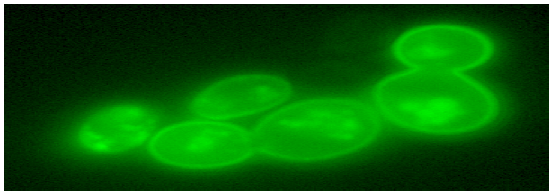

XX JORNADAS DE BIOLOGIA DE LEVEDURAS

"PROFESSOR NICOLAU VAN UDEN"

Comissão Organizadora

Isabel Sá-Correia, IBB, IST/UL
Miguel Cacho Teixeira, IBB, IST/UL
Nuno Pereira Mira, IBB, IST/UL
Margarida Palma, IBB, IST/UL
Pedro Tiago Monteiro, INESC-ID, IST/UL
Rogério Tenreiro, BioFIG, FC/UL

MENSAGEM DE BOAS-VINDAS

É com grato prazer que damos as boas vindas aos participantes nas XX Jornadas de Biologia de Leveduras "Professor Nicolau van Uden", a decorrer nos dias 11 e 12 de Julho de 2014 no campus da Alameda do Instituto Superior Técnico (IST), Universidade de Lisboa (UL). A Comissão Organizadora (CO) agradece a adesão de um número alargado de participantes, representativo da comunidade científica e tecnológica que se dedica às Leveduras em Portugal, bem como de alguns representantes da mesma comunidade oriundos de Espanha e Brasil, totalizando 101 inscritos. Como é habitual o programa iniciar-se-á com a Conferência van Uden e terminará com a Conferência Jovem Investigador correspondente ao Prémio Isabel Spencer-Martins atribuído pela Sociedade Portuguesa de Microbiologia (SPM). Conta ainda com 40 comunicações orais, organizadas em 5 sessões temáticas, apoiadas cientificamente por 8 dos grupos de trabalho da SPM. Pela primeira vez, estas Jornadas incluem ainda duas sessões de *posters*, que reúnem 73 apresentações. A melhor apresentação em *poster*, receberá um prémio, patrocinado pela STAB-VIDA. Na sua XX edição, o programa destas Jornadas abarca um público vasto, desde os que usam as Leveduras nas suas perspectivas mais aplicadas, até aos que estudam os seus processos fundamentais, desde os Microbiólogos mais tradicionais aos Micriobiologos de Sistemas e Bioinformáticos, desde investigadores reconhecidos nacional e internacionalmente aos estudantes de mestrado e doutoramento, e outros profissionais com actividade relacionada com as leveduras.

Fazemos votos de que estas XX Jornadas possam ser cientificamente produtivas, um ponto de encontro de velhos e novos amigos, e uma base para a promoção de colaborações e sinergias científicas que contribuam para a constante melhoria da qualidade do trabalho de I&D desenvolvido por uma comunidade inspirada pelo exemplo do Professor Nicolau Van Uden.

A Comissão Organizadora

PROGRAMA RESUMIDO:

Sexta-feira

11:00 Sessão de Abertura

11:30 Conferência "Professor Nicolau Van Uden"

12:30 Almoço

14:00 Session I – Yeast Physiology and Genetics

16:00 Poster Sessions I and II

17:00 Session II – Yeasts and Bioenergy, Food and Environmental Biotechnology

20:00 Jantar das Leveduras

Sábado

9:00 Session III – Yeast Diversity and Evolution

11:00 Session IV – Yeasts and Human Health

12:30 Almoço

14:00 Session V – Yeast Functional Genomics and Bioinformatics

15:45 Poster Sessions III, IV and V

17:00 Conferência "Jovem Investigador" – Tributo à Professora Isabel Spencer Martins

17:30 Sessão de Encerramento

*para informações detalhadas, por favor consultar a página do encontro em http://groups.ist.utl.pt/bsrg/XXJornadasVanUden/index.html

PROGRAMA CIENTÍFICO:

11:00 Sessão de Abertura

Presidente do IST, Presidente do IBB, Isabel Sá-Correia (Comissão Organizadora e Presidente SPM)*, Álvaro Fonseca* (Comissão Organizadora das XIX Jornadas Van Uden)

Conferência "Professor Nicolau Van Uden"

Chair: Pedro Moradas Ferreira

11:30 **Cross-talk between sphingolipid and nutrient signaling pathways: new clues to old yeast**, Vítor Costa, IBMC and ICBAS, Universidade do Porto

Session I – Yeast Physiology and Genetics

(supported by SPM working groups: Fisiologia Microbiana and Microbiologia Celular e Molecular)

Chairs: Helena Santos, Fernando Rodrigues, Maria João Sousa

14:00 **Functional characterization of putative PIPs and TIPs aquaporins of *Vitis vinifera* through heterologous expression in aqy-null *Saccharomyces cerevisiae***, Farzana Sabir, CBAA, Instituto Superior de Agronomia

14:15 **Cell wall dynamics modulate acetic acid-induced apoptotic cell death of *Saccharomyces cerevisiae***, Susana R. Chaves, Centre of Molecular and Environmental Biology, University of Minho

14:30 **Study of the intracellular trafficking of the *Saccharomyces cerevisiae* lactate transporter Jen1 using domain swap experiments**, Gabriel Talaia, Centre of Molecular and Environmental Biology (CBMA), University of Minho

14:40 **Cell-cell interactions in yeast cultures at high cell density: role of the *Saccharomyces cerevisiae* cell-wall associated protein GAPDH and physiological alterations induced in sensitive yeast cells**, Patrícia Branco, Unidade Bioenergia, LNEG

14:50 **Ammonium toxicity in prototrophic aging yeast cells**, Júlia Santos, ICVS/3B's

15:00 **Screening for new Ffz-like hexose transporters in yeasts and correlation with strain fructophilic/glucophilic behavior**, Sara Cabral, CBAA, Instituto Superior de Agronomia

15:10 **Pesticide toxicity and iron homeostasis in *Saccharomyces cerevisiae*: the case of the priority active substance alachlor**, Fátima N Gil, IBB, Instituto Superior Técnico

15:20 **Ubiquitin ligase Ufd2 is required for Yap8 stabilization upon arsenic stress**, Rita T. Ferreira, Stress and Genomics Laboratory, ITQB

16:00 Poster Sessions I and II

Chairs: José Peinado, Arlete Mendes-Faia

Session II – Yeasts and Bioenergy, Food and Environmental Biotechnology

(supported by SPM working groups: Biotecnologia microbiana and Controlo de Qualidade Microbiológica)

Chairs: Francisco Gírio, Alexandra Mendes Ferreira, Manuel Malfeito Ferreira

17:00 ***Saccharomyces cerevisiae* as a pivotal tool for the functional characterization of plant membrane transporters**, Paula Duque, Instituto Gulbenkian Ciência.

17:15 **Third generation biofuels and high value added products from the yeast *Rhodosporidium toruloides* NCYC 921: A possible approach for reducing the current fossil oil dependence**, Sofia Sousa, Laboratório Nacional de Energia e Geologia

17:30 **Physiological stress response of the yeast *Rhodospoditium toruloides* to pH changes grown on batch cultures for lipids and carotenoids production**, Carla Dias, Laboratório Nacional de Energia e Geologia

17:40 **New insights of yeast H^+ homeostasis: intracellular pH assessed by Fluorescence Ratio Imaging Microscopy (FRIM) in cells collected during wine fermentation**, Tiago Viana, CBAA, Instituto Superior de Agronomia

17:50 **The effect of chitosan in viability of *Brettanomyces bruxellensis* and 4-ethylphenol production in wines**, Miguel T Fernandes, Instituto Superior de Agronomia

18:00 **The effect of wine composition on *Brettanomyces bruxellensis* growth and volatile phenol production**, Mahesh Chandra, Centro de Botânica Aplicada à Agricultura (CBAA), Instituto Superior de Agronomia

18:10 **Isolation of lipase-producing yeasts from olive mill wastewater**, Vera Salgado, LNEG – Unidade de Bioenergia

18:20 **Pseudozyma yeasts as microbial cell factories for lignocellulosic biorefineries**, Nuno T Faria, IBB, Instituto Superior Técnico, and LNEG

18:30 **Acetic acid resistance in *Saccharomyces cerevisiae*: role of the ABC transporter Pdr18, proposed to mediate plasma membrane sterol incorporation**, Cláudia Godinho, IBB, Instituto Superior Técnico

Session III – Yeast Diversity and Evolution

(supported by SPM working group: Leveduras e Fungos Filamentosos)

Chairs: José Paulo Sampaio, Filomena Duarte

9:00 **Evolution of the mating type locus in tremellaceous yeasts: insights from comparative genomics**, Álvaro Fonseca, Centro de Recursos Microbiológicos, FCT/UNL

9:15 **A common phylogenetic origin links the DHA2, ARN and GEX transporters: phylogenetic characterization and proposal of the new DAG protein family**, Paulo Jorge Dias, IBB – Institute for Biotechnology and Bioengineering, Instituto Superior Técnico

9:30 **Elucidating the role of CUG mistranslation in *Debaryomyces hansenii* stress tolerance**, Isabel M. Miranda, Department of Microbiology, Faculty of Medicine of the University of Porto

9:45 **Experimental evolution of chromosome structure in yeast**, Lilia Perfeito, Instituto Gulbenkian de Ciência

10:00 **Global diversity and domestication in the wine and cider yeast *Saccharomyces uvarum***, Carla Gonçalves, Centro de Recursos Microbiológicos, FCT-UNL

10:15 **Population genomics of *Saccharomyces cerevisiae* in Brazil**, Raquel Barbosa, Centro de Recursos Microbiológicos, FCT-UNL

Session IV – Yeasts and Human Health

(supported by SPM working groups: Microbiologia e Saúde Pública and Infecção e Imunidade)

Chairs: Margarida Casal, Paula Ludovico, Isabel Miranda

11:00 ***TLR9* activation dampens the early inflammatory response to *Paracoccidioides brasiliensis*, impacting host survival**, João Filipe Menino, Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho

11:15 **Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease**, Sandra Tenreiro, Instituto de Medicina Molecular

11:30 **Mechanistic insights into polyphenol-mediated protection against alpha-synuclein toxicity**, Diana Macedo, Instituto de Tecnologia Química e Biológica

11:40 **A selective activator of protein kinase Cdelta discovered using a yeast-based assay**, Cláudia Bessa, REQUIMTE, Laboratório de Microbiologia

11:50 **Role of *Candida glabrata* Drug:H^+ Antiporters CgAqr1, CgQdr2 and CgTpo3 in antifungal drug resistance: from lab strains to clinical isolates**, Catarina Costa, IBB, Instituto Superior Técnico

12:00 **In Vitro Induced Resistance to Azoles, Echinocandins and Amphotericin B in *Candida glabrata***, Ana Silva-Dias, Department of Microbiology, Faculty of Medicine, University of Porto

12:10 **Unveil of the resistance mechanisms induced by voriconazole in *Candida krusei***, Elisabete Ricardo, Department of Microbiology, Faculty of Medicine, University of Porto

12:20 **The CgHaa1-dependent pathway mediates *Candida glabrata* response and tolerance to acetic acid thereby enhancing colonization of vaginal epithelium**, Ruben Bernardo, IBB, Instituto Superior Técnico

Session V – Yeast Functional Genomics and Bioinformatics

(supported by SPM working groups: Òmicas e Microbiologia Integrativa e de Sistemas)

Chairs: Claudina Rodrigues Pousada, Miguel Cacho Teixeira, Pedro Monteiro

14:00 **YEASTRACT-NET: extracting and visualizing transcription regulatory networks in *S. cerevisiae***, Pedro T. Monteiro, INESC-ID

14:15 **Sequencing and annotation of the genome of the highly acetic acid-tolerant *Zygosaccharomyces bailii*-derived interspecies hybrid strain ISA1307**, Nuno P Mira, IBB, Instituto Superior Técnico

14:30 **Screening for genes involved in the high acetic acid resistance of the *Zygosaccharomyces bailii*-derived interspecies hybrid strain ISA1307**, Margarida Palma, IBB, Instituto Superior Técnico

14:40 **Yeast protein expression profile during acetic acid-induced apoptosis in the highly resistant food spoilage *Zygosaccharomyces bailii* derived hybrid strain ISA1307**, Joana F Guerreiro, IBB, Instituto Superior Técnico

14:50 **Role of Sit4p-dependent protein dephosphorylation in the regulation of mitochondrial function and yeast lifespan**, Andreia T Pereira, IBMC

15:00 **Unveiling wine yeast performance combining phenotypic, metabolic and transcriptomic data**, Catarina Barbosa, IBB – Centre of Genomics and Biotechnology, Universidade de Trás-os-Montes e Alto Douro

15:10 **Genome-wide identification of *Saccharomyces cerevisiae* genes required for tolerance to sulphur dioxide**, Patrícia Lage, IBB – Centre of Genomics and Biotechnology, Universidade de Trás-os-Montes e Alto Douro

15:20 **Elucidating the role of *MRR1* transcription factor mutations in *C. parapsilosis* azole resistance**, Joana Branco, Department of Microbiology, Faculty of Medicine of the University of Porto

15:45 Poster Sessions III, IV and V

Chairs: Maria da Conceição Loureiro-Dias, Cecília Leão

Conferência "Jovem Investigador" – Tributo à Professora Isabel Spencer Martins

Chairs: Isabel Sá-Correia (Presidente da Sociedade Portuguesa de Microbiologia)*, Manuela Côrte-Real*

17:00 **The emerging role of autophagy/mitophagy in α-synuclein-induced toxicity: studies on the yeast chronological aging model**, Belém Sampaio-Marques, Life and Health Sciences Research Institute (ICVS), Universidade do Minho

17:30 Sessão de Encerramento (inclui a entrega do prémio de melhor apresentação em poster, patrocinado pela STAB-VIDA)

CONFERÊNCIA

"PROFESSOR NICOLAU VAN UDEN"

Cross-talk between sphingolipid and nutrient signaling pathways: new clues to old yeast

Vítor Costa[a,b]

[a]IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal. [b]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Departamento de Biologia Molecular, Universidade do Porto, Porto, Portugal.

Sphingolipids are ubiquitous structural components of cell membranes and act as regulatory molecules in signal transduction pathways, e.g. through the modulation of protein kinases or phosphatases. Bioactive sphingolipids regulate cellular processes such as cytoskeleton dynamics, endocytosis, protein turnover, cell cycle, stress responses, cell senescence and apoptosis, and have been implicated in ageing and in a wide range of diseases, including neurodegenerative disorders. Ceramide is the central core lipid in the metabolism of sphingolipids and constitutes a family of structurally distinct molecular species that can be generated in distinct subcellular compartments. In yeast, ceramide is produced by acylation of a long chain base (LCB; dihydrosphingosine or phytosphingosine) or through hydrolysis of complex sphingolipids (inositol phosphosphingolipids) mediated by Isc1p, an orthologue of mammalian neutral sphingomyelinase 2. Recent studies suggest that the interplay between sphingolipids and nutrient signaling pathways plays a key role in the regulation of redox homeostasis and chronological lifespan in yeast. Indeed, the Sch9p protein kinase and the Sit4p protein phosphatase integrate nutrient and stress signals from TORC1 (Target of Rapamycin complex 1), a major regulator of cell growth and autophagy, with sphingolipid signals. How this complex network of interacting pathways mediates the mitochondrial dysfunction, enhanced oxidative stress sensitivity and shortened lifespan exhibited by yeast mutants lacking Isc1p or Ncr1p, an orthologue of mammalian NPC1 associated with the Niemann-Pick type C disease, will be discussed.

ACKNOWLEDGEMENTS: This work was financially supported by FEDER (Fundo Europeu de Desenvolvimento Regional) through the program "Programa Operacional Fatores de Competitividade-COMPETE" and by FCT (Fundação para a Ciência e Tecnologia) through the projects PEST-C/SAU/LA0002/2013-FCOMP-01-0124-FEDER-037277 and FCOMP-01-0124-FEDER-028210.

CONFERÊNCIA "JOVEM INVESTIGADOR"

TRIBUTO À PROFESSORA ISABEL SPENCER MARTINS

The emerging role of autophagy/mitophagy in α-synuclein-induced toxicity: studies on the yeast chronological aging model

Belém Sampaio-Marques[1,2], Carolina Felgueiras[1,2], Alexandra Silva[1,2], Márcio Rodrigues[1,2], Sandra Tenreiro[3], Vanessa Franssens[4], Andreas S. Reichert[5], Tiago F. Outeiro[3,6,7], Joris Winderickx[4], Paula Ludovico[1,2]

[1]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Cell and Molecular Neuroscience Unit, Instituto de Medicina Molecular, Lisboa, Portugal; [4]Functional Biology, KU Leuven, Heverlee, Belgium; [5]Buchmann Institute for Molecular Life Sciences, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany and Mitochondriale Biologie, Zentrum für Molekulare Medizin, Goethe Universität Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany; [6]Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal; [7]Department of Neurodegeneration and Restorative Research, University Medizin Goettingen, Goettingen, Germany. (mbmarques@ecsaude.uminho.pt)

α-Synuclein misfolding and aggregation is strongly associated with both idiopathic and familial forms of Parkinson's disease (PD). Evidence suggests that α-synuclein impacts on cell clearance routes and protein quality control systems such as the ubiquitin/proteasome system and autophagy. Recent advances on the key role of the autosomal recessive Parkin and PINK1 genes in mitophagy, highlighted this process as a prominent new pathogenic mechanism. Nevertheless, the role of autophagy/mitophagy in the pathogenesis of sporadic and autosomal dominant familial forms of PD is still enigmatic. The yeast Saccharomyces cerevisiae is a powerful "empty room" model that has been exploited to clarify different molecular aspects associated with α-synuclein toxicity and that combines the advantage of being an established system for aging research. The contribution of autophagy/mitophagy for the toxicity induced by the heterologous expression of the human wild type α-synuclein and its clinical A53T mutant during yeast chronological lifespan (CLS) was explored. A reduced CLS together with an increase of autophagy and mitophagy activities were observed in cells expressing both forms of α-synuclein. Impairment of mitophagy by deletion of *ATG11* or *ATG32* resulted in a CLS extension, further implicating mitophagy in the α-synuclein toxicity. Deletion of SIR2, essential for α-synuclein toxicity, abolishes autophagy and mitophagy, thereby rescuing cells. These data show that Sir2 functions as a regulator of autophagy, like its mammalian homologue, SIRT1, but also of mitophagy. Our work highlights that increased mitophagy activity, mediated by the regulation of *ATG32* by Sir2, is an important phenomenon linked to α-synuclein-induced toxicity during aging.

This work was supported by a grant from FCT — Fundação para a Ciência e a Tecnologia Portugal (PTDC/BIA-MIC/114116/2009). B.S.M. has a fellowship from FCT (SFRH/BPD/90533/2012).

ORAL COMMUNICATIONS

SESSION I – Yeast Physiology and Genetics

O2 - Functional characterization of putative PIPs and TIPs aquaporins of *Vitis vinifera* through heterologous expression in aqy-null *Saccharomyces cerevisiae*

Farzana Sabir[1,2], Maria José Leandro[1], Maria C. Loureiro-Dias[1], Teresa F. Moura[2], Graça Soveral[2,3], Catarina Prista[1]

[1]CBAA, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal; [2]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; [3]Dept. de Bioquímica e Biologia Humana, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal. (fsabir@isa.ulisboa.pt)

Aquaporins are small integral membrane proteins present in the plasma and intracellular membranes of plant cells, where they facilitate the transport of water across the membranes. Based on their sequence similarity and sub-cellular localization, they are divided in seven subfamilies in plants: the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the nodulin26-like intrinsic proteins (NIPs), the small intrinsic proteins (SIPs), the GlpF-like intrinsic proteins (GIPs), the hybrid intrinsic proteins (HIPs) and the uncategorized X intrinsic proteins (XIPs). Studies on plant aquaporins revealed their role far beyond the membrane water transport. Besides water, they are reported to transport also other small molecules (as ammonia) and/or gases (as CO_2) of physiological importance, suggesting their versatile function in plants. Grapevines (*Vitis vinifera* L.) are the most widely cultivated and economically important fruit crop worldwide. Release of full genomic sequence of *V. vinifera* revealed the existence of 28 genes encoding putative aquaporins. Since the water status of the plant greatly influences the fruit quality and hence the characteristics of wine, it is important to study the molecular components of water transport and to characterize them individually, which can be accomplished through their heterologous expression in an aqy-null strain of *Saccharomyces cerevisiae*. In this study, we cloned six putative aquaporins tagged with GFP protein (PIPs: VvTnPIP2;1, VvTnPIP1;4 and VvTnPIP2;3 and TIPs: VvTnTIP1;1, VvTnTIP2;2 and VvTnTIP4;1) from Portuguese cultivar of V. vinifera (cv. Touriga nacional), using aqy-null strain of *S. cerevisiae* as a host strain. GFP-tagged *Vitis aquaporins* were expressed in the plasma membrane. Functional characterization for water transport was performed through stopped-flow spectroscopy and was evaluated in terms of permeability coefficient (Pf) and activation energy (Ea). Water transport activity of heterologously expressed *Vitis aquaporins* revealed that three aquaporins (VvTnPIP2;1, VvTnTIP1;1 and VvTnTIP2;2) exhibited higher Pf (7.43±0.64 X10-4 cms-1, 8.06±0.34 X10-4 cms-1 and 9.65±0.026 X10-

4 cms-1, respectively) and lower Ea (10.84±0.83, 8.8±0.77 and 8.77±0.62 kcal mol-1, respectively) than control strain (Pf = 4.30±0.28 X10-4 cms-1, Ea=14.05±0.01 kcal mol-1), hence these aquaporins were considered functional for water transport. The other three cloned aquaporins (VvTnPIP1;4, VvTnPIP2;3 and VvTnTIP4;1) showed almost equal Pf as the control (4.0±0.35 X10-4 cms-1, 5.30±0.63 X10-4 cms-1 and 3.3±0.08 X10-4 cms-1, respectively) and Ea (14.74±0.62, 14.53±0.55, 14.86±0.22 kcal mol-1, respectively) values and were assumed as non-functional for water transport. Among all cloned *Vitis aquaporins*, TIPs (VvTnTIP1;1, VvTnTIP2;2) exhibited higher water conductivity than VvTnPIP2;1. All functional aquaporins were found to be sensitive for 0.5mM $HgCl_2$, a well-known inhibitor of aquaporins. Pf of these aquaporins was reduced by 24-38% in the presence of $HgCl_2$. In addition, their sequence analysis revealed the presence of signature sequences for transport of non-aqua substrates (ammonia, boron, CO_2, H_2O_2 and urea). Our results on growth assays also showed that heterologous expression of *Vitis aquaporins* increased the susceptibility of yeast cells to externally applied boron and H_2O_2, indicating their putative involvement in the transport of these substrates.

This work is supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (Post-Doctoral fellowships SFRH/BPD/89427/2012 to F.S and SFRH/BPD/41812/2007 to M.J.L, Ciência 2007 contract to CP and Research Project "AQUAVITIS - Understanding water transport in *Vitis vinifera*: biochemical characterization of aquaporins upon their heterologous expression in yeast" (PTDC/AGR-AAM/099154/2008)).

O3 - Cell wall dynamics modulate acetic acid-induced apoptotic cell death of *Saccharomyces cerevisiae*

Flávio Azevedo[1], Ana Marta Duarte[1], António Rego[1], Maria João Sousa[1], Manuela Côrte-Real[1], Susana R. Chaves[1]

[1]Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal. (chaves.sr@gmail.com)

Saccharomyces cerevisiae is currently a well-established eukaryotic model organism used in the elucidation of molecular mechanisms of programmed cell death pathways. In particular, acetic acid-induced apoptosis is among the best-characterized yeast apoptotic pathways, due to the interest of modulating this response for applications in both biotechnology and biomedicine. Multiple proteins that control acetic acid-induced apoptosis have been identified; however, upstream events and signaling pathways regulating this process remain poorly characterized. In this study, we analyzed whether impairing Mitogenic Activated Protein Kinase (MAPK) signaling affected acetic acid-induced apoptosis. We show that the pheromone/mating response and the cell wall integrity pathways are

major mediators of acetic acid-induced cell death, especially the CWI pathway, which we characterized in detail, including a screen for downstream effectors of this pathway, namely the targets of the transcription factor Rlm1p. This screen uncovered several processes involved in resistance to acetic acid, of which a decrease in cell wall remodeling was particularly evident. Modulation of cell surface dynamics therefore emerges as a powerful strategy to increase resistance of yeast strains to acetic acid, with potential application in industrial fermentations carried by yeast. Short chain fatty acids in their unprotonated form produced by propionibacteria that reside in the human intestinal tract, such as acetate and propionate, trigger apoptosis in colorectal carcinoma cell lines. Therefore, our results also open new avenues of research into the regulation of acetate-induced apoptosis in mammals, with particular impact for the design of novel therapeutic opportunities against colorectal carcinoma based on the modulation of MAP kinases.

ACKNOWLEDGEMENTS: This work was supported by FEDER through POFC – COMPETE and by Fundação para a Ciência e Tecnologia through projects PTDC/BIA-BCM/69448/2006, FCT-ANR/BEX-BCM/0175/2012, PTDC/AGR-ALI/102608/2008, and PEst-OE/BIA/UI4050/2014, as well as fellowships to F.A (SFRH/BD/80934/2011), A.R (SFRH/BD/79523/2011) and S.R.C (SFRH/BPD/89980/2012).

O4 - Study of the intracellular trafficking of the *Saccharomyces cerevisiae* lactate transporter Jen1 using domain swap experiments

Gabriel Talaia[1], Margarida Casal[1], George Diallinas[2] and Sandra Paiva[1]

[1]Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, Braga, 4710-057, Portugal; [2]Faculty of Biology, Department of Botany, University of Athens, Athens 15781, Greece (gabriel.rocha@bio.uminho.pt)

The intracellular trafficking of plasma membrane (PM) proteins, such as receptors and transporters, in eukaryotic cells is a highly regulated process. Changing environment conditions (e.g. nutrients, substrates, hormones) can trigger endocytosis of unwanted transporters. Studies in yeast revealed that addition of ubiquitin (ubiquitylation), by the ubiquitin ligase Rsp5, plays a crucial role in the endocytic downregulation of PM transporters. This posttranslational modification acts as a signal triggering these proteins' internalization, subsequent targeting to Multi vesicular bodies (MVBs) and final degradation in the lysosome/vacuole. To study in detail these mechanisms, our group has been using the lactate transporter Jen1 of the yeast *Saccharomyces cerevisiae* as a model. It is known that glucose acts as a signal for endocytic down-regulation of Jen1 within minutes (Paiva et al, 2002), a process dependent on ubiquitination and phosphorylation (Paiva et al, 2009). Furthermore, it was shown that not only

Rsp5 but also an arrestin-like protein, Rod1, is required for Jen1p down-regulation by glucose (Becuwe et al, 2012). Moreover, Rod1 makes the bridge between glucose signalling and transporter endocytosis (Becuwe et al, 2012). In an attempt to identify domains that are important for the subcellular localization, activity and turnover of Jen1, domain swap experiments were carried out. The hybrid transporter genes carry a fusion with GFP gene which enables to study in vivo the trafficking of the proteins. The strategy involved in the construction of the chimeric transporters will be presented, as well as data on their characterization at biochemical and subcellular levels, under various physiological conditions.

REFERENCES: Paiva et al., 2002. Biochem. J. 363: 737-744; Paiva et al., 2009. J Biol Chem. 284: 19228-36; Becuwe et al., 2012. J Cell Biol. 196: 247-59; ACKNOWLEDGMENTS: This work was supported by PEst-OE/BIA/UI4050/2014 and SFRH/BD/86221/2012.

O5 - Cell-cell interactions in yeast cultures at high cell density: role of the *Saccharomyces cerevisiae* cell-wall associated protein GAPDH and physiological alterations induced in sensitive yeast cells

Patrícia Branco[1],Varongsiri Kemsawasd[2], Nils Arneborg[2], Helena Albergaria[1]

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Nissen et al. (2003) showed that *Saccharomyces cerevisiae* (Sc) cells at high densities induce the growth arrest of some non-*Saccharomyces* by a cell-cell contact-mediated mechanism. In a very recent work (Branco et al, 2013) we found that Sc secretes antimicrobial peptides (AMPs) active against several wine-related yeasts and bacteria that are derived from the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Besides, in a previous work (Albergaria et al., 2010) we also showed that *S. cerevisiae* begins to secrete those AMPs to the extracellular medium at the end of the exponential growth phase (1-2 days) in alcoholic fermentations. Since Delgado et al. (2001) showed that GAPDH is a cell-wall associated protein in Sc we wonder if GAPDH-derived AMPs could be present at the membrane of Sc cells and thus induce death of sensitive cells by a cell-cell contact mediated mechanism. In order to investigate this hypothesis, we performed assays in which Sc cells pre-grown for 12 and 48 h, respectively, at enological growth conditions were putted in direct contact with *Hanseniaspora guilliermondii* (Hg) at high cell density values (107-108 cells/ml) in a carbon-free medium. As a positive control we performed similar assays in which Sc and Hg cells were separated by a dialysis tube (pore cut-off 1000 kDa)

and as a negative control an assay where Hg cells were alone. We also tested the effect of Sc cells (pre-grown for 48 h) from single mutants deleted in each of the GAPDH codifying genes (TDH1-3). Results showed that Sc cells pre-grown for 48 h induced death of Hg cells, while both Sc cells pre-grown for 12 h and TDH1-3 mutants did not. In order to relate the death-inducing ability of Sc cells pre-grown for 48 h with the presence of AMPs we extracted the cell wall proteins of Sc cells and analyzed them by different techniques. Results revealed the presence of a peptidic fraction that induces growth inhibition of Hg. Furthermore we assessed the physiological changes induced by Sc cells pre-grown for 12 and 48 h on Hg cells, namely membrane permeability and intracellular pH (pHi). Membrane permeability was evaluated by staining cells with propidium iodide (PI) and pHi by the fluorescence ratio imaging microscopy (FRIM) technique (Guldfeldt and Arneborg, 1998). This technique gives information at a single-cell level, which allows determining the pHi of different sub-populations of cells simultaneously.

REFERENCES: Albergaria H, Francisco D, Gori K, Arneborg N, Gírio F (2010). Appl Microbiol Biotechnol 86: 65-972. Branco P, Francisco D, Chambon C, Hébraud M, Arneborg N, Almeida MG, Caldeira J, Albergaria H (2013). Appl Microbiol Biotechnol 98(2):843-53. Delgado ML, O'Connor JE, Azorín I, Renau-Piqueras J, Gil ML, Gozalbo D (2001) Microbiol 147: 411–417. Guldfeldt LU and Arneborg N (1998) Appl Microbiol Biotechnol 64: 530–534. Nissen P, Nielsen D, Arneborg N (2003). Yeast 20: 331-341.

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O6 - Ammonium toxicity in prototrophic aging yeast cells

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In grape must, suboptimal concentrations of yeast assimilable nitrogen can lead to sluggish or stuck fermentations, ammonium and/or amino acids being frequently added to avoid such problem. However, we have recently shown that the presence of ammonium can be toxic, negatively affecting the yeast chronological life span (CLS), particularly under amino acids restriction conditions. We demonstrated that decreasing the concentration of ammonium in the culture medium increases CLS of auxotrophic *Saccharomyces cerevisiae* BY4742 strain in both standard amino acids supplementation and amino acid restriction media, being particularly relevant under the later condition. In the present study we further report that the

CLS of prototrophic strains is also decreased by ammonium, in comparison to other nitrogen sources. The presence of ammonium increases loss of cell viability, whereas in medium supplemented only with glutamine as the nitrogen source, cell survival is largely extended. In these conditions, glucose consumption was measured and a delay in glucose consumption was observed in medium supplemented with glutamine, in comparison to medium supplemented with ammonium. Also in cell cycle analysis there were major differences between these two nitrogen sources, with glutamine providing for a normal cell cycle arrest contrasting with an improper cell cycle arrest observed in the presence of ammonium. When the two nitrogen sources were provided simultaneously, it seems the toxic effects of ammonium prevail, leading to cell death similar to the one observed for cells cultured in medium supplemented with ammonium alone. Ammonium also seems to inhibit the full consumption of glutamine that had been observed in medium supplemented with glutamine alone. Further studies are now necessary to clarify the impact of adding ammonium and /or amino acids to overcome sluggish or stuck fermentations.

O7 - Screening for new Ffz-like hexose transporters in yeasts and correlation with strain fructophilic/glucophilic behavior

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Several yeasts such as the main fermentative yeast *Saccharomyces cerevisiae*, prefer glucose over all the other available sugars, being therefore designated as glucophilic yeasts. On the other hand, yeasts belonging to the genus *Zygosaccharomyces*, like *Z. bailii* and *Z. rouxii*, share an opposite behavior, consuming preferentially fructose over glucose, being designated as fructophilic. These yeasts are also known by their ability to spoil food and beverages with high economic value, due to its capacity to grow under harsh conditions, such as low pH, low water activity and in the presence of weak acids. Another peculiar characteristic of these *Zygosaccharomyces* yeasts is the presence of a different type of hexose transporters, the Ffz proteins, that are phylogenetically unrelated to all hexose transporters characterized so far, that usually belong to the Sugar Porter family. These Ffz transporters seem to belong to a new family of hexose transporters closely related with the Drug/H^+ Antiporter DHA1 family. In *Z. bailii* and *Z. rouxii* a specific fructose facilitator system with high capacity and low affinity, ZbFfz1 and ZrFfz1, respectively, has been already characterized and in *Z. rouxii* another Ffz protein that transports glucose and fructose with similar capacity and affinity, ZrFfz2, was described. Recently, our group also characterized a high-affinity fructose/H^+ symporter of *Z. rouxii* that, contrary to

Ffz proteins, belong to the Sugar Porter family. As, so far, these type of transporters were only characterized in fructophilic yeasts, we hypothesized that these new Ffz transporters may exist in other fructophilic yeasts and that a possible correlation might exist between their presence and the yeast fructophilic behavior. In this work we screened by PCR with degenerate primers for the presence of FFZ-like genes in (1) several yeasts of the *Zygosaccharomyces* genus, (2) yeasts that in the past belonged to this genus but are currently re-assigned to other genera (*Z. mrakii* nowadays is denominated *Zygotorulaspora mrakii*), (3) yeasts in clades closely related with the *Zygosaccharomyces* clade, such as *Lachancea* e *Zygotorulaspora*, (4) yeasts already described as fructophilic yeasts (*C. magnoliae*) and (5) other yeasts related to fructophilic yeasts. Moreover, the glucophilic/fructophilic behavior of the strains in study was evaluated in order to possibly correlate the presence of the FFZs screened and the sugar preference of the strains.

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O8 - Pesticide toxicity and iron homeostasis in *Saccharomyces cerevisiae*: the case of the priority active substance alachlor

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Alachlor (ALA) has been a commonly used herbicide worldwide and is among the 33 EU priority substances listed in the European Water Framework Directive (2000/60/EC) for which environmental monitoring programs are required. Evidences pointing to an existing relationship between the iron regulon and the yeast response to oxidative stress have been reported in the literature [1]. In a previous transcriptomic analysis of the yeast response to sublethal moderately toxic concentrations of ALA [2], we found that genes encoding proteins involved in iron uptake through the non-reductive system mediated by siderophores (namely, *ARN1*, *ARN3*/*SIT1*, *ARN4*/*ENB1*, *FIT2*) were up-regulated [2]. On the other hand, *FTR1* and *FET3*, encoding a protein complex involved in iron uptake through the reductive system, were down-regulated. In the present work, we present results suggesting that the main regulator of iron homeostasis in the yeast, Aft1p, may play an important role in the cells response to the stress exerted by the herbicide ALA. Indeed, we found that the up-regulation of the iron-regulon

genes *ARN1*, *FIT2* and *CTH2* in the ALA-stressed cells was dependent on the expression of *AFT1*. Moreover, deletion of this iron-homeostasis regulatory gene increased yeast cells susceptibility to the herbicide. We also observed the predominant nuclear localization of a fusion protein GFP-Aft1p in the ALA-stressed cells that correlated with the observed up-regulation of the iron-regulon genes (*FIT2*, *ARN1* and *CTH2*). We also found that ALA induced ROS production in stressed yeast cells and that the observed susceptibly of *Δaft1* to ALA may be related with oxidative damage. Overall our experimental evidences suggest that the functionality of the reductive pathway for iron uptake appears to be affected in the ALA-stressed yeast cells.

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O9 - Ubiquitin ligase Ufd2 is required for Yap8 stabilization upon arsenic stress

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All living organisms respond promptly to adverse stimuli mainly through the control of gene expression. Upon arsenic stress, yeast cells arrest cycle progression, alter gene expression and activate multiple adaptation pathways in order to maintain their integrity. In *Saccharomyces cerevisiae*, the AP-1 like protein Yap8 is the major transcription factor involved in cellular response to arsenic. Although its regulatory role in the transcriptional activation of *ACR2* and *ACR3* (encoding detoxification proteins)is well characterized, very little is known about the post-translational mechanisms controlling Yap8 activity. Ufd2 belongs to the ubiquitin chain elongation class, which has a conserved domain among eukaryotes (termed the U-box), commonly associated with a degradation signal. In this study, we show that Ufd2 physically interacts with Yap8 by using the two-hybrid system and we validate the interaction of both proteins by co-immunoprecipitation. Furthermore, we observe by inhibiting protein synthesis

with cycloheximide that the Yap8 half-life is decreased in the *ufd2* mutant strain. This result indicates that Ufd2 is a positive regulator of Yap8 stability. Indeed, via quantitative RT-PCR experiments, we show that in the ufd2 mutant the mRNA levels of Yap8 targets are decreased, as well as the Yap8 transactivation potential measured by yeast one-hybrid. Using a mutant in the U-box we also show that this motif is only partially implicated in the control of Yap8 activity. However, the U-box mutant does not reveal a sensitive phenotype in contrast to the ufd2 mutant strain, in the presence of arsenic compounds. Interestingly, the *UFD2* mRNAs are highly induced by arsenic which are well corroborated with the protein levels. Our hypothesis is that this Ufd2 protein may play a role as a ubiquitin ligase in proteolysis but also preventing the degradation of a transcriptional factor. To our knowledge, this is the first report in *S. cerevisiae* pointing out to a E4 ubiquitin ligase that stabilizes a transcription factor, a fact that was also found in p63 of mammalian cells.

SESSION II – Yeasts and Energy, Food and Environmental Biotechnology

O10 - *Saccharomyces cerevisiae* as a pivotal tool for the functional characterization of plant membrane transporters

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Abiotic stress factors are responsible for a 50% reduction in crop productivity worldwide, and a fundamental understanding of plant stress tolerance at the molecular level is widely seen as crucial to secure sufficient crop yields for the growing population in a changing environment. Membrane transporters play important roles in a plant's fitness to cope with different forms of abiotic stress, such as drought or soil salinity, heavy metal tocixity and mineral deprivation. The Major Facilitator Superfamily (MFS) of transporters is found in all classes of living organisms, being the largest known group of active secondary carriers whose transport of a diverse range of small solutes is energized by electrochemical gradients. The genome of the model plant *Arabidopsis thaliana* contains more than 120 genes encoding MFS membrane proteins, but very few have been characterized to date. I will describe how heterologous expression, mutant complementation and transport activity assays in *Saccharomyces cerevisiae* have been pivotal in our recent discovery of key physiological roles for *Arabidopsis* MFS transporters, namely in root development [1,2] and tolerance to drought stress [1], phosphate starvation [3] or zinc toxicity [4].

REFERENCES: [1] Remy E, Cabrito TR, Baster P, Batista RA, Teixeira MC, Friml J, Sa-Correia I, Duque P (2013) A major facilitator superfamily transporter plays a dual role in polar auxin transport and drought stress tolerance in *Arabidopsis*. Plant Cell 25(3): 901-26; [2] Remy E, Baster P, Friml J, Duque P (2013) ZIFL1.1 transporter modulates polar auxin transport by stabilizing membrane abundance of multiple PINs in *Arabidopsis* root tip. Plant Signal Behav 8(10): doi: 10 4161/psb 25688; [3] Remy E, Cabrito TR, Batista RA, Teixeira MC, Sa-Correia I, Duque P (2012) The Pht1;9 and Pht1;8 transporters mediate inorganic phosphate acquisition by the *Arabidopsis thaliana* root during phosphorus starvation. New Phytol 195(2): 356-71; [4] Remy E, Cabrito TR, Batista RA, Hussein MA, Teixeira MC, Athanasiadis A, Sa-Correia I, Duque P (2014) Intron Retention in the 5'UTR of the Novel ZIF2 Transporter Enhances Translation to Promote Zinc Tolerance in *Arabidopsis*. PLoS Genet 10(5): e1004375.

O11 - New insights of yeast H^+ homeostasis: intracellular pH assessed by Fluorescence Ratio Imaging Microscopy (FRIM) in cells collected during wine fermentation

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Wine fermentation can be considered a complex bioprocess that leads to the chronic adaptation of wine yeasts to stressful conditions by the interaction of numerous genetic, metabolic, and environmental factors. In yeast, while fermenting acidic must, to keep an appropriate pH in the cytosol and within membrane-enclosed compartments is a constant challenge. Yeast proton homeostasis is essentially maintained by a balance between the flux of H+ pumped out by the *PMA1* ATPase and the influx of H^+ by passive diffusion through the lipid bilayer. Ethanol interferes with this delicate equilibrium by inhibiting mediated transport and enhancing passive diffusion, disturbing both intracellular pH (pHi) and proton motive force across the plasma membrane. *Saccharomyces cerevisiae* BY4741 and *Δrim101* (mutation previously selected as for conferring an improved fermentative performance) strains were studied at both population and single-cell levels, while fermenting a synthetic grape must under simulated winery conditions. Late stationary populations presented a more acidic intracellular content, and also became almost impermeable to H+, even when challenged with high ethanol concentrations (up to 16%, added in the assay). The highest H^+ efflux (1.84 and 2.11 mmol (g biomass)-1 h-1 for BY4741 and *Δrim101*, respectively) and the highest H+ influx (0.15 and 0.16 mmol (g biomass)-1 h-1 for BY4741 and *Δrim101*, respectively) were exhibited in the exponential phase. Fluorescence Ratio Imaging Microscopy (FRIM) was used to estimate pHi of individual cells and different cell subpopulations could be distinguished. Based on a comparison of the results obtained between 5(6)-

Carboxy-2',7'-dichlorofluorescein diacetate succinimidyl ester (CDCFDA-SE) and 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) probes, it was possible to identify a organelle-specific staining growth phase dependent for CDCFDA-SE but not for CFDA-SE. CFDA-SE is a strict cytosolic staining probe in simulations of a wine fermentation. *Δrim101* subpopulations remained viable and metabolically active at late stationary phase. Understanding the molecular and physiological events underlying yeast H^+ homeostasis at late stages of fermentations may provide relevant physiological knowledge for strain improvement and wine quality.

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O12 - The effect of chitosan in viability of *Brettanomyces bruxellensis* and 4-ethylphenol production in wines

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The spoilage yeast *Brettanomyces bruxellensis*, or its teleomorph *Dekkera bruxellensis*, is a major threat to the wine industry mainly due to the production at high rates of 4-ethylphenol by conversion of p-coumaric acid. The "horse sweat" taint, characteristic of this volatile phenol, causes serious economical losses in the wine industry. The control of this yeast is of great importance and chitosan as been pointed as an alternative to sulphur dioxide as a preservative in wine. Therefore, the aim of this work was to evaluate the antimicrobial action of chitosan against this yeast at different growth phases and concentrations in wine. Under laboratory conditions the effect of chitosan, at different concentrations (0, 0.05, 0.1, 0.5, 1, 2 and 3 g/L), was tested in various *Brettanomyces bruxellensis* strains in synthetic medium and wine. Viability was followed by hemocytometer count with vital staining and culturable cells by plating in GYP medium. 4-ethylphenol accumulation was determined by gas chromatography. In synthetic medium it was possible to control the growth of *B. bruxellensis* with 1 g/L of chitosan. In wine the concentration and growth phase factors were tested and in both, the necessary concentrations to control the growth were lower than in medium since it is a more stressful environment. With lower concentrations and with cells in lag phase it was possible to control, the growth of the strains tested with 0.1 g/L and 0.05 g/L. However, when chitosan is added after the exponential phase, it is only possible to control the growth with 0.5 g/L. The production of 4-ethiphenol only reached significant values, over 800µg/L, on the wine trial with adapted cells. The results in this trial show that the effect of chitosan is dependent of the strain, cellular concentration and growth stage of the cells. The production

of 4-ethylphenol was significant only in wine with adapted cells and the usage of chitosan had no effect on the induction of the viable but non culturable stage in *B. bruxellensis* cells.

O13 - The effect of wine composition on *Brettanomyces bruxellensis* growth and volatile phenol production

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The presence of *Brettanomyces bruxellensis* is increasingly common in cellars. This yeast can persist throughout the harsh winemaking process and have become a major oenological concern in recent years. The present study aimed at determining the effect of sugar, alcohol and sulfur dioxide on the growth and volatile phenol production by *B. bruxellensis* using a central composite rotatable design (CCRD). Sulfur dioxide fraction proved to have a significant (P) negative linear and quadric effect (L and Q) on growth and 4-ethyl production. However, lower limit of recommended SO_2 (20 mg L-1) led cells to enter viable but not culturable (VBNC) state, showing continuous 4-ethylphenol production without ability to grow on culture media. Elevated level of ethanol (15% v/v), however, didn't cause complete loss of culturability but ceased 4-ethylphenol production. It appears that cell exhibits different metabolisms in response to sulfur dioxide and alcohol exposure; former leads to VBNC but continued 4-ethylphenol production, while later, ceased 4-ethylphenol production but reduced culturability. Effect of ethanol on cell growth was influenced by concentration of present sugar: low sugar concentration showed less susceptibility to death by ethanol. Though, sugar was found to be a significant factor (quadratic levels) in biomass increase especially in low ethanol (12.5% or below) conditions, no direct relation between 4-ethylphenol production and glucose input was observed. This study points out the importance of combined effect of glucose, ethanol and sulfur on growth behavior of *B. bruxellensis* and usefulness of elevated level of ethanol combined with recommended sulfur dioxide level in wine to control *B. bruxellenis* growth and thereby 4-ethylphenol production.

O14 - Third generation biofuels and high value added products from the yeast *Rhodosporidium toruloides* NCYC 921: A possible approach for reducing the current fossil oil dependence

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The current use of fossil fuels contributes for the increasing Greenhouse effect as a result of pollutant gas emission, being predicted that they will be depleted in the medium term. Also, due to their extraction being both difficult and expensive, it will unquestionably lead to an increase of fuel prices. Therefore, it is imperative to search for new renewable and sustainable sources of energy that are economically viable and not harmful for the environment. Biofuels from microbes present a credible prospect from an environmental and commercial standpoint. It has been dubbed "the third generation" biofuels in order to complement or possibly surpass the traditional energy crops and lignocellulosic-waste materials. Microbial oils, also called single-cell oils (SCO), are produced by some oleaginous microorganisms such as yeast, fungi, bacteria, and microalgae. It has been demonstrated that such microbial oils can be used as feedstocks for biodiesel production. Compared to other vegetable oils and animal fats, the production of microbial oil has many advantages: short life cycle, less labor required, less affected by venue, season and climate, and easier to scale up. Therefore, microbial oils might become one of potential oil feedstocks for biodiesel production in the future. At the present most of the works focusing microbial oils use autotrophic microalgae. However, although autotrophic microalgae have faster growth rates than either oil seed crops or heterotrophic algae, their biomass volumetric productivity is much lower than heterotrophic microalgae, bacteria and yeast. In addition, autotrophic cultures are weather and season dependent, being restricted to some latitudes of the planet. Therefore, biodiesel production from microorganims other than autotrophic microalgae may have particular interest for countries located at higher latitudes such as European countries. The oleaginous yeast *Rhodosporidium toruloides* NCYC 921 has been widely reported as a potential oil producing yeast. In addition, the yeast biomass, beyond its high lipid content (that can be converted into biodiesel), is rich in high value added products such as carotenoids with commercial interest, as their commercialization may contribute to reduce the overall process cost of biofuels and carotenoid production. The present work studied the oil (for biodiesel) and carotenoid production from this yeast, grown in a 5L fed-batch fermentation. The feed was added as pulses of nutrients, or in a continuously way, using a peristaltic

pump. Both strategies were compared. A flow cytometric technique was implemented to assess (on-line) the carotenoid content and the yeast viability.

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O15 - Physiological stress response of the yeast *Rhodospoditium toruloides* to pH changes grown on batch cultures for lipids and carotenoids production

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Oleaginous microbes such as microalgae and yeasts can be used as biodiesel feedstocks. Compared to other vegetable oils and animal fats, the production of microbial oil has many advantages: short life cycle, less labor required, not affected by season and climate, and easier to scale-up. Among oleaginous microorganisms, yeasts have a few advantages over algae, as they attain higher biomass and lipid productivities. Moreover, biodiesel production from yeasts is of particular interest for countries located at higher latitudes, where the insolation is not as high as in countries near the tropics, wherein autotrophic microalgae may be more suitable. The oleaginous yeast *Rhodosporidium toruloides* NCYC 921 has been widely reported as a potential oil producer. In addition, this species, often called ''pink yeast'', has also been reported as a source of carotenoids of high commercial interest which are used as a natural food colorants and feed additive in aquaculture. The co-extraction of lipids (for biodiesel) and carotenoids with commercial value from the yeast biomass may contribute for the economic sustainability of the overall process. pH is a critical parameter that influences the lipid and carotenoid production by yeasts. The present work studied the effect of different pH on the lipid and carotenoid production by the yeast *Rhodosporidium toruloides* NCYC 921. Multi-parameter flow cytometry was used to understand the yeast mechanism response to different media pH, evaluating the cell membrane integrity, membrane potential, enzymatic activity and reactive oxygen species production, as a way to detect the yeast stress response. It was found that the best pH for cell division and lipid production was 4, while the best pH for carotenoid production was 4.5. However, pH 4 induced a high proportion of cells with permeabilised membrane. These results contradict most of the works that

used this strain for lipid and carotenoid detection, where in medium pH 5.5 was used.

O16 - Isolation of lipase-producing yeasts from olive mill wastewater

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An approximate range of 7 million up to 30 million m^3 olive mill wastewater (OMW) is annually produced in the Mediterranean Basin. The organic matter of OMW consists of a great variety of pollutants, including polysaccharides, sugars, phenolic compounds, tannins, polyalcohols, proteins, organic acids and lipids. Besides being a serious environmental problem, OMW is a potential source of lipase-producing microorganisms and also is a complex medium potentially suitable for lipase production.

Lipases, triacylglycerol hydrolases, are an important group of enzymes with applications in food, dairy, detergent and pharmaceutical industries. Additionally, lipases have an important application in the field of bioenergy. The lipase-catalyzed esterification of vegetable oils has been studied as an environmentally friendly process to produce fatty acid alkyl ester (FAME), which is called biodiesel fuel.

The purpose of the present study is to isolate novel yeast strains from OMW that are capable of producing lipases and to assess the suitability of OMW for lipase production. Sixteen yeast strains were isolated from OMW samples in YMA, PDA and CRBA media and their lipase production capacities were investigated. Screening for lipase activity on tween 20/ tributyrin/ phenol-red agar medium allowed selecting six yeast isolates using Yarrowia lipolytica as positive control. Lipolytic activity of the six isolates was determined in shake-flasks containing (w/v): yeast extract 0.5%, MgSO4 0.1%, KCl 0.1% and olive oil 0.5% as the inducer. Incubation was carried out at 30oC for 216 hours under orbital shaking (180 rpm). The isolate G was the only one able to produce lipase with maximum activity of 0.8 U.L-1 at 96h. For identification of yeast isolate G, DNA was extracted the D1/D2 region of 26S rDNA was amplified with NL1 and NL4 primers for yeast identification purposes. The effect of phenols toxicity on yeast isolate G growing in OMW was investigated. The influence of different NH_4Cl concentrations and pH values on OMW medium growth was studied to improve lipase production by yeast isolate G, according to a statistical design following the Doehlert distribution. Total lipids content was assayed by traditional

gravimetric lipid analysis and fatty acids content were monitored by gas chromatography and flow citometry.Valorisation of OMW appears to be promising as a fermentation medium for the production of lipase activity and isolation of lipase producing yeasts.

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O17 - Pseudozyma yeasts as microbial cell factories for lignocellulosic biorefineries

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Yeasts belonging to Pseudozyma genus are haploid and anamorphic basidiomycetes belonging to the family Ustilaginales [1]. Among the genus Pseudozyma, *P. antarctica* was found to produce industry-relevant extracellular lipases at high levels. In addition, under certain conditions, the genus Pseudozyma synthetizes glycolipids, designated as mannosylerythritol lipids (MEL) [2]. These biosurfactants are recognized as fine chemicals with high-value applications like bioremediation, health care, oil and food processing industries [3,4], and interesting characteristics such as low toxicity, high biodegradability, effectiveness at extreme temperatures or pH, and mild production conditions when compared to chemical surfactants [2,4]. Moreover, their production can reduce the utilization of non-renewable petrochemical resources. MEL is produced by *Pseudozyma* spp. from different substrates, preferably vegetable oils, but also sugars, glycerol or hydrocarbons. However, besides costs associated with downstream processing, the relatively high prices of raw materials currently used in MEL production inhibit its sustainable production at industrial scale.
This work was focused on the utilization of *Pseudozyma* spp. for MEL production from lignocellulosic materials towards cost-effective and sustainable processes. *Pseudozyma antarctica* PYCC 5048T or P. aphidis PYCC 5535T were able to convert pentoses (D-xylose and L-arabinose), D-xylose/D-glucose mixtures, cellulose and xylan into MEL, reaching titres of 1-9 g/l, depending on the strain, substrate and operational conditions used. While different process configurations (separated hydrolysis and fermentation and simultaneous saccharification and

fermentation) were successfully developed for MEL production from cellulosic substrates (e.g. wheat straw) combining commercial enzyme cocktails and *Pseudozyma* spp., the ability to produce own xylanolytic enzymes allowed the direct conversion of xylan into MEL.
The ability of using renewable sugar-based substrates for MEL production contributes for process sustainability at two levels: i) substrate, since lignocellulose is inexpensive, abundant and renewable raw material; ii) downstream processing, since MEL recovery from sugar-based substrates involves a single-step liquid-liquid extraction process, while the use of oil-based substrates requires a solvent intensive and low-yield downstream process. In addition to current industrial applications of *Pseudozyma* spp., it is expected that this work will potentiate the use of these yeasts as relevant cell factories for the production of advanced biofuels and other bio-based chemicals within a biorefinery concept.

REFERENCES: [1] Begerow D, Bauer R, Boekhout T (2000) Mycol Res 104:53-60; [2] Arutchelvi J, Bhaduri S, Uppara P, Doble M (2008) J Ind Microbiol Biotechnol 35:1559-1570; [3] Kitamoto D, Isoda H, Nakahara T (2002) J Biosci Bioeng 94:187-201; [4] Soberón-Chávez G (2010) Springer, New York.

O18 - Acetic acid resistance in *Saccharomyces cerevisiae*: role of the ABC transporter Pdr18, proposed to mediate plasma membrane sterol incorporation.

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Multidrug/multixenobiotic resistance (MDR/MXR) in yeast may be achieved through several mechanisms, in particular those related with the maintenance of reduced intracellular drug/xenobiotic levels. Understanding these mechanisms is crucial to struggle against the negative implications of the MDR/MXR phenomenon in the treatment of tumors and infectious diseases, food preservation, crop protection, as well as to take advantage of the positive outcomes such as the ability of industrial microorganisms to tolerate chemical stresses associated with biotechnological processes [1]. The yeast multidrug resistance ATP-binding cassette transporter Pdr18 was previously found to confer resistance to toxic concentrations of wide range of metabolites, pesticides and di-cations, such as ethanol [2], the herbicides 2,4-D, MCPA and barban, the fungicide mancozeb, and Zn^{2+}, Mn^{2+}, Cu^{2+} and Cd^{2+} [3]. Pdr18 was proposed to

mediate plasma membrane sterol incorporation in yeast and demonstrated to affect plasma membrane potential [3]. The action of Pdr18 in yeast tolerance to 2,4-D, which was found to contribute to reduce [^{14}C]2,4-D intracellular accumulation, was proposed to be indirect, given the observation that 2,4-D exposure deeply affects sterol plasma membrane composition, this effect being much stronger in a *Δpdr18* background [3]. *PDR18* expression was also found to contribute to decreased ^{3}H-ethanol intracellular concentrations and decreased plasma membrane permeabilization of yeast cells challenged with inhibitory ethanol concentrations [2]. This role of Pdr18 under ethanol-induced stress was explored to improve the performance of a *PDR18*-overexpressing strain in very high gravity fermentation [2]. In a recent screening to search for MDR transporters required for acetic acid resistance in yeast, Pdr18 was identified as an important determinant. In response to acetic acid-induced stress, yeast cells were found to exhibit increased *PDR18* transcription levels and the regulatory network behind *PDR18* expression activation by this weak acid is being examined. Additional results reinforce and detail the cellular mechanisms underlying the role of Pdr18 in acetic acid resistance, involving the mechanisms hypothesized before [3].

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SESSION III – Yeast Diversity and Evolution

O19 - Evolution of the mating type locus in tremellaceous yeasts: insights from comparative genomics

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Mating-type loci (MAT) are specialized regions of the fungal genome that determine sexual identity of haploid cells and progression through the sexual cycle. There is considerable interest in the genetic characterization of MAT loci due to their central role in fungal life cycles and their connection to lifestyle and virulence. MAT loci structure has been elucidated for some yeast species belonging to the Tremellales. The human pathogens *Cryptococcus neoformans* and *C. gattii* have a bipolar mating system with a single MAT locus that contains genes encoding homeodomain (HD) transcription factors (Sxi genes),

pheromones precursors (MF) and pheromone receptors (Ste3), as well as other genes encoding proteins that may (Ste12, Ste20) or may not be related to mating. All other tremellaceous yeasts investigated so far have tetrapolar mating systems with two unlinked loci, one contains two divergently transcribed HD genes (HD locus) and the other (P/R locus) the pheromone and pheromone receptor genes as well as Ste12, Ste20 and other genes found in the *C. neoformans* MAT locus. Besides *Cryptococcus neoformans*, *C. gattii* and *Tremella mesenterica*, whole genome sequences have been recently determined for yeast species in different clades within the Tremellales, namely *Cryptococcus flavescens*, *Dioszegia cryoxerica*, *Kwoniella heveanensis*, *K. mangrovensis*, and *Trichosporon asahii*. Access to those genome sequences enabled us to search for the respective MAT loci to determine their structure and to put forward hypotheses on the evolution of these genomic regions. We have also studied a large collection of isolates of the species *C. flavescens* and *C. terrestris* to assess their mating behavior and MAT locus structure. We will discuss our findings, including the presence of distinct MAT locus structures and their correlation with mating behavior, and the conservation of MAT gene order across species for strains with homologous alleles of the P/R locus.

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O20 - A common phylogenetic origin links the DHA2, ARN and GEX transporters: phylogenetic characterization and proposal of the new DAG protein family

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The simultaneous acquisition of resistance to a wide range of structurally and functionally unrelated cytotoxic chemicals is a widespread phenomenon in nature known as Multidrug/Multixenobiotic resistance (MDR/MXR) [1,2]. Following the release of the full genome sequence of *S. cerevisiae*, the similarity analysis of the amino acid sequences encoded in the annotated ORFs led to the separation of the genes encoding multidrug resistance transporters belonging to the Major Facilitator Superfamily (MDR-MFS) into 2 sub-families, depending on whether their protein products contained 12 or 14 transmembrane segments (TMS): the 12-spanner drug:H+ antiporter family 1 (DHA1) and the 14-spanner drug:H+ antiporter family 2 (DHA2) [1,2]. The subsequent phylogenetic analysis of these

protein sequences showed that they can be included into three major clusters, with cluster I comprising the 12-spanner MDR-MFS transporters and clusters II and III comprising the 14-spanner transporters [3]. While cluster II included the DHA2 family proteins, those in cluster III were assigned to the Unknown Major Facilitator (UMF) family [4]. After the demonstration that four UMF family members encoded siderophore transporters and that the other two UMF family members encoded glutathione exchangers (GEX), these proteins were reassigned to the new ARN (also known as the Siderophore-Iron Transporter/SIT family) [5] and GEX families, respectively [6]. In this study, we revisited the evolutionary origins of the 14-spanner MDR-MFS transporters using a comparative genomics approach. The amino acid sequence pairwise similarity network derived from the translated ORFs of 31 strains from 25 hemiascomycetous species, including 10 pathogenic Candida species, was analyzed [7]. The constraining and traversing of this network gathered ARN and GEX family members together with DHA2 transporters, suggesting the existence of a close relationship among these three phylogenetic sub-families [7]. This fact fact led to the re-classification of these 14-spanner major facilitators in a single family, denominated DAG. Gene neighbourhood analysis combined with tree construction methodologies were used to reconstruct the evolutionary history of the gathered 355 full size proteins, and 7 DHA2 gene lineages, 5 ARN gene lineages, and 1 GEX gene lineage were identified [7]. The S. cerevisiae DHA2 proteins Sge1, Azr1, Vba3 and Vba5 co-clustered in a large phylogenetic branch, the *ATR1* and *YMR279C* genes were proposed to be paralogs formed during the Whole Genome Duplication (WGD) whereas the closely related ORF *YOR378W* resides in its own lineage. Homologs of *S. cerevisiae* DHA2 vacuolar proteins Vba1, Vba2 and Vba4 occur widespread in the Hemiascomycetes. Arn1/Arn2 homologs were only found in species belonging to the Saccharomyces complex and are more abundant in the pre-WGD species. Arn4 homologs were only found in sub-telomeric regions of species belonging to the Sacharomyces sensu strictu group (SSSG). Arn3 type siderophore transporters are abundant in the Hemiascomycetes and form an ancient gene lineage extending to the filamentous fungi. Species-to-species lateral transfer was a major evolutionary force affecting the DAG lineages containing the following *S. cerevisiae* genes: *SGE1*, *VBA3*, *VBA5*, *VBA4*, *VBA2*, *GEX1*/*GEX2*, *ARN2* and *YOR378W*. Additional results also suggest that, during the evolutionary transition that originated the Dikarya fungi, selection, radiation and neofunctionalization of the initial ancestral DAG genes gave rise to their biological functions which span the MDR phenomenon, amino acid transport, boron homeostasis, siderophore transport and glutathione exchange.

[1] dos Santos SC, Teixeira MC, Dias PJ, Sá-Correia I (2014) Front. Physiol. 5: 180 [2] Sá-Correia I, dos Santos SC, Teixeira MC, Cabrito TR, Mira NP (2009) Trends Microbiol 17: 22–31 [3] Nelissen B, De Wachter R, Goffeau A (1997) FEMS Microbiol. Rev. 21: 113–134 [4] Paulsen IT, Sliwinski MK, Nelissen B, Goffeau A, Saier MH Jr (1998) FEBS Lett. 430: 116–125 [5] Yun CW, Tiedeman

JS, Moore RE, Philpott CC (2000) J. Biol. Chem. 275: 16354–16359 [6] Dhaoui M, Auchere F, Blaiseau PL, Lesuisse E, Landoulsi A, Camadro JM, Haguenauer-Tsapis R, Belgareh-Touze N (2011) Mol. Biol. Cell 22: 2054–2067 [7] Dias PJ, Sá-Correia I (2013) BMC Genomics 14: 901

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O21 - Elucidating the role of CUG mistranslation in *Debaryomyces hansenii* stress tolerance

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Debaromyces hansenii is normally found in salty environments, being extremely halo- and osmo-tolerant. These features make this yeast extremely attractive for biotechnological purposes. However, *D. hansenii* also takes part of the CTG-clade, meaning that CUG codon is ambiguously decoded as serine and leucine. Conversely to what was initially thought CUG codon is not a rare codon, 65% of *D. hansenii* ORFeome possess at least one CUG codon. Therefore, we wonder whether statistical proteome originated by CUG mistranslation functions as a stress buffering in *D. hansenii*. In order to reveal the biological role of CUG mistranslation in *D. hansenii*, we increased CUG mistranslation levels by tRNA engineering. We tested several abiotic stress conditions, comparing *D. hansenii* strains with increased and wild-type CUG mistranslation levels. Results obtained so far indicate that increased proteome plasticity affects stress response to weak acids and toxic alkali and metallic cations.

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O22 - Experimental evolution of chromosome structure in yeast

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As a unicellular eukaryote, yeast is a powerful model to study the evolution of complex features in the lab. With the well-established methodology of experimental evolution, we can observe populations as they adapt to controlled selective pressures. Moreover, we can keep a frozen fossil record that allows us to replay evolution as required and, with next generation sequencing technology, we can easily access the mutations that are responsible for phenotypic changes. Recently, we used fission yeast (*Schizosaccharomyces pombe*) to study how the order of genes in chromosomes affects fitness during asexual growth. We showed that chromosome rearrangements, like most mutations, can be deleterious or beneficial even when they do not affect the coding sequence of any gene. This implies that, despite their disadvantage during meiosis, some rearranged strains can be maintained because of their mitotic advantage. This observation gives us insight into the short term consequences of this type of chromosome alteration. We are now addressing the impact of chromosome structure on the long-term evolutionary path of populations. Towards that goal we performed two experiments. One was to propagate rearranged strains under asexual reproduction and ask how quickly they evolved in new environments and which genes were responsible for adaptation. We found that chromosome structure can have a significant impact on the evolutionary rate of populations which hints at strong epistatic effects between gene order and new mutations. The second experiment consisted in mixing a wild type with a strain carrying a large inversion and propagate the mix in a life cycle that included sexual and asexual stages. We tested whether the initial meiotic problems caused by the presence of an inversion were still present after 500 generations of evolution. We found two general strategies by which chromosome rearrangements can ameliorate their meiotic defects: avoid mating or suppress recombination. These results indicate that chromosome rearrangements can play an important role as drivers of speciation and chromosome differentiation. We propose a new model for the evolution and maintenance of chromosome structure variants in natural populations. Once a spontaneous rearrangement appears in an otherwise wild type population, it can be maintained for several generations due to its direct effect on fitness. During this time, the rearranged strain can acquire new mutations that either differentiate it further from the wild type, or that compensate for its meiotic problems. The former will lead to strain divergence and speciation while the latter will allow the chromosome variant to be kept in the population and evolve neutrally.

O23 - Global diversity and domestication in the wine and cider yeast *Saccharomyces uvarum*

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For thousands of years *Saccharomyces* yeasts have been used by mankind for the fermentation of foods and beverages. Although *S. cerevisiae* is the predominant species in most wine fermentations, its cryotolerant relative, *S. uvarum*, plays a fundamental role in the fermentation of cider and certain types of wine that are produced at low temperatures. While the global diversity and domestication processes have been studied to some extent in *S. cerevisiae*, until now nothing was known in this respect for *S. uvarum*. In this study we used a population genomics approach to investigate the global phylogeography and domestication fingerprints of *S. uvarum*, using a wide collection of 58 isolates obtained from fermented beverages and from natural environments on five continents. Three main phylogenetic clusters were detected: one grouping all the strains from the Northern Hemisphere (Holarctic) and some strains from South America, a second one grouping exclusively South American isolates and a third more divergent clade clustering the Australasian isolates, suggesting a geographical association between the strains. Additionally, we observed that the South American isolates contain more genetic diversity than that found in the Northern Hemisphere and coalescence analyses suggest that a Patagonian sub-population gave rise to the Northern hemisphere population through a recent bottleneck. Although phylogenetically clustered together, the Northern hemisphere strains from fermentation environments can be distinguished from the wild strains by the presence of multiple and extensive introgressions from its sister species *S. eubayanus*. These introgressions were identified in almost all European strains associated with human-driven fermentations. Gene ontology analyses highlighted several gene categories relevant for wine fermentation as being overrepresented in the introgressed regions. This work elucidated for the first time the global diversity of *S. uvarum* as well as important differences between wild and domesticated strains.

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O24 - Population genomics of *Saccharomyces cerevisiae* in Brazil

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The yeast *Saccharomyces cerevisiae* is employed since ancient times for the fermentation of a myriad of foods and beverages, of which beer, wine and bread and the most emblematic examples. Through the repeated use of the most suitable strains and selection of the most desirable phenotypic traits it is likely that some lineages become domesticated. In addition, recent studies focusing on the natural ecology of wild *Saccharomyces* populations have associated this yeast with temperate oak forests in the Northern hemisphere, in Europe, North America, China and Japan.

However, virtually nothing is known about the natural distribution and domestication processes of *S. cerevisiae* in tropical regions. Here we report on field studies carried out in different ecosystems in Brazil aiming at detecting wild populations of *S. cerevisiae* and on population genomics analyses involving representatives of putatively domesticated lineages from Brazil and neighboring countries, including representatives from cachaça and traditional beverages like chicha.

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SESSION IV – Yeasts and Human Health

O25 - *TLR9* activation dampens the early inflammatory response to *Paracoccidioides brasiliensis*, impacting host survival

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In the last decades, significant advances were accomplished on the study of neglected fungal diseases, mainly in defining the pathogen genetics and host risk factors. Paracoccidioidomycosis, a mycosis caused by *Paracoccidioides* species, is one of these diseases, and it is estimated to affect 10 million individuals in countries from Latin America. Over the last years, host-fungus immunological studies raised important questions, especially on the consequences that the multinucleated nature of *Paracoccidioides brasiliensis* can have on triggering host defence mechanisms. Knowing that this fungus is characterized by its multinucleated nature and that during the infection fungal cell death is likely to result in the release of large amounts of DNA, one could expect the triggering of innate immune mechanisms of the host via Toll-Like Receptor 9 (*TLR9*). Nevertheless, *TLR9* role during *P. brasiliensis* infections was never assessed. We herein demonstrate that activation of this receptor upon recognition of *P. brasiliensis* yeast cells is an event that seems to be crucial in early-times of infection. Lack of this receptor caused the premature death of the hosts (in a mouse model of infection with *P. brasiliensis* yeast cells), associated with signs of organ-pathology and high production of pro-inflammatory cytokines. One possible explanation for this profile can be the abnormal neutrophilia observed in *TLR9*-depleted infected mice. Overall, we show that *TLR9* activation is immuno-protective in early stages of *P. brasiliensis* infections, playing an important role on the development of a controlled cell-mediated response.

O26 - Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease

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Protein aggregation is a common hallmark in neurodegenerative disorders, but is also associated with phenotypic plasticity in a variety of organisms, including yeasts. Alpha-synuclein (aSyn) forms aggregates that are typical of synucleinopathies such as Parkinson's disease (PD), and is phosphorylated at S129, but the significance of phosphorylation in the biology and pathophysiology of the protein is still controversial. Exploring the power of budding yeast, we found phosphorylation reduces aSyn toxicity and the formation of inclusions and oligomeric species. Moreover, S129 phosphorylation modulates aSyn dynamics in inclusions that are larger and show FRAP heterogeneity when phosphorylation is blocked (S129A aSyn). While no colocalization was observed between the inclusions formed by WT or S129A aSyn with subcellular protein quality control compartments such as the "juxtanuclear quality control" compartment (JUNQ), or the "insoluble protein deposit" (IPOD), neither with the P-bodies, both WT or S129A aSyn colocalize with several trafficking markers from ER to plasma membrane. Endocytic trafficking studies shown a deficiency in the delivery of late endosomes carrying aSyn to the vacuole that is more pronounced in the strain expressing S129A aSyn. Blockade of aSyn phosphorylation also compromises its degradation. Upon blockade of aSyn expression, cells were able to clear the inclusions formed by WT aSyn. However, this process was much slower for the inclusions formed by S129A aSyn. Interestingly, whereas the accumulation of WT aSyn led to a marked induction of autophagy, cells expressing the S129A mutant failed to activate this protein quality control pathway. Interestingly, clearance of aSyn inclusions was reduced in cells where phosphorylation of aSyn at S129 was blocked, correlating with deficient autophagy activation. The finding that phosphorylation alters the ability of cells to clear aSyn inclusions provides novel insight into the role phosphorylation may have in synucleinopathies, and

suggests posttranslational modifications might constitute switches cells use to control the aggregation and clearance of key proteins, opening novel avenues for the development of therapeutic strategies for these devastating disorders.

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O27 - Mechanistic insights into polyphenol-mediated protection against alpha-synuclein toxicity

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The budding yeast is a powerful model in the study of the molecular basis of neurodegenerative disorders. Among these, Parkinson's disease (PD) is the most common movement disorder and is associated with the aggregation of alpha-synuclein (aSyn), the main component of Lewy bodies, the typical pathological protein inclusions found in the brains of PD patients. Oxidative stress is also a hallmark of PD and seems to trigger aSyn aggregation. Thus, the development of therapeutic agents for PD is focused either on decreasing the levels of reactive oxygen species (ROS), and/or inhibiting toxic aSyn aggregation. Among phytochemicals, polyphenols are appealing molecules in the context of PD, due to their beneficial effects against oxidative injury and neuroprotective properties. In this study, we analyzed the protective capacity of (poly)phenols extracted from leaves of *Corema album* (Portuguese crowberry), here characterized for the first time, against aSyn aggregation and toxicity, using yeast and mammalian cell models of PD. We observed that *C. album* leaves (poly)phenols prevented H_2O_2- and aSyn-induced toxicity in yeast and in human neuroglioma cells (H4). Using in vitro assays, we found the (poly)phenols inhibited recombinant aSyn fibrilization. Furthermore, we found (poly)phenols reduced aSyn inclusion formation, induced autophagy and reduced the levels of reactive oxygen species in both yeast and H4 models. Altogether, our data suggest that the beneficial

effects of (poly)phenols surpass their free-radical scavenging activity and might constitute potential therapeutic agents by preventing protein aggregation and toxicity in PD and other synucleinopathies.

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O28 - A selective activator of protein kinase Cdelta discovered using a yeast-based assay

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The Protein Kinase C (PKC) is a family of serine/threonine kinases with at least ten isoforms divided into three major subfamilies: classical (cPKCalpha, betaI, betaII and gama), novel (nPKCdelta, épsilon, eta and teta) and atypical (aPKCzeta and lambda\iota) [1]. In recent years, PKC isoforms have emerged as relevant anticancer drug targets. Particularly, PKCdelta has been the focus of intense study due to its well-known pro-apoptotic functions [1]. Therefore, the discovery of selective modulators of individual PKC isoforms has proved to be a promising strategy in anticancer treatment. In spite of this, the number of isoform-selective PKC modulators is still low due to the difficulty to carry out an independent analysis of individual PKC isoforms in mammalian cells. To circumvent this limitation, a yeast-based assay was previously developed by our group for a simpler and faster screening of isoform-selective PKC modulators [2]. In this yeast assay, PKC activators cause growth inhibition in yeast expressing a mammalian PKC isoform, without affecting the growth of control yeast (transformed with the empty vector) [2]. In the present work, *Saccharomyces cerevisiae* cells expressing individual mammalian PKC isoforms were used to study the modulatory activity of four semisynthetic diterpenic compounds (C1 - C4), from iMed. UL, on individual mammalian PKCalpha, betaI, delta, epsilon and zeta. The obtained results showed that C1 had no effect on PKCs, whereas C2 and C3 induced a significant growth inhibition in yeast cells expressing PKCalpha, betaI, delta, épsilon or zeta, without affecting the growth of control yeast. Notably, C4 only inhibited the growth of yeast expressing PKCdelta. The C4-induced growth inhibition in yeast expressing PKCdelta was associated with an increase of DNA fragmentation (apoptosis), without loss of plasma membrane integrity (necrosis). Moreover, C4 was a potent growth inhibitor of human colon

adenocarcinoma (HCT116) tumour cells, with a GI50 (concentration that causes 50% of growth inhibition) value of 0.93 ± 0.03 microM, an effect associated with late apoptosis, but not with cell cycle arrest. Finally, the selectivity and direct activation of PKCdelta by C4 was further confirmed using an in vitro PKC assay. In conclusion, a putative PKCdelta selective activator (C4) was identified in yeast. Further studies are underway in human tumour cell lines to confirm its molecular mechanism of action. Promising pharmacological applications may be therefore envisaged for C4, as the first non-peptide selective activator of PKCdelta, and as a potential anticancer agent.

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REFERENCES: [1] Reyland, M.E. (2009), Frontiers in Bioscience 14, 2386-2399. [2] Coutinho, I. et al. (2009), Biochemical Pharmacology, 78 (5), 449-459.

O29 - Role of *Candida glabrata* Drug:H^+ Antiporters CgAqr1, CgQdr2 and CgTpo3 in antifungal drug resistance: from lab strains to clinical isolates.

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The emergence of antifungal drug resistance among fungal pathogens poses a severe clinical problem. Drug resistance often results from the action of drug efflux pumps from the ATP-Binding Cassette superfamily and the drug:H+ antiporters (DHA) from the Major Facilitator Superfamily (MFS).
The systematic characterization of the DHA transporters from *Candida glabrata* is showing that most of them are indeed found to be implicated in antifungal drug resistance. For example, the expression of *CgQDR2* in *C. glabrata* was found to confer resistance to imidazole antifungal drugs. CgQdr2 was found to decrease the intracellular accumulation of ^{3}H-clotrimazole in *C. glabrata*, and to play a role in the extrusion of this antifungal from pre-loaded cells. *CgQDR2* transcript levels were further seen to be up-regulated in *C. glabrata* cells challenged with clotrimazole and quinidine, in the direct dependency of the CgPdr1 transcription factor [1]. As a second example, CgAqr1 was identified as a determinant of resistance to the antifungal agents flucytosine and, less significantly, clotrimazole. These antifungals were found to act synergistically with acetic acid

against this pathogen. Significantly, CgAqr1 expression was found to reduce the intracellular accumulation of ^{3}H-flucytosine and, to a moderate extent, of ^{3}H-clotrimazole, consistent with a direct role in antifungal drug efflux [2]. Finally, CgTpo3 was found to confer resistance to imidazole and triazole antifungal drugs, catalyzing their extrusion from within preloaded *C. glabrata* cells [3]. Interestingly, CgAqr1 and CgTpo3 were found to confer resistance to acetic acid and polyamines, respectively. Since these compounds easily accumulate to inhibitory levels in the female and male genital tracts, respectively, it is hypothesized that these transporters may play a role in *C. glabrata* persistent colonization in these specific niches. In order to assess the role of these transporters in the clinical acquisition of antifungal resistance, their expression level was inspected in a collection of clinical isolates. A statistically significant correlation between the expression of *CgQDR2*, *CgAQR1* and *CgTPO3* and the levels of azole drug resistance was registered.
Altogether, the results obtained during the ongoing systematic characterization of the DHA transporter family in *C. glabrata* are expected to improve current understanding of multidrug resistance in fungal pathogens and to guide de design of new tools for the diagnosis and treatment of *Candida* infections [4].

[1] Costa et al, 2013, Antimicrob Ag Chemother, 57, 3159; [2] Costa et al, 2013, Front Microbiol, 4, 170. [3] Costa et al, 2014, J Antimicrob Chemother, DOI:10.1093/jac/dku044; [4] Costa et al, 2014, Front Physiol, 5, 197.
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O30 - In Vitro Induced Resistance to Azoles, Echinocandins and Amphotericin B in *Candida glabrata*

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Objectives: *C. glabrata* is the second most prevalent *Candida* species colonizing humans and its incidence in systemic infection is increasing dramatically, possible due to the extensive use of antifungal therapy. The aim of our study was to evaluate the potential in vitro induction of resistance by different azoles, echinocandins and amphotericin B in a *C. glabrata* clinical isolate and its stability followed incubation without antifungal drugs.

Methods: In vitro induction of resistance assays were initiated with a bloodstream isolate of *C. glabrata*, susceptible to all antifungal drugs used in this assay. The strain was incubated daily in fresh YPD containing one of the following antifungal drugs: fluconazole, voriconazole, posaconazole, clotrimazole, caspofungin, micafungin, anidulafungin and amphotericin B. Every 5 days of incubation, Minimal Inhibitory Concentrations (MIC) values were re-determined, according to the CLSI microdilution reference protocol M27-A3 S4. Reversion of the resistance was assessed by the incubation of the resistant strains in medium without antifungal for 30 days.
Results: *C. glabrata* resistance was successfully induced to all antifungals tested. Respecting to azoles, clotrimazole was the antifungal which triggered resistance more promptly, at day 5 of induction. The second was posaconazole at day 20, followed by fluconazole, at day 45, and voriconazole, at day 55. Resistance to caspofungin, micafungin and anidulafungin was developed at day 5 and to amphotericin B at day 50. The acquisition of resistance to one azole always generate cross resistance to all other tested azoles. The same phenomenon was verified after echinocandins exposure. No reversion of resistance was noticed after 30 days of incubation without antifungal pressure.
Conclusions: Antifungal exposure confers antifungal resistance in *C. glabrata*, being echinocandins, clotrimazole and posaconazole the stronger inducers.

O31 - Unveil of the resistance mechanisms induced by voriconazole in *Candida krusei*

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C. krusei is innately resistant to fluconazole (FLC), but not to voriconazole (VRC). A kidney transplant patient was diagnosed with candiduria due to *C. krusei*. The patient was treated with VRC 200mg/2x day for 3 weeks and 5 *C. krusei* isolates were recovered from the urine: isolate CkB.VRC (isolated Before VRC therapy and considered the reference strain in these group for the molecular studies), isolates CkD.VRC9 and CkD.VRC16 (isolated During 9th and 16th days of VRC therapy,) and isolates CkA.VR9 and CkA.VRC17 (isolated After 9th and 17th days of VRC discontinuation). Clinical isolates were all genotyped using

intergenic repeat-PCR (CKRS-1) technique. Additionally, we hypothesized that exposure to suboptimal VRC concentrations could lead to VRC resistance. Therefore, we incubated five independent clinical strains of *C. krusei* susceptible to VRC in brain-heart infusion (BHI) containing VRC 0.001 mg/l, for 30 days. The minimal inhibitory concentration (MIC) to VRC of all C. krusei strains was determined according to CLSI protocol M27-A3 and M27-S4, by the Clinical Laboratory for Standards Institute (CLSI). Different approaches were used in order to uncover the VRC resistance mechanisms: microdilution susceptibility assay to VRC in the presence of FK506 100 mg/l and agar disk diffusion assay, in the presence of VRC 4 mg/l and serial 10-fold dilutions of the efflux pump blocker FK506, impregnated in paper disks ranging from 1 to 1000 mg/l. *ABC1* and *ABC2* genes, encoding for ATP dependent efflux pumps and *ERG11* gene encoding for lanosterol-14α-demethylase target enzyme are associated to azole resistance in *C. krusei*. *ABC1*, *ABC2* and *ERG11* genes expression was quantified by real time PCR. ACT1 gene, encoding for actin was used as the housekeeping gene. The VRC resistant strains presenting an increase higher than 2-fold in gene expression when compared to the respective reference susceptible strains were considered as overexpressing isolates. We also sequenced *C. krusei ERG11* gene since mutations in this gene have been previously described to be associated to azole resistance in *C. albicans*. The sequence obtained from the respective susceptible reference strains was compared to the resistant strain. The MIC to VRC of isolate CkB.VRC increased during therapy from 0.25 to 4.0 mg/l for isolates CkD.VRC9 and CkD.VRC16 and decreased after VRC discontinuation to 0.25 mg/l, represented by isolates CkA.VR9 and CkA.VRC17. We are facing a case of development of resistance in vivo, dependent on the presence of the antifungal drug, since all the strains are genetically related, presenting similar molecular patterns. Considering the five susceptible independent clinical isolates, designated CknD0, after the 30 days of incubation with VRC strains become resistant. The MICs to VRC ranged from 4 to 8 mg/l and the strains were designated CknD30, with n ranging from 1 to 5. In the microdilution assay, FK506 restored the susceptibility of all the VRC-resistant isolates, with the MIC decreasing up to 0.06 mg/l. Additionally in the test disk assay growth inhibition was registered around the disks impregnated with highest concentration of FK506, 1000mg/l in the presence of VRC. The expression of *ABC1* and *ERG11* was increased in the clinical VRC-resistant isolates CkD.VRC9 and CkD.VRC16 and went back to basal level in post-therapy isolates CkA.VR9 and CkA.VRC17. Concerning the in vitro induced strains, different gene expression profiles were obtained: Ck1/2/5D30 strains present a significant increase in *ABC1* gene expression; Ck2D30 is the only strain presenting *ABC2* gene expression higher than *ABC1* gene; strains Ck4/5D30 present a significant increase in ERG11 gene expression. All the susceptible and resistant *C. krusei* strains, presented different heterozygous alterations in *ERG11* sequence: at 642bp (C-T) and 1389bp (T-C)

resulting in synonymous SNPs; heterozygous alterations in Ck2D30 strain at 364bp (G-T; Ala-Ser), in Ck5D30 strain at 418bp (T-C; Tyr-His) resulting in non- synonymous SNPs. Most importantly strain Ck3D30 presented a missense mutation at position418 bp (T-C), translating a different aminoacid Tyr to His. This is quite interesting since this strain was the only one presenting a very low expression level of the resistance genes quantified. In conclusion, all the *C. krusei* isolates recovered from the kidney transplant patient were clonal. The synergistic effect registered between VRC and FK506 and the high gene expression levels for *ABC1* gene indicates a resistance mechanism in these strains associated to efflux pumps activity. The strains presenting a lower *ABC1* gene expression level, present a significant increase in *ERG11* gene and the strain Ck3D30 not presenting an increase in gene expression showed a mutation in *ERG11* gene, therefore this gene is definitely associated to resistance in different *C. krusei* strains either by overproduction and lowered affinity to the azoles drugs.

O32 - The CgHaa1-dependent pathway mediates *Candida glabrata* response and tolerance to acetic acid thereby enhancing colonization of vaginal epithelium

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To successfully colonize the vaginal tract *Candida glabrata* has to cope with various stresses including the presence of acetic acid at a low pH that is produced by the bacteria that co-colonize this niche. The genes/pathways involved in *C. glabrata* tolerance and response to acetic acid are largely unknown, although these are a highly interesting set of novel targets to control vaginal infections caused by this yeast. *Saccharomyces cerevisae* response and tolerance to acetic acid was found to be largely mediated by the ScHaa1 transcription factor [1,2,3]. In this work the involvement of CgHaa1 in *C. glabrata* tolerance and response to acetic acid is demonstrated. Elimination of *CgHAA1* gene from *C. glabrata* genome dramatically increased susceptibility of this pathogenic yeast to acetic acid (30 mM at pH 4.0). Around 140 genes were found to be up-regulated, directly or indirectly, by CgHaa1 in response to acetic acid stress, based on

results of a transcriptomic analysis. Functional clustering of the genes activated by CgHaa1 under acetic acid stress shows an enrichment of those involved in carbohydrate metabolism, transport, cell wall maintenance, regulation of internal pH and nucleic acid processing. At least five of the CgHaa1-regulated genes were found to increase *C. glabrata* tolerance to acetic acid including *CgGAD1*, encoding a glutamate decarboxylase; *CgTPO2/3*, encoding a drug efflux pump of the Major Facilitator Superfamily; *CgYPS1*, encoding a cell wall aspartyl protease; and *CAGL0H04851* and *CAGL0E03740*, encoding two uncharacterized ORFs. Altogether our results are consistent with the concept that the CgHaa1-signalling pathway increases *C. glabrata* tolerance to acetic acid by reducing the internal accumulation of the acid and by up-regulating the activity of the plasma membrane proton pump H^+-ATPase CgPma1, two essential features for a robust weak acid response.

The role exerted by CgHaa1 in the ability of *C. glabrata* to colonize reconstituted vaginal human epithelium (RVHE) in the presence of acetic acid (30 mM at pH 4.0) was also investigated in this work. In the absence of acetic acid wild-type and Δ*CgHaa1* mutant cells were able to colonize RVHE at a similar rate, however, in the presence of acetic acid colonization of the vaginal tissue was markedly reduced in the mutant background. The reduced colonizing capacity of Δ*CgHaa1* mutant cells was correlated with a reduced expression of the adhesin-encoding genes *EPA6*, *EPA7* and *EPA1* and with a lower adhesiveness to the extracellular matrix proteins fibronectin and vitronectin.

[1] Mira NP, Becker J and Sá-Correia I, OMICS:14, 587-601, (2010)
[2] Mira NP, Teixeira MC and Sá-Correia I, OMICS:14, 525-40, (2010)
[3] Mira NP et al., Nucleic Acids Res., 16, 6896-907, (2011)

SESSION V – Yeast Functional Genomics and Bioinformatics

O33 - YEASTRACT-NET: extracting and visualizing transcription regulatory networks in *S. cerevisiae*

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The YEAst Search for Transcriptional Regulators And Consensus Tracking (YEASTRACT - http://yeastract.com) information system is a tool for the analysis and prediction of transcription regulatory associations at the gene and genomic levels in *Saccharomyces cerevisiae*. Since its first release in 2006, YEASTRACT has been extensively used by hundreds of researchers from all over the world and receiving more than 1.500 monthly visits. YEASTRACT currently contains nearly 206.000 regulatory associations between Transcription Factors (TFs) and target genes, including 326 specific DNA binding sites for 113 characterized TFs. The last YEASTRACT update, in June 2013, revisited and annotated all existing regulatory associations with information on the experimental conditions of all associations and their associated type of regulation (activation/repression) [1]. Presently, the regulatory associations between TFs and target genes reported to the user, throughout all the existing functionalities in YEASTRACT, consider either the sets provided by the user or the whole information contained in the YEASTRACT database. The direct consequence of this procedure is that the user: should either have some a priori information about the direct regulatory influence between the TF-target gene pairs under study; or it will receive a flood of indirect regulatory influences from the whole database upon the TF/target genes under study. Here, we propose to improve the algorithms behind the YEASTRACT functionalities in two essential manners. Firstly, we propose to relax the database search, by retrieving all the regulatory association paths between a (set of) TF(s) and a (set of) target gene(s). This means that the system will be able to consider indirect influences that were previously ignored. Secondly, we propose to generalize these searches through the adoption of specific and meaningful search filters helping the user to retrieve the relevant regulatory associations for the particular system under study. Regulatory association networks of infinite length will at the limit be equivalent to consider the whole YEASTRACT information. To overcome this problem, we propose to filter these associations on the maximum length of the path to be considered, since most of the times indirect associations at length bigger than 3-4 will not be relevant. Additionally, profiting from the new annotations on most of the regulatory associations, searches could be filtered also by their environmental conditions, regulation type or evidence code, controlling the degree of confidence with the experimental methodologies used to obtain the data. Finally, new methods will be developed for the visualization of the regulatory associations selected by each functionality. The YEASTRACT system already uses D3.js, a Javascript technology that scales well for the visualization of large regulatory networks. Here, we propose to rely on this proven technology, enriching the interactions between the network nodes with the new information regarding their environmental conditions, regulation type and evidence code.

[1] Teixeira, M.C., Monteiro, P.T., Guerreiro, J.F., Gonçalves, J.P., Mira, N.P., dos Santos, S.C., Cabrito, T., Palma, M., Costa, C., Francisco, A.P., Madeira, S.C., Oliveira, A.L., Freitas, A.T., Sá-Correia, I., Nucleic Acids Research, 42: D161-D166, 2014

O34 - Sequencing and annotation of the genome of the highly acetic acid-tolerant *Zygosaccharomyces bailii*-derived interspecies hybrid strain ISA1307

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The yeast species *Zygosaccharomyces bailii* exhibits a remarkable but poorly understood intrinsic resistance to weak acids used as food preservatives, being the most significant spoilage yeast especially in acidic food products. In this work the nucleotide sequencing and annotation of the genome of the yeast strain ISA1307 strain are reported [1]. This strain was isolated from a continuous sparkling wine production plant and has been used to study different phenotypic traits of the *Z. bailii* species including fructophilicity [2] and extreme tolerance to acetic acid [3,4]. Analysis of ISA1307 genome sequence showed that this strain is, in fact, an interspecies hybrid strain between *Z. bailii* and a closely related species. Around 4,385 duplicated genes (approximately 90% of the total number of predicted genes) and 1155 single-copy genes were predicted based on the annotation of ISA1307 genome sequence. The functional categories that include a higher number of ISA1307 genes are: “Metabolism and generation of energy”, “Protein folding, modification and targeting” and “Biogenesis of cellular components”. The molecular mechanisms underlying relevant physiological traits of the ISA1307 strain and of other strains of *Z. bailii* species, such as nno Crabtree effect, fructophilicity and resistance to weak acid stress, will be discussed, based on the functional annotation of predicted genes. The knowledge of the genome sequence of the ISA1307 strain is expected to contribute to accelerate systems-level understanding of stress resistance mechanisms in *Z. bailii* and to inspire and guide novel biotechnological applications of this yeast species/strain in fermentation processes, given its high resilience to acidic stress. The availability of the ISA1307 genome sequence also paves the way to a better understanding of

the genetic mechanisms underlying the generation and selection of more robust hybrid yeast strains in the stressful environment of wine fermentations.

[1] Mira NP et al., DNA Research, 2014 (DOI: doi:10.1093/dnares/dst058)(in press); [2] Sousa-Dias et al., Microbiology, 142, 1733-8, 1996; [3] Guerreiro J et al., PROTEOMICS, 12, 2303-18, 2012; [4] Sousa MJ et al., Applied and Environmental Microbiology, 62, 3152-7, 1996

O35 - Screening for genes involved in the high acetic acid resistance of the *Zygosaccharomyces bailii*-derived interspecies hybrid strain ISA1307

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Zygosaccharomyces bailii is the most problematic food spoilage yeast species due to its exceptional capacity to resist to high concentrations of weak acids used as fungistatic preservatives at low pH. However, the mechanisms underlying its intrinsic high weak acid resistance remain poorly characterised. The identification, at the genome level, of these mechanisms and of the genes involved in *Z. bailii* resistance to acetic acid was on the focus of this study. For this, a genomic library previously prepared from the highly acetic acid resistant strain ISA1307 [1], an interspecies hybrid between *Z. bailii* and a closely related species [2], formerly considered a *Z. bailii* strain, was screened for genes responsible for acetic acid resistance by transformation of the highly acetic acid susceptible *Saccharomyces cerevisiae* deletion mutant BY4741_Δ*haa1* with the gene encoding the transcription factor Haa1, required for acetic acid resistance, deleted. The expression of 31 different ISA1307 DNA inserts was found to significantly increase the host cell resistance to acetic acid. The identification and in silico analysis of these DNA inserts was based on the recently available genome sequence of ISA1307 [2]. The acetic acid resistance phenotype of the hybrid strain could not be exclusively associated to one of the hybrid strain parental species, since the number of DNA inserts identified from each species was identical. In total, 32 complete plus 33 truncated ORFs were identified as putative determinants of acetic acid resistance. For most of the identified ORFs, with the exception of 7 ORFs, an *S. cerevisiae* gene homologue was found and clustered into functional classes according to their biological function. Based on suitable criteria, the following genes were selected and proposed as strong candidate determinants of resistance to acetic acid in the yeast strain ISA1307: *GYP8*, *END3*, *YOS9*, *WSC4* and *NPL3* (cellular transport, transport facilities and transport routes), *PMT1*, *KTR7* and *LTN1* (protein fate), *TIF3*, *RPL27A*/*RPL27B*

and *RPL1A*/*RPL1B* (protein synthesis), *PCL10* and *ILV3* (carbohydrate and amino acid metabolism, respectively) and *MSN4* (transcription). This study follows the recent release of strain ISA1307 genome sequence [2] and provides valuable indications on the cellular components, pathways and processes that should be targeted in order to diminish *Z. bailii* species resistance to acetic acid and thus to counteract its action as acidic food spoilage yeast.

REFERENCES: [1] Rodrigues F, Zeeman AM, Alves C, Sousa MJ, Steensma HY, Corte-Real M, Leão C, FEMS Yeast Res 2001, 1:67-71. [2] Mira NP, Münsterkötter M, Valada FD, Santos J, Palma M, Roque FC, Guerreiro JF, Rodrigues F, Sousa MJ, Leão C, Güldener U, Sá-Correia I, DNA Research 2014, doi: 10.1093/dnares/dst058.ACKNOWLEDGMENTS: This research was supported by "Fundação para a Ciência e a Tecnologia" (FCT) [contracts PTDC/AGR-ALI/102608/2008 and post-doctoral (MP, SFRH/BPD/73306/2010) and PhD (FCR, SFRH/BD/82226/2011 and JFG, SFRH/BD/80065/2011) grants].

O36 - Yeast protein expression profile during acetic acid-induced apoptosis in the highly resistant food spoilage *Zygosaccharomyces bailii* derived hybrid strain ISA1307

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The molecular mechanisms underlying the global adaptive response to sub-lethal concentrations of acetic acid in the highly acetic acid resistant food spoilage yeast *Zygosaccharomyces bailii* derived hybrid strain ISA1307 were previously examined using a quantitative two-dimensional electrophoresis(2-DE)-based expression proteomics approach [1]. This strain, isolated from a sparkling wine continuous production plant, was formerly considered to belong to the *Z. bailii* species, but it is an hybrid between *Z. bailii* and a closely related species [2]. It has been used in the study of *Z. bailii* physiology, in particular its high tolerance to acetic acid. A proteome-wide analysis of *S. cerevisiae* response to a pro-apoptotic concentration of acetic acid implicated the TOR signaling pathway (involved in yeast response to nutrient availability) in acetic acid-induced death [3]. However, even though acetic acid also induces apoptosis in *Z. bailii* at higher concentrations, the molecular players remain largely unknown [4]. The objective of this work was to get insights into the mechanisms involved in PCD in strain ISA1307 with special emphasis on the processes taking place in the mitochondria that plays a key role in acetic acid-induced PCD in *S. cerevisiae* using a 2-DE

based expression proteomics, following sub-fractionation of mitochondrial proteins [5]. The previous study on the proteomic response to sub-lethal concentrations of acetic acid was severely limited by the lack of ISA1307 genome sequence [1]. During this study, the genome sequence of *Z. bailii* hybrid strain ISA 1307 was obtained and annotated [2] and it was possible to use this information to identify about 92% of the proteins with altered content in acetic acid challenged cells. The proteins identified in this study as being differently expressed in apoptotic cells were clustered into functional groups and suggest the involvement of carbohydrate and energy metabolism, stress response, protein translation, amino acids and nucleotides metabolism, among other processes, in ISA1307 PCD response.

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O37 - Role of Sit4p-dependent protein dephosphorylation in the regulation of mitochondrial function and yeast lifespan

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Aging is characterized by a progressive, generalized impairment of function, resulting in an increased vulnerability to environmental alterations and an increasing risk of disease and death [1]. The study of aging mechanisms is of prime importance to develop new strategies to decrease the incidence of age-related diseases and extend the lifespan. Yeast cells have been used as a tool for identifying the genes and pathways involved in the aging process and stress response. Sit4p, the catalytic subunit of a ceramide-activated serine-threonine protein phosphatase, was identified as a key protein in these processes. *SIT4* deleted cells shows a catabolic derepression in log phase, a high resistance to hydrogen peroxide and increased longevity [2]. The aim of this work is to identify Sit4p target proteins that play a critical role in the regulation of

mitochondrial function and lifespan. We performed a phosphoproteomic analysis of proteins extracted from mitochondrial fractions of WT and *sit4Δ* cells. Proteins were separated by two-dimensional gel electrophoresis and protein phosphorylation was analyzed by Western blot, using an antibody that recognizes phosphorylated residues. The results show that nine proteins, including Por1p (voltage dependent anion-selective channel of the mitochondrial outer membrane) and Qcr2p (subunit 2 of ubiquinol cytochrome-c reductase, complex III), were hyperphosphorylated in *sit4Δ* cells. *POR1* deletion in *sit4Δ* cells decreased growth rate and oxygen consumption but not hydrogen peroxide resistance and chronological lifespan. *QCR2* deletion in *sit4Δ* cells also decreased the growth rate and abolished the oxygen consumption. However, distinctly from *POR1* deletion, absence of Qcr2p decreased the hydrogen peroxide resistance and chronological lifespan of *sit4Δ* mutants. To characterize the impact of Por1p and Qcr2p phosphorylation on the *sit4Δ* phenotypes, Ser or Thr residues phosphorylated in these proteins will be mutated into Ala (phosphoresistant) or Glu (phosphomimetic) residues. Additionally, the potential interaction of these proteins with Sit4p is being evaluated by co-imunoprecipitation. Our data suggest that Sit4p, directly or indirectly, modulate the phosphorylating patterns of mitochondrial proteins. The contribution of these alterations for mitochondrial function and lifespan is under assessment.

REFERENCES: [1] Kirkwood TB (2005) Cell 120:437–447. [2] Barbosa AD, Osorio H, Sims KJ, Almeida T, Alves M, et al. (2011) Mol Microbiol 81: 515–527; ACKNOWLEDGEMENTS: This work was financially supported by FEDER (Fundo Europeu de Desenvolvimento Regional) through the program "Programa Operacional Fatores de Competitividade-COMPETE", and by FCT (Fundação para a Ciência e Tecnologia), through the project FCOMP-01-0124-FEDER-028210. C.P. (PTDC/BBB-BQB/1850/2012) is supported by a FCT fellowship.

O38 - Unveiling wine yeast performance combining phenotypic, metabolic and transcriptomic data

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Currently, pursuing yeast strains that display both a high potential fitness for alcoholic fermentation and a favorable impact on quality is a major goal in the alcoholic beverage industry. This considerable industrial interest has led to many studies characterizing the phenotypic and metabolic traits of commercial yeast populations. In this study, twenty *Saccharomyces cerevisiae* strains from different geographical origins exhibited high phenotypic diversity when their response to nine biotechnologically relevant conditions was examined. Additionally, the

fermentation fitness and metabolic traits of eight selected strains, based on their unique phenotypic profile, were evaluated under two nitrogen regimes. The results obtained revealed phenotypic and metabolic diversity among the commercial wine yeasts and contribute with new findings on the relationship between nitrogen availability, yeast cell growth and sugar utilization. Furthermore the genome-wide transcriptomic analysis performed with phenotypically distinct wine strains provided insights in the molecular and physiological mechanisms underlying the distinctive behavior among strains with respect to fermentation fitness, nitrogen assimilation and specific growth rates.

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O39 - Genome-wide identification of *Saccharomyces cerevisiae* genes required for tolerance to sulphur dioxide

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Sulphur dioxide (SO_2) and its derivatives are widely used as preservatives of food products, beverages and pharmaceutical products. Some yeast species present during winemaking are resilient and it is often required the addition of higher levels of sulphites, near or greater than the legal limits. As for other additives, European Union is limiting these additions and established “sulphite” indication in food products since 2005, giving the increasing awareness that sulphites may induce a range of adverse clinical effects in sensitive individuals. *Saccharomyces cerevisiae* is known to tolerate relatively high concentrations of sulphites. Here we report great wine yeast strain variability in their natural resistance. The mechanisms by which SO_2 exert toxicity in yeast cells remain to be characterized. In this work a chemogenomics approach was used to systematically identify the genes required for tolerance to inhibitory concentrations of SO_2 in *S. cerevisiae*. Over 760 *S. cerevisiae* genes were found to be required for maximal tolerance to SO_2. Functional clustering of these genes revealed enrichment in the functional classes “vacuolar/lysosomal transport”, “regulation of C-compound and carbohydrate metabolism”, “protein targeting, sorting and translocation”,”transcription” and “lipid, fatty acid and isoprenoid metabolism”. Additionally, rho0 yeast cells showed significantly higher sensitivity to SO_2, compared to wild type cells, suggesting that mitochondrial function is required for yeast protection from SO_2. The results emerging from this study can be

explored to the development of more suitable and safer preservation strategies towards the use of lower concentrations of SO_2 not only in wine but also in other food products.

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O40 - Elucidating the role of MRR1 transcription factor mutations in *C. parapsilosis* azole resistance

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The incidence of *C. parapsilosis* has been rised in the last decade. This *Candida* species is mostly isolated from patients with bloodstream infections and its incidence in some countries is equal or exceeds that of *C. albicans*. For the treatment of these infections, azoles are widely used as prophylactic and therapeutic drugs. However, *C. parapsilosis* clinical azole resistance has been increasing in the last decade and little is known about the antifungal resistance mechanisms involved in the development of such resistance. A recent study describing the transcriptome alterations triggered by azole exposure and consequent development of resistance shed some light over this matter. Voriconazole (VRC) resistance mechanisms were associated with *MDR1* (encoding for a multidrug efflux pump) and its transcription factor *MRR1* overexpression. Furthermore, a mutation in the *MRR1* (A to C in the 2619 position of the nucleotide sequence) which resulted an alteration in polypeptide chain for lysine to asparagine, K873N, was firstly reported. To elucidate whether this mutation corresponded to a gain of function, MRR1_RVRC mutated gene was integrated in its native locus in a double deleted strain, Δmrr1/Δmrr1. The MRR1_RVRC complemented strain changed azole susceptibility profile, from susceptible to resistant, and also displayed an up regulation of *MRR1* and *MDR1* genes. Thus, it was shown that K873N found in *MRR1* transcription factor is a gain of function mutation.

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POSTER SESSIONS

SESSION I – Yeast Physiology and Genetics

P1 - Evaluation of impact of methanol concentration and low temperatures of culture on oxidative stress in genetically modified *Pichia pastoris*.

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Pichia pastoris is an excellent system of expression for production of recombinant proteins. The methanol used as inductor for this production has been related with formation of reactive oxygen species (ROS) during metabolization in yeast peroxisomes, otherwise the increase of temperature could also generate higher levels of ROS. The main ROS generated during the metabolization of methanol is hydrogen peroxide (H_2O_2) and the exposition of cellular membranes to ROS could induce oxidative cellular damage as lipid peroxidation. The objective of this work is to evaluate the conditions of culture, temperature and concentration of methanol on the levels of intracellular H_2O_2 and evaluate the effects on lipid peroxidation of yeast membranes during the production of scFv anti-LDL (-). Were evaluated five conditions of culture: 14°C-1% (v/v) methanol (m), 14°C- 2% (v/v) m, 18°C-1.5% (v/v) m, 22°C–1% (v/v) m and 22°C–2% (v/v) m. H_2O_2 was evaluated using the fluorescent dye 2´,7´Dichlorofluorescein Diacetate (DCFH-DA) by flow cytometry and lipid peroxidation was measured spectrophotometrically the level of thiobarbituric acid reactive species (TBARS). The results to indicate that level of H2O2 increased at low temperature and concentration of methanol (14°C and 1% (v/v) methanol), whereas that the levels of lipid peroxidation of all conditions studied decreased significantly. In conclusion, the culture of genetically modified *Pichia pastoris* at low temperature and concentration of methanol induces an increment of intracellular ROS levels, but it does not produce lipid peroxidation. Moreover, the use of low temperatures of culture during induction phase prevents oxidative damage to cell membranes.

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P2 - Evaluation of different methods of cell lysate to improve the analysis of LDH enzyme activity in yeast *Pichia pastoris*

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It is well known that yeast *P. pastoris* has a cell wall that must be penetrated in order to make the analysis of their molecules. Lysis techniques help to break the cell wall and release the cytoplasm. In mechanical lysis to release the cytoplasm have been used as micro-knife methods, poly-dimethylsiloxane (PDMS) membrane, ultra-sonication and irradiation beam laser while the chemical analysis is another important sample preparation method. This can be accomplished using buffers and lytic agents such as ammonium chloride, SDS, lysozyme, chaotropic salts, β-mercaptoethanol, and Triton-X4 that maintain the structure and function of the protein. The objective of this trial was determine the best enzymatic activity of LDH comparing different lysis techniques with glass beads and methanol. To carry out the measurement of enzyme activity were used samples which are held at -80°C which were centrifuged twice at 10,000 g for 5 min at 4°C to remove traces of medium and glycerol, then to restore the original volume of the sample is added 50 mM buffer potassium phosphate (pH 7.4). The Lactate Dehydrogenase Activity Kit (Cat . K726 -500, Biovision, USA) was used to evaluate two types of lysing methods: chemical and mechanical. The chemical method involved methanol at various concentrations, and the mechanical method involved the use of glass beads of 0.45 mm, and the use in combination of both methods, all of which correspond to: lysis with glass beads for 5 cycles (each cycle corresponds to 1 min vortexing with pearls inside the sample tube , and then placing the tube containing sample and beads for 1 min on ice), lysis with glass beads for 10 cycles, lysis with glass beads for 15 cycles, lysis with 100% methanol for 30 seconds at a final concentration of the reagent of 75% (v/v), lysis with 100% methanol for 60 seconds at a final concentration of the reagent of 75% (v/v), lysis with 100% methanol for 90 seconds at a final concentration of the reagent of 75% (v/v), lysis with 100% methanol for 120 seconds in a final concentration of the reagent of 75% (v/v), lysis with 100% methanol (final concentration of the reagent of 70% (v/v) and glass beads for 2 cycles and, lysis with 100% methanol (final concentration of reagent 100% (v/v) and glass beads for 1 cycle. The results indicate that using beads in different cycles is not very

effective in spite of these methods are found to be aggressive with the proteins of interest, mainly due to heat generation. Regarding the use of methanol, organic solvent which dissolves the cell wall and also the destabilization of this, has lower values for enzymatic activity in comparison with using only beads. However, the combination of both methods achieved a similar result to that obtained in the positive control, thereby increasing the activity analysis of the samples. In conclusion, the use of a mechanism that allows the cell wall rupture (methanol) and a method for the disintegration of the cell membrane in a sufficient amount of cycles that allow release of entire enzyme content without the consequence of the disintegration of these excess heat (glass beads for 2 cycles) allow obtaining higher values of activity in the samples.

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P3 - Regulation of the inositol transporters in *Saccharomyces cerevisiae* by hydrogen peroxide

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Regulation of the inositol transporters in *Saccharomyces cerevisiae* by hydrogen peroxide. Inositol is a precursor of several membrane phospholipids in yeast and plays a key role in the regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*. Many genes involved in phospholipid biosynthesis are regulated by inositol via the UASINO sequence, which binds the Ino2p-Ino4p DNA binding factors, which are regulated by Opi1p, a negative regulator of transcription (1). The two major routes of inositol supply in yeast are its biosynthesis from glucose-6-phosphate, and uptake from the growth medium, mediated by two inositol permeases, Itr1p and Itr2p, encoded by the *ITR1* and *ITR2* genes, respectively (2). In the yeast *S. cerevisiae*, the rate of hydrogen peroxide (H_2O_2) diffusion through the plasma membrane decreases during adaptation to H_2O_2 (exposure to low doses of H_2O_2), rendering cells more resistant to further exposure to lethal doses of H_2O_2 (3). This lower permeability is associated with an altered lipid profile of the plasma membrane and fatty acid levels (4). In these conditions, different genes containing the UASINO sequence are repressed due to the activation of the repressor Opi1p. The activation of Opi1p, corresponding to its translocation from the endoplasmic reticulum to the nucleus, may occur due to either an increase or a decrease of inositol levels (5). We have already showed that intracellular inositol levels in yeast were decreased after exposure to H_2O_2 (6). With the present work we aimed at understanding the molecular mechanism by which H_2O_2 leads to decreased cellular inositol levels. When *S. cerevisiae* cells are treated with H_2O_2

in adaptation conditions, we observed an accumulation of the inositol transporter Itr1p in the plasma membrane, concomitantly with an inhibition of the internalization of this transporter. On the contrary H_2O_2 does not affect the levels of Itr2p. The hypothesis that H_2O_2 oxidizes Itr1p, affecting its endocytosis in *S. cerevisiae* cells will be tested.

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P4 - Redox regulation of the transcription factor Opi1p by hydrogen peroxide in *Saccharomyces cerevisiae*

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Adaptation to hydrogen peroxide (H_2O_2) in the yeast *Saccharomyces cerevisiae* decreases the permeability of the plasma membrane and changes membrane composition and dynamics [1, 2]. During adaptation to H_2O_2 several genes that contain the regulatory element UASINO, and which codify for enzymes involved in phospholipid and fatty acid metabolism, are repressed. This repression is due to the translocation into the nucleus of the endoplasmic reticulum bound transcription factor Opi1p [3]. This H_2O_2-induced Opi1p translocation does not involve known mechanisms [4]. In the last years it has been established that most classical pathways of transcription factor activation are redox modulated, depend critically on oxidant signalling, and that H_2O_2 is a key molecule in these processes [5]. Redox regulation by H_2O_2 involves oxidation of critical sulfhydryl groups in proteins acting as sensors. The control of H_2O_2 intracellular concentration is made by several enzymes, namely catalases and thiol peroxidases such as peroxiredoxins (Prxs) and glutathione peroxidases (GPxs). Both types of thiol peroxidases have been implicated as sensors and probably have a role in the transfer oxidative signals to the signalling proteins and in the regulation of transcription [6]. The main objective of this study was to find whether Opi1p is oxidized by H_2O_2 leading to translocation. Also, we aimed at finding whether thiol peroxidases and thioredoxins are involved in the redox regulation of Opi1p. *Saccharomyces cerevisiae* cells were transformed with plasmid pTL212 to obtain cells expressing *OPI1-GFP*. Cells were adapted using 150 µM steady-state H_2O_2

and Opi1p oxidation was determined by Western blot. The effect of the deletion of several genes coding for glutathione peroxidases and for thioredoxins on H_2O_2-dependent Opi1p translocation was also studied by fluorescence microscopy. We found that Opi1p was oxidized during adaptation to H_2O_2. We also found that no H_2O_2-dependent Opi1p translocation occurred in gpx1delta cells and that Opi1p translocation was delayed in gpx3delta cells when compared to wt cells. This indicates that Opi1p is under redox regulation by H_2O_2 and that both Gpx1p and Gpx3p have differential roles in H_2O_2-induced redox signalling leading to Opi1p translocation.

REFERENCES: [1]. M. R. Branco, H. S. Marinho, L. Cyrne, F. Antunes, J. Biol. Chem. 279, 6501 (2004). [2]. N. Pedroso et al., Free Radic. Biol. Med. 46, 289 (2009). [3]. A. Cepeda, MSc thesis, FCUL (2010). [4]. B. P. Young et al., Science 329, 1085 (2010). [5]. H.S. Marinho, C. Real, L. Cyrne, H. Soares, F. Antunes, Redox Biol., 2, 535-562 (2014) [6]. D. E. Fomenko et al., Proc. Natl. Acad. Sci. U. S. A 108, 2729 (2011); ACKNOWLEDGEMENTS: FCT (grants PTDC/QUI-BIQ/104311/2008, PEst-OE/QUI/UI0612/2013); Carolina Camelo was a recipient of a grant from Universidade de Lisboa/Fundação Amadeu Dias. Prof. Tim Levine, (DCB, Institute of Ophthalmology, London, UK) for the kind gift of plasmid pTL212. Prof. Enrique Herrero, (University of Lleida, Spain) for the kind gift of wt and trx1deltatrx2delta strains with the W3O3 background.

P5 - Antimicrobial properties of GAPDH-derived AMPs secreted by *Saccharomyces cerevisiae* and death-inducing mechanisms (apoptosis/necrosis)

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Recently, we found (Branco et al, 2014) that *Saccharomyces cerevisiae* secretes antimicrobial peptides (AMPs) during alcoholic fermentation that are derived from the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and active against several wine-related yeasts (e.g. *Hanseniaspora guilliermondii*) and bacteria (e.g. *Oenococcus oeni*). In the present study we characterised the antimicrobial properties (e.g. MIC and IC50) of the "native" AMPs (i.e. purified from *S. cerevisiae* supernatants) and those of chemically synthesised analogues (VSWYDNEYGYSTR and ISWYDNEYGYSAR). Results showed that the antimicrobial ability of the "native" AMPs is significantly higher than that of synthetic analogues. In addition, the antimicrobial activity of the GAPDH-derived AMPs seems to depend on the complementary action of two peptides and on their relative proportion. The death mechanisms (apoptosis/necrosis) induced by these AMPs (both native and synthetic analogues) on sensitive yeast cells were also investigated by assessing molecular processes that are typical of death by apoptosis (Reiter et al., 2005),

namely: DNA fragmentation (Tunnel assay), chromatin condensation (DAPI staining), phosphatidylserine exposure at the surface of the cytoplasmatic membrane (Annexin V staining) and membrane integrity (propidium iodide staining). Results indicate that both the native AMPs and synthetic analogues induce apoptosis in sensitive yeast cells.

REFERENCES: Branco P, Francisco D, Chambon C, Hébraud M, Arneborg N, Almeida MG, Caldeira J., Albergaria H (2014) Appl. Microbiol. Biotechnol 98: 843-853. Reiter J, Herker E, Madeo F, Schmitt MJ (2005) J Cell Biol 168: 353-358; ACKNOWLEDGEMENTS: This work was financed by FEDER through POFC-COMPETE and by national funds from Fundação para a Ciência e Tecnologia, project FCOMP-01-0124-FEDER-014055. Patrícia Branco is the recipient of a PhD fellowship (SFRH/BD/89673/2012) funded by FCT. Portugal.

P6 - The role of ammonium and its interplay with amino acids in the modulation of *Saccharomyces cerevisiae* survival during aging in culture medium

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The composition of the culture medium can modulate chronological lifespan (CLS), and therefore, culturing cells in different media leads to differences in CLS. Manipulation of several single components of the culture medium is known to extend CLS, such as reducing glucose concentration (known as caloric restriction-CR) or manipulating the supply of amino acids. In previous work, we showed that ammonium ($NH4^+$), a commonly used nitrogen source, is able to regulating CLS of *Saccharomyces cerevisiae*. We have shown that cells starved for auxotrophic-complementing amino acids and aged in water are particularly sensitive to ammonium-induced cell death, this process being mediated through the regulation of the evolutionary conserved pathways PKA, TOR and SCH9 and accompanied by an initial apoptotic cell death followed by a fast secondary necrosis. We also showed that $NH4^+$ detrimental effects on the CLS of *S. cerevisiae* BY4742 strain could similarly be observed in culture medium. To further assess the role of ammonium in the modulation of CLS in culture medium, namely its role in the previously described CLS shortening induced by amino acid restriction, we accessed viability of cells cultured and aged in medium supplemented with low and high auxotrophy-complementing amino acid concentrations and in the presence or absence of ammonium. The results showed that cells grown with low concentrations of auxotrophy-complementing amino

acids and ammonium lost viability very fast displaying a very short CLS as previously described, whereas in medium without ammonium supplementation, the decrease in cell death was considerably less pronounced. In fact, cells aged in this last condition presented a CLS that was only slightly shorter that the ones observed for high concentrations of auxotrophy-complementing amino acids, either in the presence or absence of ammonium. These results show that in the absence of ammonium no significant difference in CLS is observed between the cells cultured with high or low auxotrophy-complementing amino acids concentrations, thus suggesting that the decrease of CLS generally observed in cultures under auxotrophy-complementing amino acids restriction, is mainly due to the presence of ammonium in the culture medium. Similar results were observed either for unbuffered medium or for medium buffered to pH 6.0 or 3.4. In summary, our data indicate the CLS shortening effect induced by amino acid restriction can be reverted by removing ammonium from the aging medium. Further studies are now necessary to clarify the interconnection in the regulation of ammonium and amino acids metabolism in CLS. Since NH4+-induced cell death is involved in different human disorders that are accompanied by hyperammonemia, our results may also afford new insights into the understanding of the cell molecular bases triggering cell death in such pathologies.

P7 - Mitochondria involvement in human lactoferrin-induced programmed cell death in *Saccharomyces cerevisiae*

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Human lactoferrin (hLf) is an 80 kDa non hemic iron-binding glycoprotein produced by mucosal epithelial cells, found in most exocrine fluids, with particular abundance in milk and colostrum. hLf exhibits different biological activities, namely as an antifungal agent (1). It was previously shown by our group that the antifungal activity of lactoferrin (hLf) against *Candida albicans* relies on the ability of this protein to induce programmed cell death (PCD) associated with different apoptotic markers, including phosphatidylserine externalization, nuclear chromatin condensation, DNA degradation, and involving mitochondrial dysfunctions such as accumulation of reactive oxygen

species (ROS) and changes in mitochondrial membrane polarization (2). In order to gain deeper understanding of the mechanisms underlying hLf-induced apoptosis, we choose to characterize this cell death process in *Saccharomyces cerevisiae*, which is a more genetically amenable yeast species and the most well characterized species in the yeast apoptosis field. Our results indicate that hLf induces loss of cell viability associated with nuclear chromatin condensation and preservation of plasma membrane integrity. The lethal effect of hLf is inhibited by cicloheximide and by treating cells in the absence of glucose, in the presence of 2-deoxyglucose or inhibitors of respiratory chain complexes. hLf also caused depolarization of the plasma membrane and mitochondrial dysfunction associated with mitochondrial hyperpolarization and ROS accumulation. Overexpression of the anti-apoptotic protein Bcl-XL or pre-incubation with N-acetyl cysteine reduced the intracellular level of ROS and increased resistance to hLf, confirming it is a mitochondrial ROS-mediated cell death process. Mutants deficient in the ATP synthase complex, namely in the assembly factor Atp10p and Atp2p subunit, were more resistant to death induced by hLf and, accordingly, co-incubation with the oxidative phosphorylation inhibitor oligomycin increased resistance to hLf, indicating mitochondrial energetic metabolism plays a key role in the killing effect of hLf. Mitochondrial involvement was further reinforced by the higher survival of cells lacking the yeast apoptosis inducing factor Aif1p and the heme lyase Cyc3p, required for binding of the heme group to apocytochrome c. Yeast metacaspase Yca1p is involved in hLf-induced apoptosis, and consistently leads to caspase activation in hLf-treated cells. As a whole, the results show that, like C. albicans, S. cerevisiae commits to an active mitochondrial apoptotic death in response to hLf. This study also provides new insights into a detailed understanding at the molecular level of hLf cytotoxicity, which may allow the design of new strategies to overcome the emergence of resistance of clinically relevant fungi to conventional antifungals.

REFERENCES: 1.Farnaud,S and R W Evans (2003) Mol Immunol 40:395–405. 2.Andrés M T et al. (2008) Antimicrob Agents and Chemother 52:4081–4088

P8 - Molecular characterization of new Hxt hexose transporters from *Zygosaccharomyces rouxii* CBS 732T

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Most sugar transporters belong to the Sugar Porter family, that includes facilitators (like the *Saccharomyces cerevisiae* ScHxt transporters) and sugar/H^+ symporters (like the *Saccharomyces pastorianus* SpaFsy1). More recently, a new

family of sugar transporters, the Ffz proteins, was described, phylogenetically unrelated to the Sugar Porter family, and phylogenetically closer to drug/H^+ antiporters from the DHA1 family. *Zygosaccharomyces rouxii* is a fructophilic yeast and is one of the most important food spoilage yeasts. Currently, only three hexose transporters have been characterized in this yeast, the fructose/H+ symporter ZrFsy1 and the fructose facilitators ZrFfz1 and ZrFfz2 (this last one also transports glucose). In the genome of *Z. rouxii* CBS 732T there are other five genes that encode putative protein sequences with high homology with hexose transporters from *S. cerevisiae* and *Kluyveromyces lactis*. In the present work, we intend to characterize the above mentioned *Z. rouxii* CBS 732T putative hexose transportes in terms of specificity and affinity to the substrate, as well as to verify whether they are sugar/H^+ symport or facilitated diffusion systems. All genes were cloned in a *S. cerevisiae* hxt-null strain (strain without native hexose transporters) using a plasmid with low number of copies, expressing the genes under a strong and constitutive promotor (TEF). Drop-tests in different carbon sources were used to evaluate the substrate specificity and affinity of cloned sequences and the existence of H^+ movements associated with initial sugar uptake was assessed with a pH meter. Growth assays in liquid media were also performed. So far, one of the sequences cloned encodes a high affinity transporter of fructose/glucose/mannose by a mechanism of facilitated diffusion and three of the other sequences encode fructose/glucose/mannose/galactose transporters, presumably with intermediate and low affinity. Overall, this work demonstrates that *Z. rouxii* has at least four other functional hexose transporters, similar to *S. cerevisiae* Hxts and *K. lactis* glucose transporter Hgt1.

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P9 – Yap1-mediated repression of the yeast low affinity iron transporter gene, *FET4*, confers cadmium tolerance

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Cadmium is a well-known mutagenic metal, which can enter cells via non specific metal transporters, causing several cellular damages and eventually leading to death. In the yeast *S. cerevisiae*, the transcription factor Yap1 plays a determinant role in the regulation of several genes involved in metal stress response. Recently we have shown that Yap1 negatively regulates *FET4* gene,

encoding a low affinity iron transporter [1]. In the present work we study the relevance of this repression in cell tolerance to cadmium. Our results indicate that yap1 mutant exhibits increased *FET4* protein and mRNA levels compared to the wild-type strain. This data correlates well with the increased intracellular levels of cadmium observed in the mutant, as measured by ICP-AES. These results suggest that Yap1 repression of *FET4* prevents cadmium uptake. Nevertheless, Yap1 is not a direct repressor of this gene, as no Yap1 consensus site was found in its promoter. Our previous microarray data suggested that Yap1 regulates *ROX1* expression, a well known repressor of *FET4*. Using Chromatin-Immuno Precipitation and Real-Time PCR approaches, we show that Yap1 directly regulates *ROX1*, which in turn represses *FET4*. After cadmium induction we observed an abrupt decrease of *FET4* mRNA levels in the wild-type and yap1 mutant strains. We also show that *FET4* transcript stability after stress relies on the 5'-3' exoribonuclease Xrn1. Together, our results highlight the role of Yap1 in mediating cadmium tolerance revealing a new route for cell protection.

[1] C Pimentel., SM Caetano et al., BBA 2014, 1840, 1977-1986.

P10 - A novel mechanism conferring tolerance to iron overload in yeast

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Unlike vertebrates, but similar to plants, the yeast cell vacuoles function as iron reservoirs. Ccc1 is the vacuolar transporter that mediates iron storage in the yeast *Saccharomyces cerevisiae*. In a high-Fe milieu, *CCC1* deletion is lethal and the transcription factor Yap5 regulates its expression. However, we have previously demonstrated that Yap5-mediated regulation of *CCC1* is not essential for cells to overcome iron overload[1]. Furthermore, here we show that Yap5 is degraded in the presence of iron. These new findings bring forward the hypothesis that another yet unidentified factor is regulating iron storage in yeast. As a first attempt to identify this factor, we have generated several sequential deletions extending from the promoter region into the open reading frame (ORF) of the *CCC1* gene. The resulting constructs were next used to transform a Δ*ccc1* mutant strain and the respective sensitivity towards high iron was assayed. We found that *CCC1* promoter deletions into the ORF, were able to rescue the Δ*ccc1* sensitivity to iron overload. Interestingly, Western blot analysis of HA tagged version of *CCC1* clearly indicates the presence of a small form of the protein. Together these results strongly suggest that a mechanism involving an alternative

transcription start site of *CCC1* possibly regulates yeast response to iron overload conditions. Overall, this work provides further evidence that cells avoid iron overload by using multiple pathways.

[1]C Pimentel *et al.* PLoS One (2012), 7(5): e37434

P11 - Functional analysis of AcpA, a short-chain monocarboxylic acid transporter in *Aspergillus nidulans*

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In *Aspergillus nidulans*, AcpA is a high affinity acetate transporter, essential for the use of acetate as sole carbon source [1]. The orthologue of AcpA in the yeast *Saccharomyces cerevisiae* is Ady2 which is responsible for the uptake of acetate, propionate and formate in symport with protons [2,3]. This protein is also putatively involved in ammonia export [4]. In this work, we provide direct physiological evidence that AcpA is active from the onset of conidiospore germination, peaking at the time of germ tube emergence, and dropping to low basal levels in germlings and young mycelia. AcpA is not subject to either carbon or nitrogen catabolite repression, nor to inactivation at a post-transcriptional level. The specificity of AcpA was determined by measuring relative inhibition constants of short-chain monocarboxylic acids on the transport of acetate. Benzoate, formate, butyrate and propionate reduced AcpA-mediated acetate transport with apparent inhibition constants (Ki) of 16.89 ± 2.12, 9.25 ± 1.01, 12.06 ± 3.29 and 1.44 ± 0.13 mM, respectively. We also show that although ammonia moderately stimulates AcpA-mediated acetate uptake, AcpA is not involved in ammonia export, as hypothesised for its *S. cerevisiae* homologue. Our results confirm that AcpA is a true orthologue of the *S. cerevisiae* Ady2 permease, which has been shown to be an acetate-propionate-formate/ H^+ symporter.

[1] Robellet, X., Flipphi, M., Pégot, S., Maccabe, A.P., Vélot, C. (2008). Biochem J 412: 485–93 [2] Casal, M., Cardoso, H., Leão, C. (1996). Microbiology 142 (Pt 6: 1385–90 [3] Paiva, S., Devaux, F., Barbosa, S., Jacq, C., Casal, M. (2004). Yeast 21: 201–10 [4] Palková, Z., Devaux, F., Icicová, M., Mináriková, L., Crom, S. Le, Jacq, C. (2002). Mol Biol Cell 13: 3901–14

P12 - Mechanistic insights into tolerance of vaginal *Candida glabrata* isolates to acetic acid

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Candida glabrata is a normal commensal of the human genitourinary (GI) tract. Under certain conditions the commensal colonization caused by *C. glabrata* can progress to mucocutaneous infections and, in more serious cases, to invasive candidiasis, a life-threatening disease in which the yeasts cross the bloodstream and may colonize any major organ. To successfully colonize the acidic vaginal (pH~4.2) tract *C. glabrata* needs to adapt to multiple environmental insults including the presence of acetic acid which is produced, together with other organic acids, by the co-colonizing bacterial flora. Little is known on the genes/pathways underlying *C. glabrata* ability to tolerate acetic acid at a low pH, although these represent a highly interesting set of targets that can be used for the development of novel strategies for the treatment of candidiasis. The objective of this work was to obtain mechanistic insights into the adaptive responses used by *C. glabrata* clinical isolates to cope with acetic acid at low pH. For this a cohort of vaginal *C. glabrata* clinical isolates was screened for their tolerance to 60-80 mM acetic acid (at pH 4.0). Tolerance of a cohort of *C. glabrata* isolates collected from the gastrointestinal (GI) tract (a niche that has a pH between 6.4-11) was also assessed as well as tolerance of the laboratory strains CBS138 and BG2. The results obtained demonstrate that despite the inter-strain variability observed, in general, the vaginal isolates are significantly more tolerant to acetic acid than the isolates recovered from the GI tract or the laboratory strains. The extreme tolerance of vaginal isolates to weak acid stress did not correlate with a generalized resilience to stress and, in particular, the isolates found to be more tolerant to acetic acid were not more tolerant to other environmental stressors. Vaginal *C. glabrata* isolates were found to be more tolerant to acetic acid than to butyric or propionic acids, although equally toxic concentrations of the different weak acids (concentrations that induced similar lag phases in the laboratory strain CBS138) were used. Altogether these observations suggest that during colonization of the vaginal tract *C. glabrata* evolves dedicated adaptive responses to cope with acetic acid stress. To unravel some of these adaptive responses, physiological traits described to be relevant for a robust response to weak acid stress (such as control of internal pH and capacity to reduce the internal concentration of the acid) were compared in the highly acetic acid-tolerant clinical isolates FFUL99 and FFUL216F, in the moderately susceptible isolate

FFUL281F and in the susceptible CBS138 strain. Results obtained showed that when suddenly exposed to acetic acid stress, the highly tolerant FFUL99 and FFUL216F isolates accumulate less acetic acid than the more susceptible strains suggesting that they have evolved modifications of the cell envelope that lead to reduced cell permeability to the acid. The higher tolerance to acetic acid of the FFUL99F and FFUL216F isolates also correlated with a higher rate of glucose consumption in the presence of acetic acid, in particular when compared with the susceptible laboratory strains.

SESSION II – Yeasts and Energy, Food and Environmental Biotechnology

P13 - Xylitol production by D-xylose assimilating yeasts isolated from sugarcane bagasse and filter pie of Brazilian plants ethanol.

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The use of lignocellulosic residues has been viewed as a reasonable alternative for energy generation and chemicals because of the wide availability of these materials. In the context of biorefinery, lignocellulosic biomass can be used as feedstock for the generation of different commercially valuable products such as xylitol and second generation ethanol. The aims of this study were to isolate, identify and select yeast capable to ferment D -xylose for the possible use of these microorganisms in processes to producing xylitol from hemicellulosic hydrolyzate of sugarcane bagasse. Four hundred and fifty yeasts were obtained from 100 samples of sugarcane bagasse and 60 pie filters samples collected in the alcohol plants in Minas Gerais, São Paulo and Paraíba states. Twenty-four known yeast species were isolated, and three possible new species. *Candida tropicalis* was the most frequently isolated species. *Candida tropicalis* was the most frequently isolated species, followed by *Trichosporon mycotoxinivorans* and *Candida tartarivorans*. Nine other genera were obtained, represented by species usually found in plant materials. Of the isolates, strains of *C. tropicalis*, *Lindnera bimundalis*, *Sugiyamaella smithiae* and *Zygoascus meyerae* showed production of xylitol from D-xylose fermentation in test tubes with 2 ml of medium. In xylose fermentation assays on bench scale, these isolates produced xylitol the main

product of fermentation. In tests of hemicellulosic hydrolyzate fermentation of sugarcane bagasse into flasks containing 100 ml of medium in a bench scale, *L. bimundalis* UFMG BX21 produced high concentrations of xylitol. However, in the assay hydrolyzate fermentation in a bioreactor containing 1.5 liters of medium, this strain did not reproduce similar resulats, although the production of xylitol has been checked. The results of this work show the biotechnological potential of microorganisms isolated from waste like bagasse. The use of these microorganisms in the conversion of D-xylose to xylitol by the fermentation of hemicellulose hydrolysates derived from plant biomass can be possible by optimization of fermentation conditions in future studies.

P14 - Expression of selected genes from *Zygosaccharomyces bailii* in *S. cerevisiae*. Effect on resistance to food preservatives

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From an ecological point of view, food commodities are considered a perfect habitat for microorganisms in general. Yeasts in particular play a central role in the deterioration of food products often creating negative impacts in the food and beverage industries. Specific properties of *Zygosaccharomyces bailii* have earned it the title of one of the most problematic species in this regard. In particular, its ability to proliferate in the presence of weak acids, ranging from acetic to benzoic and sorbic acids at high concentrations (usually used as food preservatives), as well as its high fermentative capacity under these conditions can cause fast and severe spoilage of a vast scope of acidic and/or high-sugar products. The means by which *Z. bailii* is able to withstand weak acids and thrive under such conditions are still very controversial, however currently four hypotheses have arisen to explain it: (I) the metabolism of weak acids as a carbon source even in the presence of high amounts of sugars, (II) the presence of acid efflux pumps and (Ii) structure modification of the envelope, limiting the entry by diffusion of these acids. In order to test if the last mentioned hypothesis is at the basis of *Z. bailii* resistance to common food preservatives, eight proteins involved in the biosynthesis of membrane and cell wall components were chosen (Erg2, Erg4, Erg6, Sur4, Gas1, Opi3, Fen1, Isc1). We cloned their coding genes into *S. cerevisiae* BY4741 with the respective gene deleted and tested the resultant transformants for their performance under weak acid stress. Drop-tests were used to evaluate the resistance of the resultant transformants to concentrations of sorbic and benzoic acids in the range of 1-6 mM, at two different acidic pHs (4.5

and 3.5). The effect of previous adaptation to benzoic or sorbic acid was also tested. Preliminary results indicate that *S. cerevisiae* cells expressing SUR4 and GAS1, improved growth in the presence of benzoic and sorbic acids, being these results most notorious under the more acidic pH. As expected, the prior adaptation of the cells to low concentrations of benzoic and sorbic acid further improved cell resistance to these acids up to concentrations above 2 mM. These results point to a putative role of the membrane and cell wall structure as an important mechanism for weak acid tolerance. Further studies on the membrane permeability modification and weak acid diffusion are being performed to evaluate more specifically the effect of these *Z. bailii* genes in *S. cerevisiae*.

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P15 - Evaluation of membrane permeability of *Saccharomyces cerevisiae* deletion strains with different wine fermentation performances

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Wine fermentation is a complex process, during which yeast cells are submitted to a number of adverse stress conditions (osmotic pressure, low pH, ethanol, nutrient limitation and starvation). Our previous results confirmed that resting cells are physiologically very different from the cells at the first stages of the fermentation process. Throughout the years many efforts to characterize the mechanisms underlying ethanol stress tolerance have been made. Although ethanol toxicity in yeasts is a complex mechanism, the main target of such stress seems to be the cell membrane. The result may be an increase in membrane fluidity and a decrease in its structural integrity with an accompanying increase of leakiness and deleterious non-mediated diffusion processes. This observation points to metabolic pathways involved in the maintenance of cell structural integrity as possible targets to improve ethanol tolerance in yeasts In order to understand the role of different lipid metabolic processes on yeast fermentative performance, cell capability of keeping H^+ out by means of an impermeable membrane was evaluated as potential mechanisms of H^+ homeostasis. Passive proton influx was estimated by recording the alkalinization of unbuffered cell suspensions collected at different stages of synthetic must fermentation (at mid-

exponential, at early and late stationary phases and at the end of fermentation). The effect of 10% ethanol on this permeability was also evaluated. We tested a set of 13 Euroscarf single-deletion mutants selected among those genes involved in cell wall integrity and signaling (Δknh1, Δslg1, Δrom2, Δlap3, Δbck1, Δspi1, Δslt2, Δura7) and in lipid, fatty acid and ergosterol metabolism (Δcyb5, Δerg2, Δerg3, Δerg4, Δopi3) The selected genes had been previously evaluated and chosen for their beneficial or deleterious effect on the fermentative performance of the cells. In general, late stationary phase cells became almost impermeable to H+ and the effect of ethanol (added in the assay) on H^+ permeability was less evident. As expected, ethanol significantly increased the H^+ permeability of cells, mainly those collected in exponential phase. Interestingly, Δrom2, Δcyb5 and Δslg1 (mutations previously selected as beneficial for yeast performance) presented higher influx rates at early stationary phase than those selected by their deleterious effect on the fermentation performance.

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P16 - Biological production of xylitol by *Scheffersomyces amazonensis*, a new yeast species isolated from the Brazilian Amazonian Forest

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Xylitol, a five-carbon sugar alcohol, is recognized as a high value-added product due to its applications in food and pharmaceutical industries. This polyol presents anti-cariogenic and cariostatic properties, an insulin independent metabolism, a sweetening strength similar to sucrose and higher than ordinary polyols, and a reduced caloric value. These properties make xylitol a good sugar substitute in controlled diets. In addition, studies have shown that xylitol prevents osteoporosis, haemolytic anaemia as well as acute otitis media. Xylitol can be produced from hemicellulosic hydrolysates of lignocellulosic wastes by either chemical reduction of xylose through hydrogenation, or biotechnology processes that use enzymatic reactions using whole microbial cells or purified enzymes. Biological production of xylitol can be performed by yeasts capable of reducing D-xylose to xylitol as the first catabolic step in D-xylose metabolism. Nevertheless, one of the greatest challenges in xylitol production by

biotechnology processes is the availability of strains able to produce high yields of this polyol in an industrial context. To meet this challenge, increased bioprospecting is needed to discover new yeast strains or species that might convert D-xylose to xylitol in an unprecedented way. In the present work, the production of xylitol by a novel D-xylose-fermenting yeast was evaluated. *Scheffersomyces amazonensis* was described in 2012 as isolated from rotting wood sampled in the Brazilian Amazonian Forest. This is the first study to show the *S. amazonensis* biotechnological potential for xylitol production. In fermentation assays conducted under two different aeration conditions, moderate or severe oxygen-limited conditions, and using D-xylose (50 gl^{-1}) as sole carbon source, the strain *Sc. amazonensis* UFMG-HMD-26.3 generate, respectively 26.4 and 34.2 gl-1 of xylitol as its maximum titer after 36 and 72 h, equivalent to productivities of 0.73 and 0.47 $gl^{-1}h^{-1}$ and yields of 0.53 and 0.70 gg^{-1}. In fermentations using rice hull hydrolysate (D-xylose 20 gl-1, glucose 4.5 gl-1, not supplemented with nitrogen sources), maximum xylitol production under moderate and severe oxygen-limited conditions was observed after 96 and 120 h, respectively, reaching 3.0 and 6.3 gl^{-1} (productivities of 0.03 and 0.05 $gl^{-1}h^{-1}$). In both assays, the highest production was observed under severe limited conditions corresponding to an oxygen transfer rate of approx. 1-2 $mMmin^{-1}$. These results appear to be correlated to the co-factor preference of xylose reductase (XR) and xylitol dehydrogenase (XDH), the enzymes responsible for reducing D-xylose to xylitol and oxidizing xylitol to D-xylulose, which in this species are exclusive NADPH- and NAD^+-dependent, respectively. The co-factor imbalance generated by these two reactions is thought to be responsible for promoting a greater xylitol production than ethanol or other metabolites. This study demonstrates the promising use of *Sc. amazonensis* in xylitol production from D-xylose and hemicellulose hydrolysates.

P17 - A chemogenomics analysis on *Saccharomyces cerevisae* tolerance to itaconic acid

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The establishment of efficient processes for microbial conversion of biomass into add-value chemicals that could be used by the chemical industry in replacement of oil derivatives is essential for the sustainable implementation of biorefineries. Itaconic acid, a C5-dicarboxylic acid, is among the chemicals considered to have

a higher potential as a building block molecule [1]. Industrial processes for production of itaconic acid exploring different filamentous Fungi have been implemented; however, the yields obtained are significantly below those theoretically predicted [2]. This reduced yield is largely attributed to the toxic effect exerted by the acid on the producing cells, especially at the later stages of the fermentation when the weak acid accumulates in the acidic broth at high concentrations.

In this work we have taken advantage of *Saccharomyces cerevisae* as an experimental system and a eukaryotic model to identify, at a genome-wide scale, the genes/pathways required for maximal tolerance to itaconic acid stress. For this, the Euroscarf haploid mutant collection, comprising 5000 individual mutants deleted for all non-essential yeast genes, was screened in liquid MMB growth medium (at pH 3.5) supplemented with 500 mM itaconic acid. Thirteen mutant strains were found to be more tolerant to itaconic acid than the parental strain BY4741, while 430 mutants exhibited a higher susceptibility. The vast majority (around 76%) of the genes identified as yeast determinants of resistance to itaconic acid were only required to reduce the duration of the adaptation period to the acid having little or no effect in the growth rate of adapted cells. Nevertheless, a subset of 100 genes whose expression increased growth rate of yeast cells during growth in the presence of 500 mM of itaconic acid were also identified. Functional clustering of the itaconic acid-resistance genes identified in this work shows that the classes having a higher number of genes are: lipid metabolism, in particular phospholipid biosynthesis; cell cycle and DNA processing; transport; carbohydrate metabolism and energy generation, transcriptional regulation, protein synthesis and stress response. A model of the mechanisms by which itaconic acid becomes toxic for the yeast cells and of the underlying adaptive responses triggered by the cells to cope and surpass the deleterious effects of the acid stress will be discussed in light of the results obtained in the chemogenomics screening. Around 50% of the genes that were found to contribute for maximal yeast tolerance to itaconic acid have robust homologues in *Aspergillus terreus* and *Aspergillus niger*, the two host strains more commonly used for microbial production of itaconic acid [2]. It is expected that the knowledge gathered in this work could be used to guide the development of more robust industrial strains for the production of itaconic acid, in a reverse engineering perspective.

[1] Sauer M et al., Trends in Biotechnology, 26:100-8, (2008); [2] Steiger M et al., Frontiers in Microbiology, 14:14-23, (2013)

SESSION III – Yeast Diversity and Evolution

P18 - PYCC - a repository of Mediterranean yeast diversity

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PYCC is a research biological collection committed to the preservation, distribution and study of yeasts (http://pycc.bio-aware.com/). It was founded in 1952 and currently holds approximately 3000 strains, representing ca. 800 species and 140 genera. About 1300 strains are unique to PYCC and for those isolates with information on isolation and/or ecology, molecular sequence data has been obtained and is available through PYCC website. One of PYCC's goals is to become an internationally recognized repository of yeast diversity from the Mediterranean region. Presently, 42% of PYCC holdings for which accurate ecological data is available, correspond to yeast strains found in Mediterranean ecosystems or related regional fermented foods and beverages. The Mediterranean yeast lineages currently maintained at PYCC include starter cultures associated with the bread and wine varieties that make part of the Mediterranean diet, recently recognized by UNESCO as an Intangible Cultural Heritage of Humanity. PYCC also maintains a considerable collection of natural isolates mainly from plant substrates or soils collected from different Mediterranean ecosystems, for which relevant ecological and molecular information (e.g. DNA-barcodes) is available online. Molecular information on PYCC strains is available and searchable through MycoBank (www.mycobank.org).

P19 - Elucidating the molecular determinants of the homothallic sexual behavior of the astaxanthin-producing yeast, *Phaffia rhodozyma*.

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Sexual behavior in Fungi can be heterothallic (requiring genetically distinct and compatible partners) or homothallic (requiring no partner). In most basidiomycetous yeasts the heterothallic sexual cycle is initiated by mating of two compatible strains of distinct mating types. The first step is regulated by pheromones and G protein-coupled pheromone receptor genes (P/R) that mediate cell-cell recognition leading to cell fusion. The progression through the sexual cycle is then controlled by homeodomain (HD) transcription factors. In homothallic basidiomycetes the presence, absence or function of these genes has not yet been characterized at the molecular level. Using as a model the yeast *Phaffia rhodozyma*, this study aims to provide a better understanding of homothallic sexual behavior in basidiomycetes. Due to its biotechnological relevance as an astaxanthin producer, particular life cycle and its amenability to genetic transformation, *Phaffia* represents an exceptional model for this task. Using a draft genome sequence from *P. rhodozyma* strain CBS 7918T, we identified putative mating type genes, representing both MAT loci. Two putative pheromone (*MFA1* and *MFA2*) and pheromone receptor genes (*STE3-1* and *STE3-2*) were identified, as well as a pair of divergently transcribed homeodomain genes (*HD1* and *HD2*). Deletion mutants of HD and P/R genes where constructed and their ability to undergo sexual reproduction was evaluated. The results indicate that the putative MAT genes identified play an active role in the homothallic life cycle of *P. rhodozyma*. The two pheromone receptor genes seem to be redundant but necessary for sporulation, while the homeodomain proteins encoded by the two HD genes may to be able to form a functional Hd1-Hd2 complex. Interestingly the sole presence of the Hd1 transcription factor may be sufficient to support completion of the sexual cycle albeit very inefficiently. The presence of only one pair of pheromone and receptor genes (*STE3-1* and *MFA1* or *STE3-2* and *MFA2*) renders the mutant unable to sporulate by itself. These results provide the first insight into the molecular determinants of the homothallic life cycle of the basidiomycetous yeast *P. rhodozyma*.

P20 - Molecular characterization of the mating system of *Leucosporidium scottii* sets tetrapolarity as a transversal feature common to all Basidiomycota

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In Basidiomycetes, sexual reproduction relies on the conjugation of two haploid cells of compatible mating types/sexual identities. Two classes of proteins define mating type in this phylum: (1) lipopeptide pheromone (P) and complementary plasma membrane pheromone receptors (R) mediate recognition of a compatible mating partner, while (2) homeodomain transcription factors 1 (HD1) and 2 (HD2) form heterodimers that control post-mating behavior. The regions encoding these proteins are called MAT loci: "PR" and "HD" respectively, and according to their arrangement in the genome, may establish a particular mating system. When PR and HD regions are in linkage they constitute a bipolar system, which is typically biallelic and therefore has the potential to specify two mating types. On the other hand, if the MAT loci are not in linkage, they create a tetrapolar system, which is characterized by the possibility to originate four mating types out of each mating cross, and which is usually multiallelic for at least one of the PR or HD genes. Yet a third mating system has been described in members of the order Sporidiobolales, the so called pseudobipolar system [1], which is intermediate between the other two, as it is not strictly bipolar, but rather allows occasional events of independent segregation of the PR and HD loci and, thereby, the generation of more than two mating types. Within the subphylum Pucciniomycotina, which contains bipolar and pseudobipolar species, *Leucosporidium scottii* appears as the only saprophytic yeast species described to exhibit a tetrapolar mating system [2]. The present work aims at providing molecular evidence for this tetrapolarity, through the analysis of the diversity and distribution of molecular mating types in an extended collection of wild isolates, as well as the identification of the genomic localization and organization of its PR and HD loci. Sequence analysis of the STE3, HD1 and HD2 MAT genes of a collection of 27 natural isolates of *L. scottii* revealed the existence of only two STE3 allxheles, instead of three, as had been previously reported. A great diversity of HD alleles was, in contrast, found among the strains studied: 13 alleles were identified. Strikingly however, we found no evidences for random assortment of the two MAT loci, and thus for the lack of genetic linkage that characterizes tetrapolar species. Genomic organization of the MAT loci from two *L. scottii* strains of different mating types was then assessed, both by analysis of whole genome sequencing data and by chromoblot inspection, having unequivocally confirmed *L. scottii*'s tetrapolarity. Although some aspects of the ecology of this species are very difficult to ascertain, like their frequency of mating, we are currently trying to understand if the linkage disequilibrium found in the distribution of MAT loci in natural isolates may be explained by loss of viability and/or fertility of certain meiotic progenies or, if it is rather due to insufficient sampling among the collection of strains studied. Together, our results bring full proof support to the classification of *L. scottii* as a tetrapolar organism, helping to show that mating tetrapolarity is a feature shared even by the early diverged Pucciniomycotina lineage of Basidiomycota. Importantly, the

study of this yeast's mating system also appears as a demonstration of how tetrapolar species might be easily mistaken for bipolar.

[1] Coelho M.A., Sampaio J. P., Gonçalves P., PLoS Genet., 6(8), e1001052. 2010. [2] Fell, J. & Tallman, S. A. Curr. Microbiol., 7, 213-216. 1982.

P21 - Evolution of sex determination in red yeasts: exploring the impact of genomic structural variation

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Sexual reproduction is ubiquitous and extant in all of the major groups of the eukaryotic tree of life and defines a key evolutionary innovation by which species transmit and admix genetic material. Although the core features of sexual reproduction seem to be conserved, namely ploidy changes and cell-cell recognition between compatible mating partners, the increasing number of completed genomes have made apparent that the evolution of sex-determining systems in the fungal kingdom is highly dynamic. Within Fungi, basidiomycetes have developed a complex mating system involving two sets of genes: (i) lipopetide pheromones and their cognate receptors (P/R) that coordinate cell-cell recognition leading to cell fusion, and (ii) compatible homeodomain transcription factors (HD) that regulate the progression through the sexual cycle. When both MAT genes are determining sexual identity, their genomic organization defines the mating system as bipolar (if P/R and HD are located the same chromosome and genetically linked) or tetrapolar (if the genes are unlinked, e.g. located in different chromosomes). Transitions between the two systems have been observed in at least two major lineages of basidiomycetes (Ustilaginomycotina and Agaricomycotina) and are assumed to be influenced by the relative importance of inbreeding and outbreeding in the lifestyle. Aside from the bipolar and tetrapolar systems, our previous studies in the saprobic red-pigmented yeast *Sporidiobolus salmonicolor* (Pucciniomycotina) have revealed a mating configuration that deviates from these classic mating systems by the observation of two concurrent features: firstly, we found multiple HD alleles in natural isolates of this species, but each allele was always associated with only one of the two P/R alleles; and secondly, we found that recombination may occur between P/R and HD genes (albeit rarely) as assessed in the progeny of a laboratory cross between *S. salmonicolor* compatible strains. Using comparative genomics and

electrophoretic karyotype analyses, we are currently exploring the evolutionary trajectory of MAT loci in red yeasts and investigating the impact of intraspecific genomic structural variation in both sex determination and reproductive isolation.

P22 - Identification and characterization of yeasts associated with decaying wood of the Atlantic Forest

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Due to the depletion of oil reserves and environmental concerns related to the increasing greenhouse gas emissions from the intensive use of non-renewable fuel sources, it is urgent to search for alternative and renewable sources of energy for the transportation sector. Lignocellulosic ethanol is in the frontline of the advanced and sustainable biofuels to be commercialized. However, this technology still faces some obstacles, including those associated with the microbial conversion process. Yeasts associated with lignocellulosic ethanol should preferentially: (i) efficiently ferment D-xylose to ethanol; (ii) tolerate inhibitors present in lignocellulose hydrolyzates resulting from biomass pretreatment; (iii) produce enzymes able to hydrolyze cellulose and/or hemicellulose. The aim of this study was to isolate yeast present in decaying wood from the Atlantic Rain Forest (Brazil) directly in medium containing sugarcane hydrolyzate, and to test them for their ability to ferment D-xylose and to produce extracellular xylanases. Thirty hundred and eight two yeasts were isolated, and in the screening tests conducted so far, 61 (15.96%) isolates produced xylanases and 54 (14.14%) fermented D-xylose producing ethanol. Most xylanase-producing yeast isolates were identified as belonging to the genus Sugiyamaella. Most D-xylose-fermenting yeasts were identified as belonging to the genus Scheffersomyces. *Candida (Lodderomyces) tropicalis*, *Candida (Pichia) pseudolambica*, *Debaryomyces nepalensis*, *Kazachstania unispora* and *Meyerozyma guilliermondii* were frequently isolated. Furthermore, 18 isolates are probably new yeast species, three of which produced xylanases and six fermented D-xylose. The isolation of new yeast species that produce xylanases and ferment D-xylose in the Atlantic Rain Forest, shows the potential of this woody habitats to provide new yeasts with properties of interest for the production of lignocellulosic ethanol.

SESSION IV – Yeasts and Human Health

P23 - Inhibition of formation of α-synuclein inclusions by mannosylglycerate in a yeast model of Parkinson's disease

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Protein aggregation and accumulation in the brain is a central hallmark in many neurodegenerative diseases. In Parkinson's disease, the most prevalent neurodegenerative movement disorder, α-synuclein (α-Syn) is the major component of the intraneuronal inclusions found in the brains of patients. Current therapeutics is merely symptomatic, limiting our ability to alter the course and impact of the disease. Previously, we showed that mannosylglycerate (MG), a compatible solute typical of marine microorganisms thriving in hot environments, is highly effective in protecting a variety of model proteins against thermal denaturation and aggregation in vitro. Here, we demonstrate the effect of MG as an inhibitor of the formation of α-Syn inclusions in living cells [1]. To this end, a *Saccharomyces cerevisiae* strain, expressing eGFP-tagged α-Syn, was further engineered to produce MG. The mutant accumulated around 80 µmol of MG/g of dry weight and showed a reduction of 3.3 fold in the number of cells with α-Syn foci, in comparison with the control (no MG). By western blot analysis we confirmed that MG has no affect in the expression levels of α-Syn or its degradation rate. Moreover, MG did not induce the synthesis of molecular chaperones (Hsp104, Hsp70 and Hsp40), suggesting the implication of direct mechanisms for α-Syn stabilization. This is the first demonstration of the anti-aggregating ability of MG in the intracellular milieu. Our results strongly indicate that MG acts as a chemical chaperone, and that the stabilization mechanism involves direct solute/protein interactions. This work also reinforces the view that MG plays a physiological role in the stabilization of proteins in the natural host organisms, which thrive optimally in hot environments where the deleterious effects of heat on macromolecule structures have to be offset efficiently.

[1] Faria C, Jorge CD, Borges N, Tenreiro S, Outeiro TF, Santos H (2013). Inhibition of formation of α-synuclein inclusions by mannosylglycerate in a yeast model of Parkinson's disease. BBA - General Subjects, 1830:4065-4072.

P24 - Prevalence of *Candida parapsilosis* antifungal resistance

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The first fungal epidemiological study carried out in a Portuguese hospital unveiled a high fluconazole resistance among nosocomial fungal isolates which was associated with high mortality rates. Following *C. albicans*, *C. parapsilosis* was the most common yeast species isolated from patients with bloodstream infections. Yet this situation is not restricted to Portugal. In other European countries and in Latin America and Asia, *C. parapsilosis* is commonly found. Azoles, namely fluconazole, are widely used as prophylactic and therapeutic drug; however *C. parapsilosis* acquires azole resistance in a rapid and stable manner. This finding suggests that an emergent of C. parapsilosis azole resistance may be imminent. This study aims to asses and characterize the antifungal susceptible profile of *C. parapsilosis* sensu strico etiologic agent of human infections from two major Portuguese hospitals, Centro Hospitalar de Coimbra and Centro Hospitalar São João. Clinical isolates (n=88) isolated from respiratory tract (bronchoalveolar lavage fluids, nasopharyngeal aspirates and sputum), urine, central venous catheter, blood, stools and skin were assesed regarding the antifungal susceptibility profile to azoles, namely fluconazole (FLC), voriconazole (VRC), posaconazole (PSC), and echinocandins, such as caspofungin (CSF), micafungin (MCF) and anidulafungin (ANF). The minimal inhibitory concentration (MIC) of each antifungal drug was determined according to the M27-A3 protocol and M27-S4 supplement of the Clinical and Laboratory Standard Institute (CLSI). Among C. parapsilosis isolates, 10% were resistant to FLC; 6% were resistant to VRC; 2% were resistant to PSC. The incidence of susceptible–dose-dependent found was 6% for FLC and 10% for VRC. For echinocandins, no resistance was found. In order to characterize molecular mechanisms triggered by azole resistance development, gene expression profile of the clinical resistant isolates were carried. Interestingly, *MDR1* (encoding multidrug efflux pump) and *MRR1* (transcription factor that regulates *MDR1* expression) were overexpressed up to 9,5 fold and 2 fold, respectively. Our study showed that prevalence of azole *C. parapsilosis* resistant isolates has increased

dramatically since the last survey carried out in 2009. Azole resistance was mainly associated with increased ability to expel the drug.

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P25 - In vivo reversion of fluconazole resistance by ibuprofen: a new hope for fungaemia patients

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Candida represents the most frequent isolated yeast from fungaemia patients. The economic cost of bloodstream fungal infections and its associated mortality, especially in debilitated patients, remains unacceptably high. These microorganisms are highly adaptable, developing resistance to antifungal drugs whenever under its pressure. Antifungal prophylaxis or antifungal treatment displays favorable conditions for the emergence of antifungal resistance. The major mechanism responsible for high level of azole resistance is the overexpression of cell membrane efflux pumps which are accountable for lowering the accumulation of azoles inside the yeast cell by active translocations compounds across cell membrane. The main strategy to reduce efflux impact involves the maintenance of a high antifungal concentration inside the cell, at its site of action. The knowledge of the mechanism of antifungal resistance brought by the genomic era supports the development of therapeutic strategies in order to bypass drug resistance. Ibuprofen has been described to act synergistically with fluconazole. In our previous research, in *C. albicans* expressing CDR efflux pumps, the presence of ibuprofen increased azole intracellular accumulation, changing the resistant phenotype to susceptible1,2. However the in vivo combination of fluconazole and ibuprofen needed to be clarified. In this study a *C. albicans* resistant (R) strain to fluconazole was obtained by subculturing with serial concentrations of fluconazole a susceptible strain (S) during 30 days. Minimal inhibitory concentrations (MIC) to fluconazole was determined in the presence of 100µg/ml of ibuprofen (IBU), an efflux pump blocker1,2. Ibuprofen decreased azole MIC values, changing the resistant phenotype to susceptible. The in vivo study was carried out according to the murine candidiasis model. Female BALB/c mice via the lateral tail vein with the susceptible strain or the resistant

strain. Antifungal therapy was administered intraperitoneally with fluconazole or ibuprofen or the combination of both 3 hours after microbial challenge and repeated once a day for a total of four days. Mice weight was daily registered and at day 4 post-infection mice were euthanized and the kidneys were aseptically removed. The results showed that ibuprofen potentiates fluconazole antifungal activity, reducing drastically fungal burden and morbidity. A characterization of the molecular mechanisms triggered by ibuprofen-fluconazole synergistic effect to reverse antifungal resistance was pursued by transcriptome analysis. Microarray analysis identified 836 and 1517 genes with differential expression in CaRFLC (*C. albicans* resistant strain incubated with fluconazole) and CaRFLCIbu strains (*C. albicans* resistant strain incubated with fluconazole plus ibuprofen), respectively. The CaRFLC strain showed overexpression of *CDR11*, *ERG251*, *CDR4*, *CDR1* and the transcription factor *UPC2*. Interestingly, *MDR1*, *CDR4*, *NAG3* and *NAG4* genes were upregulated while *CDR11*, *ERG251* and *UPC2* genes were downregulated in the presence of ibuprofen (CaRFLCIbu strain). These results stress the fact that ibuprofen can inhibit efflux pumps by blocking access to the binding site or by down-regulating the transcription of efflux genes. Further studies are being addressed in order to uncover the main mechanism of ibuprofen on yeast cell physiology as well as to assess its influence in the dynamics of the antifungal resistance induction. By allying anti-inflammatory and analgesic properties, ibuprofen in combination with fluconazole might play a relevant role in a future therapeutic strategy for severe fungal infections. The proved in vivo synergic effect between fluconazole and ibuprofen confirmed a new approach for a better management of antifungal therapeutics to revert resistance conferred by efflux pump overexpression.

Pina-Vaz, C., et al. J Antimicrob Chemother 2005, 4: 678-85 Ricardo, E., et al. FEMS Yeast Res 2009, 4: 618-25

P26 - The implications of alternative oxidase in Amphotericin B liposomal susceptibility

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Amphotericin B (AmB) targets to ergosterol, the principal sterol in the fungal cytoplasmic membrane. This drug exhibits fungicidal activity against *Candida* spp. at very low concentrations. The acquisition of AmB resistance remains

extremely rare. Nonetheless, in spite of the observed high in vitro susceptibility, the in vivo response to AmB is often reduced. This antifungal drug tolerance can result from metabolic adaptation, in particular in respiratory mitochondrial pathway. In response to environmental changes some species of Candida express an alternative respiratory pathway (ARP). Enzyme alternative oxidase (AOX) is a terminal oxidase which inserts a branch point in the respiratory electron transport chain. AOX may reduce the generation of reactive oxygen species (ROS) that can result in oxidative damage to the mitochondria and other cellular components. Since expression of the alternative respiratory pathway occurs in response to antimicrobials, we intend to explore the implications of AOX inhibition in *Candida* species when exposed to amphotericin B liposomal (AmB-L). In order to understand the role of AOX in the ROS production, a kinetic study was performed in *Candida albicans*, *C. glabrata*, *C. krusei*, *Saccharomyces cerevisiae* (AOX negative control) and *Debaryomyces hansenii* (AOX positive control) by flow cytometry. Cells were incubated with 2,7-dichlorofluorescin diacetate (DCFH-DA, an oxidative stress marker) for 30 min. After that, cells were treated with AmB-L 3 µg/ml during 1.5, 3, 6 and 24h. All treatments were repeated following the preincubation of yeast cells with salicylhydroxamic acid (SHAM, an inhibitor of the AOX). As control cells were exposed to oxygen peroxide and menadione (AOX inducers). Fluorescence intensity (FI) values were determined on FACSCalibur cytometer at 530 nm. Susceptibility profile to AmB-L in presence of SHAM was also determined by checkerboard assay as report before by Moody et al. The results showed that the ROS generation was increased in all strains, after 6 hours of incubation with AmB-L. In presence of SHAM, ROS production has a more significant increase (1.7x), except in *S. cerevisiae* (AOX negative control). Checkerboard assay showed that the addition of SHAM decreased MIC values for AmB-L, resulting in a synergic effect in *C. albicans*, *C. krusei*, *C. glabrata* and *D. hansenii*. An indifferent effect was detected for *S. cerevisiae*. The inverse relation between the AOX expression and the intracellular ROS production suggest that the alternative respiratory pathway keep endogenous ROS at low levels, leading to greater tolerance to AmB-L.

P27 – Identification of protein kinases/phosphatases involved in the phosphoregulation of Bax-dependent cell death

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Apoptosis dysfunctions underlie multiple human pathologies, such as degenerative diseases and cancer. Apoptosis is thus tightly regulated, mainly by the Bcl-2 protein family. The major pro-apoptotic member of this family is Bax, which plays a central role in controlling outer mitochondrial membrane integrity in response to apoptotic stimuli, and therefore ultimately apoptosis. Bax can be regulated by phosphorylation/dephosphorylation, though it is not clear how, and only a few Bax residues and kinases/phosphatases involved in Bax phosphoregulation have been identified. In order to identify novel kinases/phosphatases involved in phosphorylation of Bax, we heterologously expressed human Bax in yeast cells lacking non-essential kinases/phosphatases and determined whether there were differences in the Bax phosphorylation profile. With this approach, we found putative kinase/phosphatase candidates as well as one yeast kinase (Rim11p) and two yeast phosphatases (Pph21p/22p and Pct4p), which have as human orthologs GSK3 kinase, PP2A and WIP1 phosphatases, respectively. These human kinases/phosphatases have been previously described as involved in the phosphorylation of Bax. Therefore, the proposed approach will allow assessing the consequences of this post-translation modification on Bax function, and provide novel insights on phosphoregulation of this key apoptosis regulator. Since protein kinases/phosphatases are potential drug targets, this study provides a basis for further therapeutic strategies against diseases involving apoptotic dysfunctions.

P28 - Protective effects conferred by *A. unedo* digested polyphenols against oxidative injury in *Saccharomyces cerevisiae* oxidative stress model.

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The yeast *Saccharomyces cerevisiae* has become a valuable eukaryotic cellular model to study human diseases extensively used as a model for the ageing process. Nevertheless budding yeast is a very important model in the identification of key pathways and genetic regulation mechanism of human cells, since these pathways have remarkably similarity to the higher eukaryotes and most of yeast proteins are functionally interchangeable with its orthologous in human [1]. More recently have been developed platforms based on in vivo assays

using the yeast *S. cerevisiae* for the bioprospection of phytochemicals from plants and identify health promoting bioactivities. Plants synthesize a staggering variety of secondary metabolites based on numerous backbone structures and functional group combinations. This chemodiversity provides a pristine pool of high-value compounds with potential application in human health. The phenolic compounds, one of the largest classes of phytochemicals, are described as possessing the ability to prevent biochemical phenomenon that occur in cells responsible for degeneration processes [2,3,4]. These classes of compounds have being described as having a key role in the prevention of chronic diseases, such as cancer, neurodegenerative diseases and cardiovascular disease, that highly increase the mortality and morbidity in Europe and Western societies [5,6]. However for a physiological relevant evaluation of dietary metabolites effect, the chemical alterations that occur in the gastro-intestinal tract and its influence in its bioaccesability, bioavailability and bioactivity must be considered. Therefore in vitro digestion methods are very important to mimic physic and chemical transformations that occur during digestion and allowing accessing bioactivities of polyphenol digested metabolites [3]. In this study an yeast model of oxidative stress in *Saccharomyces cerevisiae* strains (BY4741 (WT) and *Δyap1* (essential transcriptional factor in oxidative stress)) were used to evaluate the protective effect of polyphenols metabolites from A. unedo, after being submitted to an in vitro digestion, against the oxidative injury by H_2O_2. Thus, parameters such as viability, metabolic capacity and redox homeostasis alterations, were evaluated in both strains after being submitted to the *A. unedo* metabolites. In this study was used both fruit and leaf metabolites as nutraceutical approach and a chemical characterization was performed before and after the in vitro digestion process. The results obtained indicate that although after digestion *A. unedo* total phenols and in vitro antioxidant potential are decreased, the *A. unedo* metabolites presents a cytoprotective effect against an oxidative injury. In this work was also detected that this metabolites have the capacity of induce phenotype amelioration in *Δyap1* mutant and increase its metabolic capacity for levels near to WT strain. Phenolic compounds have been described to protect cells against oxidative stress by directly scavenging of ROS. However the results obtained from ROS quantification and GSH/GSSG ratio suggests that the mechanisms by which this compounds ameliorate cells viability is not through direct scavenging of ROS but by other molecular mechanisms. It was also performed a transcriptomic analysis that suggests the existence of several metabolic mechanisms protecting cells against oxidative injury. Oxidative stress is under influence of multifactorial alterations, leading to a fast and transient change in all metabolism of cell. Further studies should be performed to disclose the importance of mitochondria since this organelle have a central role in viability and survival of cells.

1.Lushchak, V. L., (2006), Acta Biochim Pol 53(4), 679-684 2.Tackling Chronic disease in Europe. Strategies, interventions and challenges, European observatory on health systems and policies, Observatory studies series Nº 20; 3.Xiao et al., (2011) Mini Rev Med Chem 11, 169; 4.Ebrahimi, A. et al., (2012). Ageing Res Rev, 11(2): 329-345. 5.Tavares, L. et al., (2012). Food Chem, 131(4): 1443-1452. 6.Gao, X. et al., (2012) Neurology, 78(15): 1138-1145.

P29 - Epidemiological survey of infections caused by Candida species in the largest Hospital Center of the Lisbon area

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Fungal infections, in particular those caused by species of the *Candida* genus, are among the leading causes of nosocomial infections having associated high rates of mortality and morbidity. Establishing and maintaining surveillance programs for fungal infections, both at a national basis and within individual care centers, is a critical activity to improve our understanding of the magnitude of the burden posed by these infections, to enable prioritization of research, and to design more specific strategies for prevention and preemptive therapy. The present work shows the results of a prospective surveillance started in January 2014 and focused on the frequency of isolation of *Candida* spp. in patients hospitalized at Centro Hospitalar de Lisboa Central (CHLC), the largest Hospital Center of the Lisbon area that includes 6 large hospitals.

Since the beginning of the study 45 isolates of the *Candida* genus were identified, 24 collected from urinary tract, 11 from the blood, 5 from sterile fluids, 5 from the vaginal mucosa, three from lower respiratory tract and one from recovered from esophageal biopsy. *C. albicans* was the predominant infecting species, being identified in 51% of the isolates gathered in our study. The frequency of isolation of non-*albicans* species (NCAS) reached 49%, in line with results obtained in other surveys carried out in other major Portuguese Healthcare facilities [1,2] and support the concept NCAS are relevant in fungal infections in Portugal. *C. tropicalis* was the more frequent NCAS identified in our setting (10 isolates, corresponding to 22.2% frequency of isolation), followed by *C. glabrata* (7 isolates, 15.6%), *C. krusei* (3 isolates, 6.7%), *C. lusitaniae* (2.2%, one isolate), and *C. parapsilosis* (2.2%, one isolate). Candidemia in the patients hospitalized at CHCL reached 20%, and the highest number of cases was associated to *C. albicans*, although we had also identified patients with invasive candidiasis caused by *C. glabrata*, *C. krusei*, and *C. tropicalis*. The crude mortality rate of

patients with candidemia was of 22%, however the death of the patients could not be directly correlated with the fungal infection.

[1] Costa-de-Oliveira S et al., Eur J Clin Inf Dis., 27, 365-74, (2008)
[2] Sabino R, Med Mycology, 48, 346-54, (2010)

SESSION V – Yeast Functional Genomics and Bioinformatics

P30 - Towards an improved set of expression vectors for *Paracoccidioides* spp.

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Paracoccidioides brasiliensis is the etiologic agent of paracoccidioidomycosis (PCM), one of the most prevalent systemic mycoses in Latin America that represents a critical health problem, mainly in populations from rural areas. This fungus exists in a non-pathogenic mycelial form at environmental temperatures, shifting to the pathogenic yeast form at the mammalian host temperatures. The yeast form of *P. brasiliensis* is characterized by its multibudded nature, with cells presenting several nuclei, and by polymorphic cell morphology. However, the molecular bases of this differential pattern are far from being understood. The currently available genetic tools to uncover the molecular and cellular biology of this fungus are based on *Agrobacterium tumefaciens*-mediated transformation (ATMT) using T-DNA binary vectors, which allows for genomic integration of a single copy of genetic constructs for gene silencing or gene overexpression. Taking into account that *P. brasiliensis* is thermodymorphic and that the infectious process comprises a temperature-dependent morphological shift, it reveals of major importance to use phase-specific promoters for genetic manipulation. In this sense, a novel panel of promoters was selected from gene expression data in *Paracoccidioides* spp., including those of genes that showed either constitutive high expression in yeast and mycelium, as well as those that were most active in either the yeast or mycelial form. T-DNA binary vectors were constructed with the green fluorescent protein (GFP) under the control of these new promoters, and *Paracoccidioides* spp. subsequently transformed by ATMT. These included promoters of constitutively highly expressed genes heat shock

protein 70 (HSP70) and ribossomal protein L35, yeast specific genes for nuclear protein YL1 and GTPase Rho1 and mycelium specific gene for aminopeptidase Y (APE3). GFP expression in both yeast and mycelium form was then evaluated by epifluorescence microscopy observation of cells, flow cytometry analysis of the fluorescent populations and quantitative real-time PCR of GFP transcripts. This analysis will allow identifying the best promoters to be used for studies in yeast, mycelium, or both forms, and should improve modification of *Paracoccidioides* spp. phenotypes by e.g. gene overexpression and gene silencing, as well as gene tracking of fluorescently-labelled proteins and cells in vitro and in vivo.

P31 - Membrane proteome-wide response to the antifungal drug clotrimazole in *Candida glabrata*: role of the transcription factor CgPdr1 and the Drug:H^+ Antiporters CgTpo1_1 and CgTpo1_2

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Azoles are widely used antifungal drugs. This family of compounds includes triazoles, mostly used in the treatment of systemic infections, and imidazoles, such as clotrimazole, often used in the case of superficial infections. *Candida glabrata*, which is the second most common cause of candidemia worldwide, presents higher levels of intrinsic azole resistance when compared to *Candida albicans*, thus being an interesting subject for the study of azole resistance mechanisms in fungal pathogens.

Since resistance often relies on the action of membrane transporters, including drug efflux pumps from the ATP-Binding Cassette superfamily or from the Drug:H^+ Antiporter (DHA) family [1,2], an iTRAQ-based membrane proteomics analysis was performed to identify all the proteins whose abundance changes in *C. glabrata* cells exposed to the azole drug clotrimazole. Proteins found to have significant expression changes in this context were clustered into functional groups, namely: glucose metabolism, oxidative phosphorylation, mitochondrial import, ribosome components and translation machinery, lipid metabolism, multidrug resistance transporters, cell wall assembly and stress response, comprising a total of 53 proteins. Among these, the DHA transporter CgTpo1_1 was identified as overexpressed in the *C. glabrata* membrane in response to clotrimazole. Functional characterization of this putative drug:H^+ antiporter, and of its homolog CgTpo1_2, allow the identification of these proteins as localized to the plasma membrane and conferring azole drug resistance in this fungal

pathogen. The assessment of their role in drug extrusion and polyamine resistance is underway. This membrane proteomics approach was also used to assess the overall role of the transcription factor CgPdr1 in the observed expression patterns, highlighting the existence of additional unforeseen targets of this transcription factor, recognized as a major regulator of azole drug resistance in clinical isolates.

[1] Costa et al. 2013. *Antimicrob Agents Chemother*, **57**(7): 3159-67; [2] Costa et al. 2014. *J Antimicrob Chemother*, in press.
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P32 - Evolution of the 12-spanner drug:H^+ antiporter family 1 (DHA1) in pathogenic Candida species: phylogenetic and comparative genomic analyses of Mdr1 and Flu1 proteins

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Candida albicans and other pathogenic Candida species can develop resistance to fluconazole and other clinical fungicides through different molecular mechanisms. Among them is the drug export activity mediated by multidrug efflux pumps [1,2]. In particular, those encoded by *MDR1* and *FLU1* genes are members of the drug:H^+ antiporter family 1 (DHA1) [1,2,3]. The goal of this study was to extend the reconstruction of the evolutionary history of the hemiascomycete DHA1 genes to the CTG phylogenetic complex species. The DHA1 proteins encoded in the genomes of 31 hemiascomycetous strains from 25 species were identified by constraining and traversing a pairwise similarity network at different e-values [4]. A phylogenetic tree was built using 468 full size DHA1 amino acid sequences. The genomes of the pathogenic Candida species, *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. parapsilosis*, were found to encode 18, 10, 17, 20 and 30 DHA1 genes, respectively [4]. Gene neighbourhood analysis allowed the reconstruction of sixteen DHA1 lineages conserved during the evolution of the CTG complex species [4]. The evolutionary history of *CaMDR1*, *CaFLU1*, *CdMDR1*, *CtMDR1* and *CpMDR1* genes, associated with the development of fluconazole resistance in Candida clinical strains, was detailed. Results suggest that gene duplication and loss are major mechanisms underlying the evolution of the DHA1 genes in Candida species. Their genomes show an abundant number of *MDR1* and *FLU1* homologs but the chromosome environment where *MDR1* homologs reside was poorly conserved during evolution [4]. The genomes of *C. albicans* and *C. dubliniensis* encode a

FLU1 paralog whose origin was traced to a local duplication event occurring after the divergence of *C. tropicalis* species [4]. Considering that the physiological role of the majority of the DHA1 proteins encoded in the genomes of the pathogenic Candida species is still unclear, one main objective of this study was to uncover new clues that can guide the functional analysis of these transporters in the pathogenic Candida species.

[1] dos Santos SC, Teixeira MC, Dias PJ, Sá-Correia I (2014) Front. Physiol. (issue on Membr. Physiol. Membr. Biophysics). 5: 180 (doi: 10.3389/fphys.2014.00180) [2] Costa C, Dias PJ, Sá-Correia I, Teixeira MC (2014) Frontiers Physiol. - Membr. Physiol. Membr. Biophysics. 5 (doi: 10.3389/fphys.2014.00197) [3] Dias PJ, Seret M-L, Goffeau A, Sá-Correia I, Baret PV (2010) OMICS. 14: 701-710. [4] Dias PJ, Sá-Correia I (2014) Genomics (accepted for publication).

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P33 - A genomic analysis on resistance of the human pathogen *Candida glabrata* to azoles and echinocandins

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Fungal infections, especially those caused by members of the *Candida* genus, are among the leading causes of hospital-acquired infections having associated high rates of morbidity and mortality. An alarming increase in the incidence of infections caused by *Candida glabrata* has been reported in the last years this being mostly attributed to the increased resistance of this species to azoles, the frontline therapy in the treatment of candidiasis. In this work we have assessed the incidence of resistance to azoles (fluconazole and voriconazole) and echinocandins (anidulafungin and caspofungin) in 58 *C. glabrata* clinical isolates recovered in a main hospital of Lisbon. The highly standardized method of microdilutions, established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), was used to determine the Minimum Inhibitory Concentration (MIC) of each antifungal tolerated by the clinical isolates. Eight *C. glabrata* isolates were found to be resistant to voriconazole, 7 of these isolates also exhibiting resistance to fluconazole. The percentages of *C. glabrata* resistance to voriconazole (around 14%) and fluconazole (around 16%)

obtained in our study are in line with those reported for this yeast species in other European countries. One of the isolates found to be resistant to voriconazole and fluconazole (designated FFUL887) also exhibited increased tolerance to caspofungin being able to grow in the presence of 0.25 mg/L of this antifungal, compared to the remaining isolates that were only able to tolerate 0.125 mg/L. None of the isolates tested exhibited resistance to anidulafungin but the reference strain *C. glabrata* CBS138 was able to grow in the presence of 0.06 mg/L of anidulafunin, the MIC breakpoint value; while all the isolates tested only tolerated 0.03 mg/L.

To unravel the genetic adaptive mechanisms underlying the acquisition of resistance to azoles and echinocandins in *C. glabrata* we have obtained the genome sequences of the FFUL887 isolate and compared it with the publicly available genome of the CBS138 strain. The genomic sequence determined for the FFUL isolate includes 12.2 Mb, this corresponding to 98% of the total genome size of the strain estimated by flow cytometry. Comparison of genome sequence of FFUL887 and CBS732 strains revealed the existence of around 6300 single nucleotide polymorphisms distributed by 855 deletions, 1072 insertions and 4383 single nucleotide variations. The functional implications of the reported SNPs in the coding genome of the two *C. glabrata* strains tested will be discussed as well as their implications for increased tolerance and resistance of this pathogenic yeast to azoles and echinocandinds.

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