). The phenotypic growth variations of yeast cells showed that expression of *Vitis* NIPs increased susceptibility to the externally applied substrates, suggesting their possible transport through *Vitis* NIPs.

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O24

Functional characterization of the Human Copper Transporters hCTR1 and hCTR2 in the yeast *Saccharomyces cerevisiae*

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Copper (Cu) is an essential trace element for both eukaryotic and prokaryotic organisms. It plays a crucial role as co-factor of metalloenzymes that participate in important cellular processes, such as growth, development and physiology. Although its importance in maintaining cell health, high level of this ion is extremely toxic (Wang et al., 2011). Therefore, cells possess tight regulated systems to conserve copper homeostasis. One of these mechanisms includes the endocytosis of the Copper Transporter 1 (Ctr1) at high Cu levels, a process already verified in yeast and human cells (Liu et al., 2007; Maryon et al., 2013). Besides these new advances, the molecular mechanisms that are behind the intracellular trafficking of the hCtr1 protein are still poor understood. So, to get new insights into this mechanism, an heterologous expression system was created using the yeast *Saccharomyces cerevisiae* as host (Pereira et al., 2016)). Human *CTR1* and *CTR2* genes tagged with GFP were cloned into pYPKpw plasmid and transformed into a *S. cerevisiae* strain disrupted for copper transporters. Importantly, phenotypic assays demonstrated that human Ctr1 complemented the yeast ctr-mutant strain for the ability to grow in a medium containing non-fermentable carbon sources. Moreover, hCtr1 and hCtr2 were localized at the plasma membrane and intracellularly. Data will be presented regarding the expression of hCTRs in different conditions.

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O25

The relationship between structure and function in the Acetate transporter family

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The AceTr transporter family is a group of Acetate Transporters with six predicted transmembrane segments. It has homologues in fungi, bacteria, archaea and protozoa. As acetate transporters, these proteins play a crucial role on metabolism being involved in the capacity of cells to adapt accordingly to the availability of nutrients. These membrane transporters may also transport other substrates, i.e. succinate, formate, and propionate [1]. To better understand the mechanism of acetate transport, we have investigated functional and structural features of this family. We have performed site-directed mutagenesis in highly conserved residues, or residues possibly involved in substrate binding of the *Saccharomyces cerevisiae* acetate permease Ady2. We have characterized acetate transport, growth on different carboxylic acids as sole carbon and energy source, and protein localization of these mutants. Several bacterial members of the AceTr family were cloned and its expression were evaluated in different *Escherichia coli* expression strains. The uncharacterized homologue from *Methanosarcina acetivorans* (MA4008) presented satisfactory levels of protein production and was then purified and used for crystallization trials. Also, functional studies have revealed that MA4008 is able to uptake acetate with a similar affinity when compared to the already characterized SatP from *E. coli* [2]. Furthermore, molecular docking studies for Ady2 and MA4008 using as template the structure of the *Citrobacter koseri* SatP [3] enabled the identification of the residues responsible for the interaction with its substrate revealing the relationship between structure and function.

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O26

Yeast as model organism to study plant solute transporters

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Saccharomyces cerevisiae is the most studied eukaryotic model organism in molecular and cell biology. Basic cellular mechanisms of DNA replication and recombination, cell division and death, stress response, autophagy and membrane transport are well conserved between yeast and higher eukaryotes, including plants. Plants are full of solutes, both inorganic ions and low-molecular-mass organic molecules and expend high amounts of energy in acquiring, synthesizing and transporting them. Our group studies membrane proteins involved in cell/plant response to environment, including sugar (MSTs, SUCs, SWEETs, polyol transporters) and mineral transporters (COPT/Ctr copper uptake transporters, Na(+) and Ca(+)/H(+) antiporters), water channels (aquaporins) and H(+) pumps. The heterologous expression of such plant solute transporter genes in the yeast model has provided useful information regarding the function and intracellular localization of the encoded proteins. For instance, a his-tagged grapevine aquaporin was heterologously expressed, solubilized and purified to homogeneity from yeast ER membranes and its reconstitution in liposomes confirmed its water channel activity by stopped-flow spectroscopy. The unlocking of the mechanisms of such solute transport systems, and their regulation in response to environmental cues, has an important basic and applied relevance, and yeast has been an essential tool in this quest.

Acknowledgments

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Apresentações *FLASH*

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F1

Understanding the molecular mechanisms of homothallism in the order Cystofilobasidiales

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In the phylum Basidiomycota, heterothallic fungi can only undergo sexual reproduction by mating with a strain of opposite mating type. Mating type is defined by the MAT loci, that contain genes coding for pheromones (MFA) and pheromone receptors (STE3), and for the homeodomain transcription factors HD1 and HD2. Given a compatibility between MFA and STE3 and, later, between HD1 and HD2 from two strains, the completion of the life cycle can be achieved for these species.[1] Homothallic fungi, however, can reproduce sexually and complete their life cycle without mating with a second strain. As a rare sexual behaviour, homothallism is still under study for many species and can occur through different molecular mechanisms.[1] The Cystofilobasidiales order constitutes a lineage with a high variety of sexual behaviours, in some cases at the species level, comprising a higher proportion of homothallic species than what is observed in other lineages in Basidiomycota [2] From this order, the species *Phaffia rhodozyma* [3] and *Cystofilobasidium capitatum* are being studied with the main objective of understanding the molecular mechanisms that are responsible for their homothallic behaviour, focussing on the interactions between HD1 and HD2. To assess these interactions, the Yeast Two-Hybrid System will be used, constructing fusion proteins between the homeodomain transcription factors and two domains of Gal4 activator for expression in *Saccharomyces cerevisiae*. For these constructions, the synthesis of HD1 and HD2 cDNA is being tested. If these can't be obtained, synthetic genes will be designed to be used in these constructions and proceed with the study of the interaction between HD1 and HD2 in these species.

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F2

High-throughput screening of oleaginous yeasts by using innovative fluorimetric and spectroscopic approaches

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Oleaginous microorganisms are capable of accumulating large quantities of lipids in the form of triacylglycerols, under certain growth conditions. In particular, yeasts represent the most competent microorganism for the production of lipid droplets, and their potential has been widely studied at several levels, with emphasis on the food, energetic, pharmaceuticals and bio-cosmetic sectors. The use of volatile fatty acids (VFAs) generated from anaerobic fermentation of several organic wastes, has been exploited as potential carbon source for lipid production, being acetic acid an ideal substrate for lipid synthesis by yeasts. In the present work, a screening of about 400 yeast isolates was accomplished, by using two approaches, different from conventional ones: 1) yeast cultivation in solid mineral medium, using acetic acid 15 g/L as carbon source; 2) lipid quantification inside cells by fluorescence method, with the lipophilic dye Nile red. We observed a correlation between the relative fluorescence units (RFU) of samples, detected by fluorimetry, and the lipid content of yeast cells, confirmed by the gravimetric method. Results allowed the identification of several yeast species as oleaginous, not yet reported as such in the literature. Another major result was the association of the yeast oleaginous character to the strain level, contrarily to the species-level linkage, as usually referred. Four best producer yeasts were further characterised by spectroscopy, in particular ATR-FTIR, in order to correlate spectral differences with results obtained previously by gravimetry and fluorimetry. Our work showed that the implementation of fluorescence methodology presents numerous advantages for screening high number of oleaginous yeasts since it is rapid, inexpensive, reliable and accurate.

Work supported by the European project VOLATILE-Biowaste derived volatile fatty acid platform for biopolymers, bioactive compounds and chemical building blocks (H2020-NMBP-BIO-2016 Grant agreement No. 720777), and by UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P., by the ERDF through the COMPETE2020.

F3

Volatile fatty acids as carbon source for single cell oil production by oleaginous yeasts

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The possibility of waste utilization, both from industry and other human activities, has been increasing importance over the last decades. In this context, volatile fatty acids (VFA), resulting from the anaerobic digestion of organic wastes, can be used as carbon sources by oleaginous yeasts for the production of single cell oil (SCO), thus contributing to the process of circular economy. Following a screening of 400 yeast isolates, using mineral medium with acetic acid 15 g/L, four strains were selected based on their ability to accumulate lipids and their biomass yields. Our objective was to investigate the profile of lipid production and carbon source consumption along growth, as well as detail their fatty acid composition. At pH 6.9, acetic acid was completely exhausted from the medium after 90h of growth and high amounts of lipids were detected after 120h, the highest lipid accumulation occurring in the stationary phase and after acetic acid exhaustion. At pH 5.5, only two strains were able to grow after a prolonged lag phase and, in both cases, acetic acid was not completely exhausted from the medium. Generally, the yeasts achieved high percentages of lipid accumulation, with one of the strains attaining 64% lipids by dry cell weight and a lipid yield of 0.4 (g/g C). In relation to the fatty acid composition of lipids, it was also affected by the medium pH, with emphasis in C18:1n9c and C16 production. Globally our results showed that these strains are potential candidates for application for SCO production at a larger scale.

Work supported by the European project VOLATILE (Call H2020-NMBP-BIO-2016 Grant agreement No.720777) and by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P., by the ERDF through the COMPETE2020.

F4

***Candida albicans* Cht3 as a novel recombinant subunit antigen against fungal infections: characterisation and liposomal encapsulation**

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Fungal infections, such as those caused by *Candida albicans*, are a major current health problem requiring the development of novel preventative therapies and treatments, of which vaccines will have an important role. Our previous studies investigating *C. albicans* cell wall surface proteins (CWSP) as antigens with DODAB:MO liposomes as carriers and adjuvants led to a protection of 67% of the mice in an intravenous lethal infection. This protection was shown to be mainly due to the production of antibodies specific to the C*. albicans* cell wall chitinase Cht3. In the present study we developed approaches for the production and purification of Cht3 so as to enable its physicochemical characterisation and the optimisation of its encapsulation in DODAB:MO liposomes. The *CHT3* gene was amplified and cloned in the methanol inducible expression vector pPIC9K for extracellular production in *Pichia pastoris* GS115, and a simple two step non-chromatographic approach developed for its efficient purification. The kinetic parameters for enzyme activity (K_M of 14.5 mg.mL^{-1} and V_{max} of 2.2 µmol.min^{-1}.mg^{-1} with colloidal chitin) and optimal conditions for activity (optimal temperature of 40-50 °C and optimal pH of 3.5-5.5) and stability were assessed with the Bernfeld reducing sugar assay. The internalisation studies of Cht3p in DODAB:MO liposomes indicated that lower protein concentrations (2.5 and 5 µg/mL) led to higher nanoparticle homogeneity as compared to higher protein concentrations (10 to 50 µg/mL). Two different encapsulation methods were investigated, incubation and hydration, with the latter presenting higher liposomal homogeneity, a mean size of approximately 200nm, positively charged surfaces (+40mV), and colloidal stability (at least 2 weeks stability).

Acknowledgments

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F5

Microbiome shift in the context of recurrent vulvovaginal candidosis (RVVC): a new biomarker for recurrence?

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RVVC, caused by *Candida* spp., is a condition that affects women worldwide; it is generally defined as the occurrence of four episodes per year. In this study we aim to shed *light* on the molecular epidemiology of RVVC in the context of the vaginal microbiome. Twenty symptomatic women attending a private clinic (2008-2014) were enrolled in the study; the samples used for diagnosis were afterwards included in this study. *Candida* spp. was isolated from a vaginal swab collected by standard techniques. Species identification was performed by Vitek; and molecular typing was performed by pulsed-field gel electrophoresis. In addition, for two RVVC patients, two sets of vaginal washes were performed for each patient. Species identification of vaginal *Lactobacillus* spp. was performed by multiplex-PCR of the swabs and the vaginal washes. Only 12 women had at least four episodes per year (Group A). Of these, the great majority of cases were associated with *C. albicans* (8/12), but *C. glabrata* (3/12) and *S. cerevisiae* (1/12) were also found. Genotypes of consecutive isolates were highly related. The remaining 8 women had a single episode per year (Group B); interestingly, *C. albicans* was recovered initially from this women; but recurrence was in almost all cases (7/8) caused by another species (*C. glabrata* and *S. cerevisiae*). In addition, different *Lactobacillus* spp. were identified in the samples of the patients. In group A we were able to identify *L. iners* and in group B we identified *L. iners*, *L. gasseri* and *L. rhamnosus*. Our results indicate that recurrence might be caused either by a new infection or re-infection of isolates in the vaginal mucosa. *Lactobacillus* spp usually associated with an healthy vaginal environment were found in cases with less frequent recurrence of yeast infection; nonetheless, the finding that *L. iners* was present in the same context as *Candida* spp in both cases might be considered as a biomarker for the predisposition of recurrence.

F6

Quantitative assessment of DNA damage in the industrial ethanol production strain *Saccharomyces cerevisiae* PE-2

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Lignocellulosic hydrolysates remain one of the most abundant substrates for the sustainable production of second generation fuels and chemicals with *Saccharomyces cerevisiae*. Fermentation inhibitors such as acetic acid, furfural and hydroxymethylfurfural are formed in varying amounts depending of the hydrolysis conditions which cause slow or stuck fermentations, a topic that has garnered much research. Some fermentation inhibitors such as furfural are also genotoxic agents that can cause genetic instability of the production strain. We present a novel dominant DEL cassette (dDEL) by which DNA damage can be quantified in wild-type or industrial yeast strains. The ethanol production strain *S. cerevisiae* PE-2 was more resistant to 4 mM hydrogen peroxide (H_2O_2) for 5-20 min or up to 20 mM furfural for 17 h compared to the laboratory DEL strain RS112. The PE-2 showed a low tendency for recombination consistent with efficient DNA protection compared to the laboratory strain. Measuring genetic stability quantitatively with the dDEL assay could aid in the selection of robust yeast strains or processes strategies using second generation raw materials.

Posters

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P1

Molecular mechanisms of chronological lifespan modulation

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Aging, the cumulative incorporation of imbalances at the genomic and proteomic levels over the course of time, is a multidimensional biological process that results in decreased cellular fitness. A growing body of evidence suggests that, in *Saccharomyces cerevisiae*, chronological lifespan can be regulated by the intracellular and extracellular concentration of some low molecular weight transmissible factors, such as glycerol, trehalose, amino acids or fatty acids. It is postulated that spatial and temporal shifts on the concentration of these molecules might regulate the cellular pathways that are widely recognized as aging modulators. However, the relevance and the role of these molecules on yeast longevity is still poorly explored. Herein, our goal was to explore if molecules released from yeast aged cells were able to modulate the longevity of younger yeast cells, and more specifically to determine the classes/nature of the transmissible factors involved in this process. Preliminary results suggest that cells have distinct secretory profiles throughout chronological lifespan, releasing different amounts of lipids, proteins and nucleic acids into the extracellular milieu. Moreover, it seems that such molecules can modulate the longevity of younger yeast cells. Altogether, this data supports the hypothesis that some molecules can act as low molecular weight transmissible factors and bring about global changes to cell functionality and as a consequence, modulate aging progression.

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P2

The versatile role of mannitol in central metabolism of the fructophilic yeast *Starmerella bombicola*

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Mannitol is one of the most abundant polyols in nature and can be found in bacteria, plants, lichens but specially in fungi (Solomon, et al 2007; Wyatt et al 2014). Fructophilic behavior is a relatively rare trait that consists in the preference for fructose over other hexoses as energy and carbon source. Although this characteristic is uncommon, this seems to be widespread in yeasts belonging to the *Wickerhamiella/Starmerella* clade to which the yeast *Starmerella bombicola* belongs (Gonçalves et al., 2018). In *Starmerella bombicola*, we found that mannitol is produced from fructose through the enzyme mannitol dehydrogenase (Mdh) which uses NADPH as a cofactor. In order to understand the role of mannitol in this yeast, the two genes that encode Mdh enzymes were disrupted using genetic approaches. When grown at high temperatures the wild-type strain exhibits higher intracellular mannitol concentrations. Moreover, in *MDH* deletion mutants biomass yield is apparently lower at higher temperatures, suggesting that mannitol has a role as a thermal protector in *Starmerella bombicola*. At the highest temperature tested (32.5°C) the mutants were unable to grow in low sugar concentrations. However, in some cases the inclusion of other polyols in the growth medium rescued growth of the *mdh* deletion mutants suggesting that other polyols can also act as thermal protectors. However, we also show that in spite of increased intracellular mannitol concentrations, most mannitol seems to be excreted by wild type cells, which suggests that conversion of fructose to mannitol may also play a role in redox homeostasis.

Acknowledgments

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P3

Semen supports growth of *Candida albicans*: a possible role of this pathogen in infertility?

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The impact of the presence of bacteria in semen has recently been linked to infertility. Specifically, an increase in hyperviscosity and/or a decrease in the number of viable sperm cells have been associated with the presence of bacteria in this fluid. *Candida albican*s is a skin fungal commensal that can colonize the male genital area; to assess the possible role of *C. albicans* in male infertility, in this study, we aim to evaluate the effect of human semen in the promotion of the growth of this opportunistic pathogen. The disposable amount of forty-one samples of semen obtained from infertility patients attending the Unit of Reproductive Medicine of Centro Hospitalar Cova da Beira, Covilhã, Portugal, were included in this study, with informed consent. The spermogram and physical characteristics of the samples were performed at the Unit; this information was provided with anonymity of the samples. Samples were inoculated with a calibrated suspension of *C. albicans* ATCC 10231 in culture media. After the incubation time*, C. albicans* CFU/ml were determined. We found that semen samples supplemented with culture medium were able to support *C. albicans* growth; using statistical analysis we observed the distribution of two different subgroups ($p<0.05$), that differed in their ability to support *C. albicans* growth: Group A: < 5 CFU/ml; Group B: > 5 CFU/ml. However, we found no relation between these two subgroups and the physical characteristics of the samples namely: discrepancies in pH, processing time after ejaculation, sexual abstinence time and presence of colonizing *Candida* spp in the semen sample. Noteworthy, the increase in viscosity of the semen (n=5) impaired significantly *C. albicans* growth; but the two subgroups were still observed in the samples with normal viscosity (n=36), indicating that a yet unknown factor, might explain the differences observed. Further studies are needed to elucidate the role of *C. albicans* in male infertility.

P4

Exposure of recurrent vulvovaginal *Candida* spp isolates to azole compounds prevents biofilm formation?

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The capacity to form biofilms is considered one of the most important virulence factors associated to persistence of *Candida* spp, in the vaginal mucosa of women, with consequent recurrence of vulvovaginal candidosis (RVVC) in many cases. We intended to verify the importance of biofilm formation for recurrence of *Candida* spp. in the vaginal area of 13 women that had at least 4 episodes of vulvovaginal candidosis per year. The susceptibility to fluconazole and clotrimazole was assessed for 57 sequential isolates (identified by Vitek) using the liquid microdilution test. We assessed the capacity of strains to form biofilms by the microtitulation plate assay, in which the biofilm biomass was quantified. The strains were genotyped by RAPD (random amplification of polymorphic DNA). We found that recurrence was caused mainly by *C. albicans* (10 women) and *C. glabrata* (3 women); and the genotype of sequential strains was highly related, indicating that recurrence is caused by the same strain. The minimum inhibitory concentration (MIC) to azole compounds increased along time in almost all cases for *C. albicans* (9/10 women) and *C. glabrata* (1/3 women). Interestingly, in half of these cases, sequential strains had also an increased capacity to form biofilm, which is an indication that the resistance to treatment could be due to the expression of this particular virulence factor. On the other hand, when the MIC to azole compounds increased along time, the capacity to form biofilm was impaired in as many cases. Therefore, in these cases treatment with azole compounds probably contributes to the reduction of the biofilm formation capacity of the strains. Our results indicate that, to persist in the vaginal mucosa, *Candida* spp. modulates its ability to form biofilm to adapt to external pressures like antifungal therapy.

P5

Functional characterization of *Cyberlindnera jadinii* carboxylate transporters in *Saccharomyces cerevisiae*

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The wide applicability of organic acids for direct use as commodity chemicals and as polymer building blocks has evidenced their importance into diverse types of industries. As organic acids, carboxylic acids (CAs) are underlined as top value products by the US Department of Energy (DOE), which has included nine organic acids among the future top 12 value-added platform chemicals.
In the yeast *Saccharomyces cerevisiae*, two permeases are responsible for the uptake of carboxylates at the plasma membrane. With affinity for lactate, pyruvate acetate and propionate, the *JEN1* gene encodes a monocarboxylate proton symporter that belongs to the Major Facilitator Superfamily (TC 2.A.1.12.2). The *ADY2* gene encodes an acetate permease, a member of the Acetate Uptake Transporter (AceTr) Family (TC 2.A.96.1.4).
In the yeast *Cyberlindnera jadinii*, a close relative of *Candida utilis*, different uptake systems for CAs were functionally characterized however until now the genes encoding these transporters remain unidentified. In this work, carboxylic acid transporter homolog genes from *C. jadinii* were identified and expressed in *Saccharomyces cerevisiae*. The *C. jadinii ScJEN1* and *ScADY2* homologs were identified through sequence alignment, secondary structure prediction and phylogenetic analysis of putative transporters.
In *C. jadinii*, 6 genes homolog to *ScJEN1* (Cjj23088, Cjj21966, Cjj22358, Cjj21989, Cjj21602, Cjj25129) and 4 genes homolog to *ScADY2* (Cja24587, Cja20823, Cja20690, Cja20822) were identified. These genes were expressed under the control of a GPD constitutive promoter, in a S. cerevisiae *jen1*Δ*ady2*Δ strain, that presents no activity for plasma membrane carboxylate permeases. GFP-fusions versions were performed revealing the localization of *C. jadinii ScJEN1* and *ScADY2* homolog transporters mainly at the plasma membrane. The complete characterization of these new plasma membrane transporters in *C. jadinii* is ongoing.

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P6

Towards a genetic toolkit for *Sporothrix brasiliensis* analysis

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Fungi of the *Sporothrix schenckii* complex are the causative agents of sporotrichosis, a chronic subcutaneous infection that affects humans and other mammals. Particularly, infections caused by *S. brasiliensis* are a major health problem, mostly due to the highly aggressive phenotype of the disease, difficulties in its treatment and its zoonotic transmission. Virulent factors of *S. brasiliensis* still uncharacterized and molecular manipulation techniques for this fungus are scarce. Our aim is to contribute for the development of molecular tools that allow genetic manipulation of *S. brasiliensis*. Although *S. schenckii* ploidy has been set as diploid [1], our results from flow cytometry analysis and next generation sequencing data have suggested a haploid profile for fungi of the complex, including *S. schenckii* and *S. brasiliensis*. Additionally, we have establish a transformation method for *S. brasiliensis* using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) system. Overall, our results point to advances for the molecular manipulation of *S. brasiliensis*.

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P7

The Ady2 "NPAPLGL(M/S)" motif is critical for acetate uptake and binding

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Organic acids, such as acetate, are recognized as one of the most prevalent compounds in ecosystems and the transport, biosynthesis, and assimilation of these molecules represent an adaptive advantage for organisms. The majority of AceTr family members so far characterized were associated with the active transport of acetate. AceTr proteins have six predicted transmembrane segments (TMS), sharing the conserved motif NPAPLGL(M/S) located at the beginning of the first TMS. Gpr1 from *Yarrowia lipolytica* was the first AceTr family member identified in yeasts, being involved in acetic acid sensitivity, cell and colony morphology, yeast-to-hyphae transition and cell lifespan. The *Saccharomyces cerevisiae* has 3 homologous genes, *ADY2*, *FUN34* and *ATO3* was latter characterized as an acetate proton symporter. In this work, we identified the residues of the conserved motif NPAPLGL(M/S) as essential for substrate uptake and binding, but not for membrane targeting. Furthermore, we demonstrated that the Gpr1 from *Y. lipolytica* is an acetate permease and the *S. cerevisiae* Fun34 does not transport acetate. Phylogenetic analysis of AceTr family shows that it is dispersed in the tree of life. In eukaryotes, however, it is almost limited to microbes, though reaching a prevalence close to 100% in fungi, where it may play an essential role in fungal survival.

P8

Assessing yeast diversity in Portuguese honey samples

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Yeasts can colonize different types of foods and beverages and when their activities and numbers become delectable they can be detrimental to food quality, being thus regarded as food spoilage microorganisms. Some yeasts are osmophilic, i.e., have evolved adaptations to thrive environments with high osmotic pressures, namely high sugar concentrations [1]. Osmophilic yeasts can be found in the genus *Zygosaccharomyces*. The species classified in this genus can be physiologically divided into to three subgroups: extreme resistance to acid-weak food preservative; preference for slow growth at cooler temperatures; and extreme osmotolerance [2]. In this project we explore the yeast diversity found in Portuguese honey samples, an environment with a high sugar concentration (95-99% of the honey solids), with fructose (32-40%) and glucose (27-35%) being the most abundant sugars [3], and low water activity (a_w≈0.6) [4]. Using an isolation protocol that employed a medium with 30% w/v glucose [5], 76 yeast strains were obtained from 20 honey samples. Yeast strains were identified down to the species level using molecular methods, namely sequence analyses of the D1/D2 domains of the rDNA region. The vast majority of isolates were assigned to the genus *Zygosaccharomyces* and belonged to the species *Z. favi*, *Z. gambellarensis*, *Z. mellis* and *Z. siamensis*. Two strains were found to represent an unknown species in this genus and their detailed characterization is currently under way.

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P9

Fostering the utilization of Non-*Saccharomyces* yeasts as co-adjuvants of wine and beer fermentations through the exploration of comparative genomics data

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Fermentation of wine or beer is a process traditionally conducted by *Saccharomyces cerevisiae*, however, the essential contribution of Non-*Saccharomyces* yeasts (NSYs) in determining the properties of the end-product obtained is being increasingly acknowledged. The benefits of utilizing NSYs include the improvement in the aroma profile of the product obtained, reduction in alcoholic level, among others. In our laboratories the influence of *Candida glabrata* UTAD68 and *Saccharomycodes ludwigii* UTAD17, two isolates recovered from wine must, in fermentations undertaken with *S. cerevisiae* is being examined. Since little is known on the biology and physiology of NSYs, the genomic sequence of this isolates was disclosed and fully annotated. Besides contributing to advance the utilization of these isolates as co-adjuvants, the results obtained from these comparative genomic analyses will also provide relevant insights into the adaptation and ecology of this species since *C. glabrata* is mostly known due to its clinical relevance while *S. ludwigii* is considered as a wine spoilage agent. Overall, the sequencing and analysis of these two NSYs genomes will enable a better understanding of their oenological relevance, particularly in their interference in wine aroma or spoilage.

P10

Saccharomyces cerevisiae as a model system to uncover target and off-target effects of fungicides of environmental concern

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Agriculture is a human activity with high impact in the environment. Among other substances, pesticide and fertilizer use is widespread and frequent in order to warrant yield and prevent crop diseases. Many of these substances reach the aquatic environment, where they are frequently detected. The use of fungicides is of particular concern, both in terms of the number of available active ingredients used as well as the magnitude (in weight or area) and frequency of application. Recent evidence has shown that some fungicides can affect non-target fungi, including decomposers and microparasites/pathogens, thus affecting important ecological processes. This has been overlooked so far, and there is evidence that the current risk assessment framework for fungicides may not be sufficiently protective of these key drivers in ecosystem functioning. This worked aims to assess molecular targets of pesticides commonly used in viticulture in the Portuguese North Region. For this purpose, we used the *Saccharomyces cerevisiae* model system, and assessed growth inhibition and cell viability in response to tebuconazole, metalaxyl, cymoxanil, myclobutanil and azoxystrobin. The azole fungicides tebuconazole and myclobutanil were cytostatic, while cymoxanil was cytotoxic. Therefore, conditions for a genome-wide screen to assess cymoxanil mechanisms of action, which are poorly understood, were optimized.

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P11

Exploring yeast as a tool to study the human pro-apoptotic protein Bax

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Apoptosis is a type of regulated cell death, which plays a fundamental role eliminating harmful cells and contributing to organ development and tissues homeostasis [1]. However, despite its beneficial biological role, unbalanced apoptosis can result in several disorders [2]. In mammalian apoptosis, the permeabilization of the outer mitochondrial membrane and the subsequent release of apoptotic factors, including cytochrome *c*, have been described as crucial events to engage cells into this cell death process. Members of the Bcl-2 proteins family, such as Bax, have been shown to be very important in these processes. Bax is a pro-apoptotic protein that is activated and translocated to the mitochondria upon a cell death stimulus, where it plays its role in favour of cell death [3]. Yeast has been used throughout the years as a valuable tool to study apoptosis and associated dysfunctions because, besides its well-known advantages, it also lacks obvious homologous of the Bcl-2 family proteins [4]. This feature along with the possibility to heterologously express these proteins, which retain their molecular and biochemical functions, allows the understanding of the mechanisms underlying the activation and regulation of human Bax without the interference of other mammalian players. In this work, we used the yeast *Saccharomyces cerevisiae* and optimised the conditions to express and characterize human Bax (either wild type or an activated form) to then perform further studies concerning its activation, function and regulation.

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P12

Evaluation of decolourisation of textile dyes by selected yeasts

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Environmental pollution is one of the most important problems of actuality. The textile sector is a large and worldwide business that produces high volumes of effluents that causes environmental problems concerning contamination of water in rivers. The discharge of effluents contain a wide range of dyes that are persistent in water and are responsible for toxicity and mutagenic effects to the aquatic life [1, 2]. Biological methods are generally considered more environmentally friendly than classic methods (that are little effective, expensive and can generate toxic by-products), since they can lead to mineralization of organic pollutants. Differences between the types of produced molecules and their levels of metabolization may occur. [3]. In the present work, yeast isolates and respective consortia were tested for their ability to decolourise reactive Everzol dyes used in textile industries. Loss of colour was quantified by UV-visible absorbance measurements. Selected yeasts and consortia were able to quickly and effectively decolourise the dyes tested, although it was possible to detect differences in the mechanisms that contributed to the loss of colour.

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P13

Polyphasic identification of a yeast isolate with dye decolourisation ability

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The textile industry generates large volumes of effluents with toxic, coloured, surfactant and chlorinated compounds and salts, thus transforming wastewater into one of the main sources of water pollution (Mansour et al, 2012; Chequer et al, 2013). The wastewaters are characterized by fluctuations in parameters such as the BOD, COD, pH, colour and salinity (Chequer et al, 2013). Dyes, visible in concentrations lower than 1 ppm, can cause toxic and mutagenic effects to the aquatic habitat (Wang et al, 2011), thus it is relevant the optimization of the wastewater treatment processes to reduce the environmental impact. LIVST11 is a yeast isolate from wastewater with the ability to decolourise several dyes. Physiological, biochemical and molecular biology assays were performed currently placing it in the genus *Candida*. Molecular biology assays are still underway to identify the strain to the species level. The intracellular and extracellular extracts both presented dye decolourisation abilities, however the extracellular extract showed a better decolourisation. The enzymatic characterization tests of the fractions obtained by FPLC is currently underway.

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P14

Role of vacuolar membrane proteins in acetic acid-induced cell death

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The yeast *Saccharomyces cerevisiae* can undergo a regulated cell death process in response to acetic acid treatment that, among other events, involves the translocation of the vacuolar protease Pep4p from the vacuole to the cytosol leading to mitochondrial degradation. Cathepsin D, the human ortholog of the yeast Pep4p, is equally released from the lysosomes to the cytosol upon acetate treatment in colorectal cancer cells. However, the mechanisms underlying the selective permeabilization of vacuolar/lysosomal membrane (VMP or LMP) are still not completely elucidated. To fill this gap of knowledge, we aimed to identify vacuolar membrane proteins involved in the permeabilization of the yeast vacuole membrane and subsequent release of Pep4p. For this purpose, a set of complementary approaches, including classical biochemistry, molecular biology and analytical methods, such as fluorescence microscopy and flow cytometry, were performed. A phenotypic characterization of yeast deficient mutants allowed the identification of three vacuolar membrane proteins with a potential role in VMP/translocation of Pep4p. Moreover, the hints provided by this study can also contribute to unveil the mechanisms underlying mammalian lysosomal membrane permeabilization with potential application in the therapy of colorectal cancer, and of diseases associated with lysosomal-dependent cell death.

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P15

Unveilling the *Candida cylindracea* genome to study the role of the translational machinery on gene evolution

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The genetic code is degenerate allowing for introduction of species-specific codon utilization biases. How those biases arise and are maintained in genes has been intensively studied, but it is not yet clear whether changes in codon usage and context arise from G+C pressure only or whether the translational machinery also shapes it. To address this question we took advantage of a unique genetic code alteration that occurred in the fungal *Saccharomycotina subphylum*, the so called CTG clade. These species have the unique characteristic of translating leucine CUG codons as serine, using a novel transfer RNA (tRNACAGSer). The atypical serine CUG codons are rarely used among the CTG clade species and *Candida cylindracea* is the exception to the rule as it uses CUGs at high level, it is the most frequently used of the 6 serine codons. To gain further insight on how the usage of this codon diverged so dramatically between *C. cylindracea* and the other CTG clade species, we conducted whole genome sequencing, assembling and annotation using MAKER1 of *C. cylindracea* genome, together with RNA sequencing for transcriptomics analysis (in a collaboration with the Génolevures Program).A detailed study of open reading frames using the software package ANACONDA2 from the annotation of *C. cylindracea* genome has showed distinct codon context and amino acid preference patterns from other species of the *Saccharomycotina subphylum*, which probably arose after the CUG reassignment. According to the phylogeny reconstruction using PhyML 3.03 *C. cylindracea* has a basal position in the CTG clade.

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P16

The role of ER-mitochondria contact sites in acetic acid-induced cell death

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Zones of close membrane apposition function as signaling hubs for interorganelle communication, sustaining organelle homeostasis and exchange of cellular cues. Particularly, endoplasmic reticulum-mitochondria contact sites (ER-MCS) have been the subject of recent scientific interest since the discovery that these structures are involved in several pathologies such as cancer and neurodegenerative diseases [1,2]. Interestingly, these diseases are also characterized by apoptotic dysregulation [3]. Until recently, the only known tethering complex between ER and mitochondria of *Saccharomyces cerevisiae* was the ER-mitochondria encounter structure (ERMES). We have previously shown that this organism commits to an apoptotic-like mitochondria-dependent cell death process in response to acetic acid [4,5]. Thus, in the present work we aimed to assess the role of the ERMES complex in the mediation of acetic acid-induced apoptosis. Preliminary analysis revealed a remarkable role of ERMES in the acid stress response [6] and prompted us to further pursue a phenotypical characterization of the cellular alterations underlying this process. We were able to unveil ERMES as a regulator of cell death. In all, this work will aid in the understanding of molecular pathways not yet fully disclosed in apoptosis, namely mitochondrial outer membrane permeabilization.

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