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Editorials

Dr. Graham H. Fleet (1946-2015)

The yeast research community is in shock with the news of the sudden death of Dr. Graham Fleet, Professor Emeritus at the University of New South Wales, in Australia, past Chair of the International Yeast Commission and friend to many of us. Graham attended ISSY 32 in Perugia and seemed in top form. He presented a wonderful plenary lecture on the microbiology of chocolate, proudly reporting the observation that yeasts, and not lactic acid bacteria, are mainly responsible for the universally loved taste of chocolate. Graham suffered a fatal stroke while in his home in Sydney, Australia. A group of Latin American researchers has prepared a tribute for this issue of the Yeast Newsletter and an obituary will appear in the next issue.

Dr. Ivan Yu. Chernov (1959-2015)

We are stunned also by the death of Dr. Ivan Chernov. Dr. Chernov was a dedicated soil microbiologist and yeast ecologist. Student of Inna Babjeva and mentor to Andrey Yurkov, he currently served as Head of Soil Biology at Lomonosov Moscow State University. I remember Ivan from his keen participation in one of the international yeast courses run by Nico van Uden in Oeiras, Portugal. Ivan died suddenly while on an expedition in Vietnam. Andrey Yurkov kindly provided an obituary.

MA Lachance, Editor

I Russian Collection of Microorganisms (VKM), Institute for Biochemistry and Physiology of Microorganisms, Pushchino, 142290, Russia - http://www.vkm.ru. Communicated by WI Golubev <wig@ibpm.pushchino.ru>.

The following papers from the VKM were recently published.

1 Golubev WI. 2015. Mycocinogeny in methylotrophic yeast. Vestnik biotechnologii i phyzico-chemicheskoy biologii 11(1):5-9 (in Russian).

A strain of *Ogataea pini* (Holst) Yamada *et al*. was revealed to secrete thermolabile fungicidal killer toxin (mycocin) with molecular mass about 8 kDa. Its activity is expressed between pH 3.5 and 5.0. The mycocin had maximum activity at pH 4.5 and in the presence of 3% NaCl in glucose-peptone medium. Probably, mycocin determinants are chromosomally inherited as the mycocinogenic strain retains its activity after "curing" treatments (growth at elevated temperature and irradiation with UV). Basidiomycetous yeasts of 13 genera and *Schizosaccharomyces spp*. are insensitive to this mycocin. It is active against representatives of the Saccharomycetales. Among methylotrophic yeasts *O. pini* mycocin inhibits growth of *Candida maris, C. nitratophila, C. pini, C. succiphila, Kuraishia molishiana, O. angusta, O. cecidiorum, O. glucozyma, O.polymorpha* and *O. wickerhamii*. The strains of *C. boidinii, Komagataella pastoris, Kuraishia capsulata, O. nonfermentans* and *O. pini* were variable in their reaction to the mycocin studied. The latter acts also against many species of genera *Ambrosiozyma* and *Nakazawaea* related phylogenetically to the above-listed methylotrophic yeasts. In addition, some species of the families Metschnikowiaceae, Pichiaceae, Saccharomycopsidaceae, Saccharomycetaceae and Wickerhamomycetaceae are sensitive (as a rule weakly) to *O. pini* mycocin.

2 Golubev WI. 2015. Antifungal activity of *Wickerhamomyces silvicola.* Microbiology (Moscow) 84(5):610-615.

Wickerhamomyces silvicola strain VKM Y-178 was shown to secrete a mycocin with a fungicidal effect. It exhibits the highest activity at pH 4.5 and elevated osmotic pressure. Over 140 species belonging to 45 genera of ascomycetous yeasts were sensitive to the mycocin, while basidiomycetous species were resistant. Taxonomically homogeneous species usually exhibit a homogeneous response to mycocins.

3 Golubev WI. 2015. Mycocinogeny in methylotrophic yeast *Ogataea nonfermentans.* Mykologia i Phytopathologia 49(5) (in press) (in Russian).

The strain VKM Y-2517 of *Ogataea nonfermentans* was revealed to secrete fungicidal mycocin active at pH of the medium within the range 3.5-6.5. The spectrum of its action is narrow and includes only several species of methylotrophic yeasts in the genera *Ogataea*, *Candida* and also some representatives related to them phylogenetically in the genera *Ambrosiozyma* and *Nakazawaea*. Strain heterogeneity of species (such as *Ogataea minuta* and *Candida boidinii*) in sensitivity to mycocin is indicative of their taxonomic heterogeneity.

II State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIgenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by GI Naumov and ES Naumova <gnaumov@yahoo.com>.

We were glad to have had in our lab Taiwanese colleague Lee Ching-Fu (National Hsinchu University of Education) for a short visit in September 2015. Many thanks to William Brown (University of Nottingham) for fruitful discussion on the population genetics of *Schizosaccharomyces pombe* during his stay in Moscow.

The following are papers published or in press for 2015.

1 Naumov GI. 2015. The yeast *Komagataella*: a genetic genus in accordance with interspecies hybridization. Microbiology (Moscow). 84 (4): 538–543. © Pleiades Publishing, Ltd.

2 Naumov GI, Naumova ES, Lee Ch-Fu. 2015. Towards reinstatement of the yeast genus *Zygowilliopsis* Kudriavzev (1960). Microbiology (Moscow). 84(5):587–594. © Pleiades Publishing, Ltd.

3 Naumov GI, Kondratieva VI, Meshcheryakova EV, Naumova ES. 2016. Genetic study of methylotrophic yeast genus *Komagataella*: a new biological species *K. kurtzmanii*. Rus J Genet 52(2) (in press).

Genetic hybridization analysis revealed that industrially important species *Komagataella kurtzmanii* has reproductive post-zygotic isolation from *K. pastotis*, *K. phaffii, K. populi, K. pseudo-pastoris*, *K. ulmi*. Therefore, it represents a new biological species of the genus *Komagataella.* The genetic data are in perfect agreement with the molecular taxonomy of the genus *Komagataella.*

4 Naumova ES, Boundy-Mills K, Naumov GI. 2015. Phylogenetics, ecology and iogeography of *Komagataella* yeasts: molecular and genetic analysis. Book of abstracts, ISSY 32, "Yeast biodiversity and biotechnology in the twenty-first century". September 13–17, 2015, Perugia, Italy, p. 7.

5 Naumov GI, Lee Ch-Fu, Naumova ES. 2015. Heterogeneity of the genus *Barnettozyma*: towards reinstatement of *Zygowilliopsis* Kudriavzev (1960). Book of abstracts, ISSY 32, "Yeast biodiversity and biotechnology in the twenty-first century". September 13–17, 2015, Perugia, Italy, p. 90.

6 Shalamitskiy MYu., Naumova ES, Martynenko NN, Naumov GI. 2015. Phylogenetic analysis of pectinase genes *PGU* in the yeast genus *Saccharomyces*. Book of abstracts, ISSY 32, "Yeast biodiversity and biotechnology in the twenty-first century". September 13–17, 2015, Perugia, Italy, p. 219.

III Biology Department, Brooklyn College, Brooklyn, New York 11210. Communicated by Nasim A. Khan <nasim.khan4@verizon.net>.

Letter to the Editor
An update on the genotype of strain 1403-7A in *Saccharomyces cerevisiae*

Strain 1403-7A, carrying the *MAL4* gene constitutive for maltase (EC 3.2.1.20), was first described by Khan and Eaton (1971). Both genetic and molecular evidence have shown that genetic alteration in the regulatory gene of the *MAL4* complex is responsible for the constitutive phenotype of this strain (Khan, 1979, Charron and Michels 1987). This strain has been extensively used in basic research , and it is a desirable strain for industrial use in the process of fermentation (Badotti et.al. 2008, Alves Jr. et.al. 2014). The known genotype of strain 1403-7A, is as follows: *MAT a gal3 gal4 MAL4 MGL3 trp1 ura3*.*

Strain 1403-7A, ferments maltose, sucrose and α-methylglucoside but not galactose. Sucrose is fermented by maltase and there is no evidence for a functional *SUC* gene in this strain (Khan et. al. 1973). It is not known if the *MGL3* gene is allelic to any of the known *IMA* genes (*IMA1*-*IMA5*) characterized by Teste et. al. 2010. The *IMA* genes are responsible for isomaltase (EC 3.2.1.10) formation, an enzyme responsible for the hydrolysis of isomaltose, sucrose and α-methylglucoside.

Sucrose is rapidly fermented by strain 1403-7A, without the presence of a classical invertase. As long as sucrose can penetrate into the cell, it is hydrolysed. Several permease genes may be involved for the uptake of sucrose and other alpha-glucosides. The permease functions are provided by the *AGT1* gene and other *MALx1* genes. For example Badotti et.al. 2008 have shown that strain 1403-7A has *MAL21*, *MAL31* in addition to the *MAL41.*

*Nutritional markers in strain 1403-7A, such as *trp1* and *ura3* are very useful in genetic analysis (Khan 1979)

References:

1 Alves Jr. SL, Thevelein, JM and Stambuk, BU. 2014. Expression of *Saccharomyces cerevisiae*

under different growth conditions. Braz J Chem Eng 31(1)
2 Teste MA, Francois JM, and Parrou JL. 2010. Characterization of a new multigene family encoding isomaltases in the yeast *Saccharomyces cerevisiae*, the IMA family. J Biol Chem 285: 26815-26824.
3 Badotti F, Dario MG, Alves Jr. SL, Cordioli ML, Miletti, LC, Araujo PS de, and Stambuk BU. 2008. Switching the mode of sucrose utilization by *Saccharomyces cerevisiae*. Microbial Cell Factories, 7:4
4 Charron MJ, Michels CA. 1987. The constitutive, glucose-repression-insensitive mutation of the yeast MAL4 locus is an alteration of the MAL43 gene. Genetics 116:23-31.
5 Khan NA. 1979. Genetic control of maltase synthesis in yeast. IV. Function of the *MAL4* gene: extragenic suppression of a maltase negetive mutant. Mol Gen Genet 172(3):281-285.
6 Khan NA, Zimmermann FK and Eaton NR. 1973. Genetic and biochemical evidence of sucrose fermentation by maltase in yeast. Mol Gen Genet 123 (1):43-50
7 Khan NA, Eaton NR. 1971. Genetic control of maltase formation in yeast. I. Strains producing high and low basal levels of enzyme. Mol Gen Genet 112 (4):317-22.

IV Laboratory of Genetics, Genome Center of Wisconsin, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, J.F. Crow Institute for the Study of Evolution, University of Wisconsin, Madison, WI 53706, USA. Communicated by Chris Todd Hittinger <cthittinger@wisc.edu>.

Recent publications.

1 Baker E, Wang B, Bellora N, Peris D, Hulfachor AB, Koshalek JA, Adams M, Libkind D, Hittinger CT. 2015. The genome sequence of *Saccharomyces eubayanus* and the domestication of lager-brewing yeasts. Mol Biol Evol 32: 2818-31. doi: 10.1093/molbev/msv168.

The dramatic phenotypic changes that occur in organisms during domestication leave indelible imprints on their genomes. Although many domesticated plants and animals have been systematically compared with their wild genetic stocks, the molecular and genomic processes underlying fungal domestication have received less attention. Here, we present a nearly complete genome assembly for the recently described yeast species *Saccharomyces eubayanus* and compare it to the genomes of multiple domesticated alloploid hybrids of *S. eubayanus* × *S. cerevisiae* (*S. pastorianus* syn. *S. carlsbergensis*), which are used to brew lager-style beers. We find that the *S. eubayanus* subgenomes of lager-brewing yeasts have experienced increased rates of evolution since hybridization, and that certain genes involved in metabolism may have been particularly affected. Interestingly, the *S. eubayanus* subgenome underwent an especially strong shift in selection regimes, consistent with more extensive domestication of the *S. cerevisiae* parent prior to hybridization. In contrast to recent proposals that lager-brewing yeasts were domesticated following a single hybridization event, the radically different neutral site divergences between the subgenomes of the two major lager yeast lineages strongly favor at least two independent origins for the *S. cerevisiae* × *S. eubayanus* hybrids that brew lager beers. Our findings demonstrate how this industrially important hybrid has been domesticated along similar evolutionary trajectories on multiple occasions.

2 Alexander WG, Peris D, Pfannenstiel BT, Opulente DA, Kuang M, Hittinger CT. 2015. Efficient engineering of marker-free synthetic allotetraploids of *Saccharomyces*. Fungal Genet Biol - doi: 10.1016/j.fgb.2015.11.002.

Saccharomyces interspecies hybrids are critical biocatalysts in the fermented beverage industry, including in the production of lager beers, Belgian ales, ciders, and cold-fermented wines. Current methods for making synthetic interspecies hybrids are cumbersome and/or require genome modifications. We have developed a simple, robust, and efficient method for generating allotetraploid strains of prototrophic *Saccharomyces* without sporulation or nuclear genome manipulation. *S. cerevisiae x S. eubayanus*, *S. cerevisiae x S. kudriavzevii*, and *S. cerevisiae* × *S. uvarum* designer hybrid strains were created as synthetic lager, Belgian, and cider strains, respectively. The ploidy and hybrid nature of the

strains were confirmed using flow cytometry and PCR-RFLP analysis, respectively. This method provides an efficient means for producing novel synthetic hybrids for beverage and biofuel production, as well as for constructing tetraploids to be used for basic research in evolutionary genetics and genome stability.

3 Hittinger CT, Rokas A, Bai FY, Boekhout T, Gonçalves P, Jeffries TW, Kominek J, Lachance MA, Libkind D, Rosa CA, Sampaio JP, Kurtzman CP. Genomics and the making of yeast biodiversity. Curr Opin Genet Dev - in press.

Yeasts are unicellular fungi that do not form fruiting bodies. Although the yeast lifestyle has evolved multiple times, most known species belong to the subphylum Saccharomycotina (syn. Hemiascomycota, hereafter yeasts). This diverse group includes the premier eukaryotic model system, *Saccharomyces cerevisiae*; the common human commensal and opportunistic pathogen, *Candida albicans*; and over 1,000 other known species (with more continuing to be discovered). Yeasts are found in every biome and continent and are more genetically diverse than angiosperms or chordates. Ease of culture, simple life cycles, and small genomes (~10-20 Mbp) have made yeasts exceptional models for molecular genetics, biotechnology, and evolutionary genomics. Here we discuss recent developments in understanding the genomic underpinnings of the making of yeast biodiversity, comparing and contrasting natural and human-associated evolutionary processes. Only a tiny fraction of yeast biodiversity and metabolic capabilities has been tapped by industry and science. Expanding the taxonomic breadth of deep genomic investigations will further illuminate how genome function evolves to encode their diverse metabolisms and ecologies.

4 Zhou X, Peris D, Hittinger CT, Rokas A. *in silico* Whole Genome Sequencer & Analyzer (iWGS): a computational pipeline to guide the design and analysis of *de novo* genome sequencing studies. BioRxiv preprint server. doi: http://dx.doi.org/10.1101/028134

The availability of genomes across the tree of life is highly biased toward vertebrates, pathogens, human disease models, and organisms with small and streamlined genomes. Recent progress in genomics has enabled the de novo decoding of the genome of virtually any organism, greatly expanding its potential for understanding the biology and evolution of the full spectrum of biodiversity. The increasing diversity of sequencing technologies, assays, and de novo assembly algorithms have augmented the complexity of de novo genome sequencing projects in non-model organisms. To reduce the costs and challenges in de novo genome sequencing projects and streamline their experimental design and analysis, we developed iWGS (in silico Whole Genome Sequencer and Analyzer), an automated pipeline for guiding the choice of appropriate sequencing strategy and assembly protocols. iWGS seamlessly integrates the four key steps of a de novo genome sequencing project: data generation (through simulation), data quality control, de novo assembly, and assembly evaluation and validation. The last three steps can also be applied to the analysis of real data. iWGS is designed to enable the user to have great flexibility in testing the range of experimental designs available for genome sequencing projects, and supports all major sequencing technologies and popular assembly tools. Three case studies illustrate how iWGS can guide the design of de novo genome sequencing projects and evaluate the performance of a wide variety of user-specified sequencing strategies and assembly protocols on genomes of differing architectures. iWGS, along with a detailed documentation, is freely available at http://as.vanderbilt.edu/rokaslab/tools.html.

V VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland. Communicated by Brian Gibson <brian.gibson@vtt.fi>.

Recent publications.

1 Alff-Tuomala S, Salusjärvi L, Barth D, Oja M, Penttilä M, Pitkänen JP, Ruohonen L, Jouhten P. 2015. Xylose-induced dynamic effects on metabolism and gene expression in engineered *Saccharomyces cerevisiae* in anaerobic glucose-xylose cultures. Appl Microbiol Biotechnol. Oct 10. [Epub ahead of print].

Xylose is present with glucose in lignocellulosic streams available for valorisation to biochemicals. *Saccharomyces cerevisiae* has excellent characteristics as a host for the bioconversion, except that it strongly prefers glucose to xylose, and the co-consumption remains a challenge. Further, since xylose is not a natural substrate of *S. cerevisiae*, the regulatory response it induces in an engineered strain cannot be expected to have evolved for its utilisation. Xylose-induced effects on metabolism and gene expression during anaerobic growth of an engineered strain of *S. cerevisiae* on medium containing both glucose and xylose medium were quantified. The gene expression of S. cerevisiae with an XR-XDH pathway for xylose utilisation was analysed throughout the cultivation: at early cultivation times when mainly glucose was metabolised, at times when xylose was co-consumed in the presence of low glucose concentrations, and when glucose had been depleted and only xylose was being consumed. Cultivations on glucose as a sole carbon source were used as a control. Genome-scale dynamic flux balance analysis models were simulated to analyse the metabolic dynamics of *S. cerevisiae*. The simulations quantitatively estimated xylose-dependent flux dynamics and challenged the utilisation of the metabolic network. A relative increase in xylose utilisation was predicted to induce the bi-directionality of glycolytic flux and a redox challenge even at low glucose concentrations. Remarkably, xylose was observed to specifically delay the glucose-dependent repression of particular genes in mixed glucose-xylose cultures compared to glucose cultures. The delay occurred at a cultivation time when the metabolic flux activities were similar in the both cultures.

2 Wiebe MG, Nygård Y, Oja M, Andberg M, Ruohonen L, Koivula A, Penttilä M, Toivari M. 2015. A novel aldose-aldose oxidoreductase for co-production of D-xylonate and xylitol from D-xylose with *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol. 99:9439-47. doi: 10.1007/s00253-015-6878-5.

An open reading frame CC1225 from the *Caulobacter crescentus* CB15 genome sequence belongs to the Gfo/Idh/MocA protein family and has 47 % amino acid sequence identity with the glucose-fructose oxidoreductase from *Zymomonas mobilis* (Zm GFOR). We expressed the ORF CC1225 in the yeast *Saccharomyces cerevisiae* and used a yeast strain expressing the gene coding for Zm GFOR as a reference. Cell extracts of strains overexpressing CC1225 (renamed as Cc aaor) showed some Zm GFOR type of activity, producing D-gluconate and D-sorbitol when a mixture of D-glucose and D-fructose was used as substrate. However, the activity in Cc aaor expressing strain was >100-fold lower compared to strains expressing Zm gfor. Interestingly, *C. crescentus* AAOR was clearly more efficient than the Zm GFOR in converting in vitro a single sugar substrate D-xylose (10 mM) to xylitol without an added cofactor, whereas this type of activity was very low with Zm GFOR. Furthermore, when cultured in the presence of D-xylose, the *S. cerevisiae* strain expressing Cc aaor produced nearly equal concentrations of D-xylonate and xylitol (12.5 g D-xylonate l(-1) and 11.5 g D-xylitol l(-1) from 26 g D-xylose l^{-1}), whereas the control strain and strain expressing Zm gfor produced only D-xylitol (5 g l^{-1}). Deletion of the gene encoding the major aldose reductase, Gre3p, did not affect xylitol production in the strain expressing Cc aaor, but decreased xylitol production in the strain expressing Zm gfor. In addition, expression of Cc aaor together with the D-xylonolactone lactonase encoding the gene xylC from *C. crescentus* slightly increased the final concentration and initial volumetric production rate of both D-xylonate and D-xylitol. These results suggest that *C. crescentus* AAOR is a novel type of oxidoreductase able to convert the single aldose substrate D-xylose to both its oxidized and reduced product.

3 Knoshaug E, Vidgren V, Magalhães F, Jarvis E, Franden M, Zhang M, Singh A. 2015. Novel transporters from *Kluyveromyces marxianus* and *Pichia guilliermondii* expressed in *Saccharomyces cerevisiae* enable growth on l-arabinose and d-xylose. Yeast 32:615-628.

Genes encoding L-arabinose transporters in *Kluyveromyces marxianus* and *Pichia guilliermondii* were identified by functional complementation of *Saccharomyces cerevisiae* whose growth on L-arabinose was dependent on a functioning L-arabinose transporter, or by screening a differential display library, respectively. These transporters also transport D-xylose and were designated KmAXT1 (arabinose–xylose transporter) and PgAXT1, respectively. Transport assays using L-arabinose showed that KmAxt1p has Km 263mM and Vmax 57 nM/mg/min, and PgAxt1p has Km 0.13mM and Vmax

18 nM/mg/min. Glucose, galactose and xylose significantly inhibit L-arabinose transport by both transporters. Transport assays using D-xylose showed that KmAxt1p has Km 27mM and Vmax 3.8 nM/mg/min, and PgAxt1p has Km 65mM and Vmax 8.7 nM/mg/min.Neither transporter is capable of recovering growth on glucose or galactose in a *S. cerevisiae* strain deleted for hexose and galactose transporters. Transport kinetics of *S. cerevisiae* Gal2p showed Km 371mM and Vmax 341 nM/mg/min for L-arabinose, and Km 25mM and Vmax 76 nM/mg/min for galactose. Due to the ability of Gal2p and these two newly characterized transporters to transport both L-arabinose and D-xylose, one scenario for the complete usage of biomass-derived pentose sugars would require only the low-affinity, high-throughput transporter Gal2p and one additional high-affinity general pentose transporter, rather than dedicated D-xylose or L-arabinose transporters. Additionally, alignment of these transporters with other characterized pentose transporters provides potential targets for substrate recognition engineering.

VI Yeast Molecular Genetics Laboratory, Institute of Molecular Biology "Acad. Rumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev str., 1113 Sofia, Bulgaria. Communicated by George Miloshev <miloshev@bio21.bas.bg> - http://www.chromatinepigenetics.com

The following are abstracts of recently published papers or attended conferences of the group.

1 Milcheva D, Serkedjiev M, Zagorchev P, Georgieva M, Miloshev G. 2015. Yeast chromatin remodeling mutants show features of accelerated ageing. Comptes rendus Acad bulgare Sci 68(7):877-882.

Studying the intricate effects of chromatin dynamics on ageing has brought the need to delve deeper into the molecular processes of the aged organism. Applying the concept of “the sum of its parts” we prospect the idea of accelerated ageing induced by the inefficiency in higher-order chromatin structure maintenance. The aberrant effects of ageing are multifactorial in nature and it is difficult to credit all of them in a single research. Therefore, in the current work we present results that follow the flow of already collected information on the subject by presenting a series of results summing some patterns of ageing of *Saccharomyces cerevisiae* chromatin remodeling mutants. Our experiments elucidate the role of the linker histone, Hho1p and Arp4p, a component of chromatin remodeling complexes in chromatin dynamics by collecting data from aged and UVC irradiated yeast mutants.

2 Milcheva D, Serkedjiev M, Staneva D, Zagorchev P, Georgieva M, Miloshev G. 2015. Epigenetic significance of chromatin structure in cellular ageing. Sci Technol 5(1) 13-17.

Ageing is a process characterized by accumulation of structural and metabolic aberrations which gradually reduce resistance of cells to internal and external stress conditions. Chromatin is a DNA-protein complex that represents the compaction of DNA in the eukaryotic nucleus and is the platform for all epigenetic processes in the cell. There are lots of data showing the basic epigenetic role of chromatin, especially at its higher levels of compaction in cellular ageing. In the current experiments we have followed the role of the linker histone H1 and Arp4p, which is a component of three chromatin remodeling complexes, in chromatin dynamics during ageing. We have observed decelerated cellular growth and changes associated with cellular morphology of the studied mutants in the time course of ageing.

3 Georgieva M, Staneva D, Milcheva D, Serlkedjiev M, Uzunova K, Efremov T, Zagorchev P, Miloshev G. 2015. Epigenetic significance of linker histones in ageing and stress resilience. Anniversary conference of the Roumen Tsanev Institute of Molecular Biology 5-6 October 2015, Sofia, Bulgaria.

Certainly, everyone is tempted to slow the process of growing old and to live longer, however, only few probably understand that ageing is a very intricate and quite individual process. Apparently, ageing is governed by many interconnected factors, genetic and environmental and therefore is a podium for extensive research. Among the numerous model organisms which are used in ageing-related studies the yeast *Saccharomyces cerevisiae* is famous for its many advantages. It is a unicellular eukaryote with very well-known genetics and biology. Moreover, it is an organism which permits separate studying of

chronological and replicative ageing. We will present our recent results on the ageing of yeast double mutants lacking the gene for the linker histone in combination with a point mutation in Arp4p (actin-related protein 4), an important subunit of several chromatin modifying complexes. By compromising higher-order chromatin structures we have followed the chronological ageing in these mutants. A complex interplay between H1 and Arp4p emerged which in turn was imperative for the ageing of the cells. Moreover, the observed interaction between these proteins resulted in altered ability of the cells to withstand different stress conditions.

VII Department of Food Science and Technology, Oregon State University, Corvallis, OR, USA. Communicated by AT Bakalinsky <alan.bakalinsky@oregonstate.edu>.

Recent publications.

1 Ding, J, Holzwarth G, Bradford S, Cooley B, Yoshinaga AS, Patton-Vogt J, Abeliovich H, Penner MH, Bakalinsky AT. 2015. *PEP3* overexpression shortens lag phase but does not alter growth rate in *Saccharomyces cerevisiae* exposed to acid stress. Appl Micro Biotech 99:8667-8680 - doi:10.1007/s00253-015-6708-9.

In fungi, two recognized mechanisms contribute to pH homeostasis: the plasma membrane proton-pumping ATPase that exports excess protons and the vacuolar proton pumping ATPase (V-ATPase) that mediates vacuolar proton uptake. Here, we report that overexpression of *PEP3* which encodes a component of the HOPS and CORVET complexes involved in vacuolar biogenesis, shortened lag phase in *Saccharomyces cerevisiae* exposed to acetic acid stress. By confocal microscopy, *PEP3*-overexpressing cells stained with the vacuolar membrane-specific dye, FM4-64 had more fragmented vacuoles than the wild-type control. The stained overexpression mutant was also found to exhibit about 3.6-fold more FM4-64 fluorescence than the wild-type control as determined by flow cytometry. While the vacuolar pH of the wild-type strain grown in the presence of 80 mM acetic acid was significantly higher than in the absence of added acid, no significant difference was observed in vacuolar pH of the overexpression strain grown either in the presence or absence of 80 mM acetic acid. Based on an indirect growth assay, the *PEP3*-overexpression strain exhibited higher V-ATPase activity. We hypothesize that *PEP3* overexpression provides protection from acid stress by increasing vacuolar surface area and V-ATPase activity and, hence, proton-sequestering capacity.

2 Osborn RA, Almabruk KH, Holzwarth G, Asamizu S, LaDu J, Kean K, Karplus PA, Tanguay RL, Bakalinsky AT, Mahmud T. 2015. *De novo* synthesis of a sunscreen compound in vertebrates. eLife 2015;4:e05919.

Ultraviolet-protective compounds, such as mycosporine-like amino acids (MAAs) and related gadusols produced by some bacteria, fungi, algae, and marine invertebrates, are critical for the survival of reef-building corals and other marine organisms exposed to high-solar irradiance. These compounds have also been found in marine fish, where their accumulation is thought to be of dietary or symbiont origin. In this study, we report the unexpected discovery that fish can synthesize gadusol de novo and that the analogous pathways are also present in amphibians, reptiles, and birds. Furthermore, we demonstrate that engineered yeast containing the fish genes can produce and secrete gadusol. The discovery of the gadusol pathway in vertebrates provides a platform for understanding its role in these animals, and the possibility of engineering yeast to efficiently produce a natural sunscreen and antioxidant presents an avenue for its large-scale production for possible use in pharmaceuticals and cosmetics.

3 Ding J, Holzwarth G, Penner MH, Patton-Vogt J, Bakalinsky AT. 2015. Overexpression of acetyl-CoA synthetase in *Saccharomyces cerevisiae* increases acetic acid tolerance. FEMS Micro Lett 362:1-7.

Acetic acid-mediated inhibition of the fermentation of lignocellulose-derived sugars impedes development of plant biomass as a source of renewable ethanol. In order to overcome this inhibition, the capacity of *Saccharomyces cerevisiae* to synthesize acetyl-CoA from acetic acid was increased

by overexpressing *ACS2* encoding acetyl-coenzyme A synthetase. Overexpression of *ACS2* resulted in higher resistance to acetic acid as measured by an increased growth rate and shorter lag phase relative to a wild-type control strain, suggesting that Acs2-mediated consumption of acetic acid during fermentation contributes to acetic acid detoxification.

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Recent publication.

1 Vanek T, Halecky M, Paca J, Zapotocky L, Gelbicova T, Vadkertiova R, Kozliak E, Jones K. 2015. A two-stage combined trickle bed reactor/biofilter for treatment of styrene/acetone vapor mixtures. J Environ Sci Health, Part A 50:1148–1159.

Performance of a two-stage biofiltration system was investigated for removal of styrene-acetone mixtures. High steady-state acetone loadings (above Cin Ac D 0.5 g.m-3 corresponding to the loadings > 34.5 $g.m^{-3}.h^{-1}$) resulted in a significant inhibition of the system's performance in both acetone and styrene removal. This inhibition was shown to result from the acetone accumulation within the upstream trickle-bed bioreactor (TBR) circulating mineral medium, which was observed by direct chromatographic measurements. Placing a biofilter (BF) downstream to this TBR overcomes the inhibition as long as the biofilter has a sufficient bed height. A different kind of inhibition of styrene biodegradation was observed within the biofilter at very high acetone loadings (above Cin Ac D 1.1 $g.m^{-3}$ or 76 $g.m^{-3}.h^{-1}$ loading). In addition to steady-state measurements, dynamic tests confirmed that the reactor overloading can be readily overcome, once the accumulated acetone in the TBR fluids is degraded. No sizable metabolite accumulation in the medium was observed for either TBR or BF. Analyses of the biodegradation activities of microbial isolates from the biofilm corroborated the trends observed for the two-stage biofiltration system, particularly the occurrence of an inhibition threshold by excess acetone.

2 Paulovičová L, Paulovičová E, Karelin AA, Tsvetkov YE, Nifantiev NE, Bystricky S. 2015. Immune cell response to Candida cell wall mannan derived branched a-oligomannoside conjugates in mice. J Microbiol Immunol Infect 48:9–19.

Background: Constructs composed of cell wall mannan-derived moieties conjugated to immunogenic proteins could be promising agents for induction of protective anti-Candida immune responses. Methods: This report is focused on the cellular immune response differences induced by BSAbased conjugates bearing synthetic a-1,6-branched oligomannosides. For monitoring of the immune responses following active immunization we evaluated changes in the frequencies of T and B lymphocytes and their activation status in the blood and spleen. We compared the immunization -induced changes of co-stimulatory molecules CD80 and CD86 expression on blood neutrophils and Th1/Th2 polarization of the immune response based on IFN-g, TNF-a (pro-Th1), IL-4, and IL-10 (pro-Th2) cytokines levels and induction of IL-17. Results: The results pointed out a comparable effect of the conjugates on the modulation of T and B lymphocytes frequencies in blood and spleen. Both conjugates induced upregulation of CD25 surface antigen on CD4ţ T lymphocytes, independently on the structural differences of oligosaccharides. The differences in structure of oligomannoside antigens or conjugate constructs were reflected in the increase of co-stimulatory molecules CD80 and CD86 expression on neutrophils, and in induced cytokine response. M5eBSA conjugate induced only a slight increase in CD80 expression but a significant increase in IFN-g, TNF-a, and IL-10. M6eBSA conjugate induced a significant increase of CD80 expression and increase of TNF-a, IL-4, and IL-10.

3 Paulovicova E, Bujdakova H, Chupacova J, Paulovičova L, Kertys P, Hrubiško M. 2014. Humoral immune responses to *Candida albicans* complement receptor 3-related protein in the atopic subjects with vulvovaginal candidiasis. Novel sensitive marker for *Candida* infection. FEMS Yeast Res 15: 1–8.

In vitro evaluation of specific anti-*Candida albicans* sera antibodies based on synthetically prepared complement receptor 3-related protein (CR3-RP) mimicking the structure of native complement receptor 3 in a cohort of 72 patients with atopy and recurrent *Candida* vulvovaginitis (RVC) revealed effective humoral response against *Candida* CR3-RP. The most significant have been IgM and IgA isotype antibodies (33 and 47% positive cases, respectively). The quantitative evaluation of anti-CR3RP isotype antibodies was confronted with results of commercial ELISA anti-*C. albicans* antibodies diagnostics based on *C. albicans* cell wall mannan and *β*-glucan antigens, the most significant correlation being observed with anti-CR3-RP IgM and anti-*β*-D-glucan IgM ($r2 = 0.624$) followed by isotype IgA ($r2 = 0.381$). The immunogenicity and immunoreactivity of CR3RP antigen in RVC patients' sera had been evaluated with regard to the results reached by ounterimmuno electrophoresis and heterogeneous enzyme immunoassay. Obviously, synthetically prepared CR3-RP mimicking the *Candida* cell-wall-derived structure moiety represents a promising immunological tool not only for *Candida* serodiagnostics, but also prospectively for follow-up of targeted antifungal therapy and as promising *Candida* vaccine candidate.

4 Nemcová K, Breierová E, Paulovičová E. 2015. Influence of copper ions on the yeast diversity associated with grapes and must Chem. Listy 109:456-462.

The diversity of yeasts and yeast microorganisms on grapes and, consequently, in musts may be strongly influenced by using Cu2+ products. The effect of Cu2+ concentration on the growth of native yeast strains isolated from grapes and grape must of three grape varieties was studied. The growth of *H. uvarum*, *P. kluyveri*, *P. kudriavzevii*, *C. californica*, *F. elegans*, *R. bacarum* and *Sc. Crataegensis* was inhibited in the presence of a minimum concentration of 0.5 mM Cu2+. The growth of *S. cerevisiae*, *Cr. magnus*, *R. glutinis* and *Sp. pararoseus* depended on the strain tested. The strains of *A. pullulans*, *M. pulcherrima*, *C. oleophila*, *P. terricola*, *R. nothofagi* and *R. minúta* were growing at increasing Cu2+ concentrations but their growth was reduced by prolongation of the lag phase compared with their growth in the absence of Cu2+. The growth reduction of ethanol-tolerant yeast strains at higher concentration of Cu ions shows the effect of the prolonged action of wine fermentation. Autochthonous yeast strains resistant to higher Cu2+ concentrations (*C. oleophila*, *M. pulcherrima*, *R. minuta*) appear to be an appropriate solution to reduction of Cu residues in the fermenting must.

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Recent publications.

1 Sidari R, Caridi A, Howell KS. 2014. Wild *Saccharomyces cerevisiae* strains display biofilm-like morphology in contact with polyphenols from grapes and wine. International Journal of Food Microbiol 189:146-152.

Polyphenols are a major component of wine grapes, and contribute to color and flavor, but their influence upon yeast growth forms has not been investigated. In this work we have studied the effect of polyphenols on the ability of natural isolates of wine-related *Saccharomyces cerevisiae* strains to form biofilms attaching to plastic surfaces, to grow as mat colonies, to invade media, and to display filamentous growth. The use of carbon- and nitrogen-rich or deficient media simulated grape juice fermentation conditions. The addition of wine polyphenols to these media affected biofilm formation, and cells exhibited a wide variety of invasiveness and mat formation ability with associated different growth and footprint patterns. Microscopic observation revealed that some strains switched to filamentous phenotypes which were able to invade media. The wide range of phenotypic expression observed could have a role in selection of strains suitable for inoculated wine fermentations and may explain the persistence of yeast strains in vineyard and winery environments.

2 Caridi A, De Bruno A, Piscopo A, Poiana M, Sidari R 2015. Study of the inheritability of the yeast trait "interaction with natural antioxidant activity of red wine" in four generations of *Saccharomyces cerevisiae* and its enhancing by spore clone selection and hybridization. European Food Research and Technology 240:1059-1063.

The aim of this research was to study the inheritability of the yeast trait "interaction with natural antioxidant activity of red wine" and to enhance this trait by spore clone selection and hybridization of wine yeasts. This research was carried out using as a model three strains of *Saccharomyces cerevisiae* (wild types), 24 derived spore clones, three hybrids obtained crossing the derived spore clones and 34 spore clones derived from the three hybrids. Using these yeast strains, micro-winemaking trials were carried out utilizing grape must of the black varieties, *Cabernet* and *Magliocco*: the wines showed significant differences, due to the wine starter used, both for Folin–Ciocalteu's index and for DPPH analysis. Data validate the main role that wine yeast selection plays to enhance red wine content in antioxidant compounds. Data also demonstrate that using spore clone selection and hybridization, it is possible to significantly enhance the natural antioxidant activity of wines, so improving their quality and stability.

3 Caridi A, Sidari R, Kraková L, Kuchta T, Pangallo D 2015. Assessment of color adsorption by yeast using grape skin agar and impact on red wine color. Journal International des Sciences de la Vigne et du Vin (in press).

Aim: Evaluating *Saccharomyces cerevisiae* strains for their color adsorption aptitude by using *Grape Skin Agar* in order to protect the phenolic compounds responsible for the color of red wines; proposing a suitable and innovative medium to be included among the tests currently used for wine strain selection. Methods and results: The strains were identified by fluorescence-Internal transcribed spacer (f-ITS) PCR and PCR-Restriction fragment length polymorphism (RFLP), confirmed by sequencing of ITS fragment, and tested for the parameter "aptitude to adsorb polyphenolic compounds" on the innovative chromogenic medium *Grape Skin Agar*. Laboratory-scale fermentations were carried out in must with and without SO_2. The SO_2 determined a decrease in tint, color intensity, and total polyphenol content. The strains M2V CHU7 and M2F CHU9 produced wines with the lowest color intensity, with and without SO_2, respectively. By contrast, the strains M2F VUP4 and M2V CHU1, with and without SO_2, respectively, produced wines with the highest color intensity, and therefore, they could improve the production of red wines. Conclusion: The study highlights great variability and significant differences among strains in regard to their aptitude to modulate wine color. *Grape Skin Agar* should be a useful medium to be included in the selection tests currently performed for *S. cerevisiae* strains. Significance and impact of the study: Our study confirms that yeast strains can modulate the chromatic properties of red wines according to their aptitude to adsorb polyphenols, as tested on *Grape Skin Agar*. Combining colored polyphenolic compound adsorption assay on Petri plate and laboratory-scale fermentation trials provides an effective way to test yeasts for their capability to improve the chromatic quality of the wines.

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The following papers have been recently published or are in press.

1 Santiago IF, Soares MA, Rosa CA, Rosa LH. 2015. Lichensphere: a protected natural microhabitat of the non-lichenised fungal communities living in extreme environments of Antarctica. Extremophiles **19**:1087-1097.

We surveyed the diversity, distribution and ecology of non-lichenised fungal communities associated with the Antarctic lichens *Usnea antarctica* and *Usnea aurantiaco-atra* across Antarctica. The phylogenetic study of the 438 fungi isolates identified 74 taxa from 21 genera of *Ascomycota*, *Basidiomycota* and *Zygomycota*. The most abundant taxa were *Pseudogymnoascus* sp., *Thelebolus* sp., *Antarctomyces psychrotrophicus* and *Cryptococcus victoriae*, which are considered endemic and/or highly adapted to Antarctica. Thirty-five fungi may represent new and/or endemic species. The fungal communities displayed high diversity, richness and dominance indices; however, the similarity among the communities was variable. After discovering rich and diverse fungal communities composed of symbionts, decomposers, parasites and endemic and cold-adapted cosmopolitan taxa, we introduced the term "lichensphere". We hypothesised that the lichensphere may represent a protected natural microhabitat with favourable conditions able to help non-lichenised fungi and other Antarctic life forms survive and disperse in the extreme environments of Antarctica..

2 Gonçalves VN, Cantrell CL, Wedge DE, Ferreira MC, Soares MA, Jacob MR, Oliveira FS, Galante D, Alves TMA, Zani CL, Murta S, Romanha AJ, Kroon EG, Oliveira JG, Gomez-Silva B, Galetovic A, Rosa CA, Rosa LH 2015 Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. Environ Microbiol: doi:10.1111/1462-2920.13005, in press.

This study assessed the diversity of cultivable rock-associated fungi from Atacama Desert. A total of 81 fungal isolates obtained were identified as 29 *Ascomycota* taxa by sequencing different regions of DNA. *Cladosporium halotolerans*, *Penicillium chrysogenum* and *Penicillium* cf. *citrinum* were the most frequent species, which occur at least in four different altitudes. The diversity and similarity indices ranged in the fungal communities across the latitudinal gradient. The Fisher-α index displayed the higher values for the fungal communities obtained from the siltstone and fine matrix of pyroclastic rocks with finer grain size, which are more degraded. A total of 23 fungal extracts displayed activity against the different targets screened. The extract of *P. chrysogenum* afforded the compounds α-linolenic acid and ergosterol endoperoxide, which were active against *Cryptococcus neoformans* and methicillin-resistance *Staphylococcus aureus* respectively. Our study represents the first report of a new habitat of fungi associated with rocks of the Atacama Desert and indicated the presence of interesting fungal community, including species related with saprobes, parasite/pathogen and mycotoxigenic taxa. The geological characteristics of the rocks, associated with the presence of rich resident/resilient fungal communities suggests that the rocks may provide a favourable microenvironment fungal colonization, survival and dispersal in extreme conditions.

2 Morais CG, Lara CA, Oliveira ES, Péter G, Dlauchy D, Rosa CA. 2015. *Spencermartinsiella silvicola* sp. nov., a yeast species isolated from rotting wood. Int J Syst Evol Microbiol: doi: 10.1099/ijsem.0.000764

Three strains of a new xylanase-producing yeast species were isolated from rotting wood samples collected in the Atlantic Rain Forest of Brazil. The sequences of the ITS region and D1/D2 domains of the large subunit of the rRNA gene showed that this new yeast species belongs to the genus *Spencermartinsiella*, and its closest relatives among the recognized species are *S. europaea* and *S. ligniputridi*. The novel species *Spencermartinsiella silvicola* sp. nov. is proposed to accommodate these isolates. The type strain is UFMG-CM-Y274T (= CBS 13490T). The MycoBank number is MB 813053. In addition, *Candida cellulosicola* is reassigned to the genus *Spencermartinsiella* as a new combination.

3 Guamán-Burneo MC, Dussán KJ, Cadete RM, Cheab MAM, Portero P, Carvajal-Barriga E, da Silva SS, Rosa CA. 2015. Xylitol production by yeasts isolated from rotting Wood in the Galápagos Islands, Ecuador, and description of *Cyberlindnera galapagosensis* f.a., sp. nov. Antonie van Leeuwenhoek **108**: 919-931.

This study evaluated d-xylose-assimilating yeasts that are associated with rotting wood from the Galápagos Archipelago, Ecuador, for xylitol production from hemicellulose hydrolysates. A total of 140 yeast strains were isolated. Yeasts related to the clades *Yamadazyma, Kazachstania, Kurtzmaniella, Lodderomyces, Metschnikowia* and *Saturnispora* were predominant. In culture assays using sugarcane bagasse hemicellulose hydrolysate, *Candida tropicalis* CLQCA-24SC-125 showed the highest xylitol production, yield and productivity (27.1 g L^{-1} xylitol, $Y_{p/s}^{xyl}$ = 0.67 g g^{-1}, Q_p = 0.38 g L^{-1}. A new species of *Cyberlindnera,* strain CLQCA-24SC-025, was responsible for the second highest xylitol production (24 g L^{-1}, $Y_{p/s}^{xyl}$ = 0.64 g g^{-1}, Q_p = 0.33 g L^{-1} h^{-1}) on sugarcane hydrolysate. The new xylitol-producing species *Cyberlindnera galapagoensis* f.a., sp. nov., is proposed to accommodate the strain CLQCA-24SC-025^T (=UFMG-CM-Y517^T; CBS 13997^T). The MycoBank number is MB 812171.

4 Freitas LFD, Barboa R, Sampaio JP, Lachance MA, Rosa CA. 2015. *Starmera pilosocereana* sp. nov., a yeast isolated from necrotic tissue of cacti in a sandy coastal dune ecosystem. Int J Syst Evol Microbiol dói: 10.1099/ijsem.0.000596, in press.

Two strains of a new cactophilic yeast species were isolated from the columnar cactus *Pilosocereus arrabidae* in a sand dune ecosystem in Rio de Janeiro, Brazil. Phylogenetic analysis of sequences of the large subunit rRNA gene D1/D2 domains showed that the strains represent a sister species to *Starmera caribaea*, from which it differs by 21 nucleotide substitutions and two indels. The new species is heterothallic and the asci are deliquescent with the formation of two to four hat-shaped ascospores. The name *Starmera pilosocereana* sp. nov. is proposed to

accommodate the species. The type strain of *S. piloso-cereana* sp. nov. is UFMG-CM-Y316T (= CBS 13266T) and the allotype is UFMG-CM-Y346a (=CBS 13265). The Mycobank number is MB 810683. *Candida stellimalicola* belonging to the *Starmera* clade, is reassigned to *Starmera* as a new combination.

5 Lopes MR, Ferreira MC, Carvalho TFC, Pagnocca FC, Chagas RA, Morais PB, Rosa LH, Lachance MA, Rosa CA. 2015. *Yamadazyma riverae* sp. nov., a yeast species isolated from plant materials. Int J Syst Evol Microbiol doi: 10.1099/ijsem.0.000597, in press.

Nine strains of a novel yeast species were isolated from rotting wood, tree bark, ant nests or living as endophytes in leaves of *Vellozia gigantea*. Analysis of the sequences of the internal transcribed spacer (ITS) region and the D1/D2 domains of the large subunit rRNA gene showed that this species is related to *Candida insectorum* in the *Yamadazyma* clade. The new species differs from its closely related species by 10 and 11 substitutions in the ITS region and the D1/D2 domains of the large subunit of the rRNA gene, respectively. The species is heterothallic and forms asci with one to two hat-shaped ascospores. The name *Yamadazyma riverae* sp. nov. is proposed. The type strain of *Yamadazyma riverae* sp. nov. is UFMG-CM-Y444T (= CBS 14121) and the allotype strain is TT12 (= CBS 14098 = UFMG-CM-Y577). The Mycobank number is MB 813221.

6 Pulschen AA, Rodrigues F, Duarte RTD, Araújo GG, Santiago IF, Paulino-Lima IG, Rosa CA, Kato MJ, Pellizari VH, Galante D. 2015. UV-resistant yeasts isolated from a high-altitude volcanic área on the Atacama Desert as eukaryotic models for astrobiology. Microbiol Open 4:574-578.

The Sairecabur volcano (5971 m), in the Atacama Desert, is a high-altitude extreme environment with high daily temperature variations, acidic soils, intense UV radiation, and low availability of water. Four different species of yeasts were isolated from this region using oligotrophic media, identified and characterized for their tolerance to extreme conditions. rRNA sequencing revealed high identity (>98%) to *Cryptococcus friedmannii*, *Exophiala* sp., *Holtermanniella watticus*, and *Rhodosporidium toruloides*. To our knowledge, this is the first report of these yeasts in the Atacama Desert. All isolates showed high resistance to UV-C, UV-B and environmental-UV radiation, capacity to grow at moderate saline media (0.75–2.25 mol/L NaCl) and at moderate to cold temperatures, being *C. friedmannii* and *H. watticus* able to grow in temperatures down to −6.5°C. The presence of pigments, analyzed by Raman spectroscopy, correlated with UV resistance in some cases, but there is evidence that, on the natural environment, other molecular mechanisms may be as important as pigmentation, which has implications for the search of spectroscopic biosignatures on planetary surfaces. Due to the extreme tolerances of the isolated yeasts, these organisms represent interesting eukaryotic models for astrobiological purposes.

7 Cardoso VM, Borelli BM, Lara CA, Soares MA, Pataro C, Bodevan EC, Rosa CA. 2015. The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Res Int 69: 331-340.

The occurrence and effects of the dry and rainy seasons on yeast populations in traditional Serro Minas cheese, one of the most popular cheeses produced from raw milk in Brazil, were studied over the course of 60 days of ripening. Enzymatic activity exhibited by these yeast isolates was also studied. A total of 19 yeast species were identified via sequence analysis of the D1/D2 domains of the large subunit of the rRNA gene. Fourteen yeast species were obtained from cheese produced during the dry season, and fifteen species were obtained from cheese produced during the rainy season. High diversity indices for the yeast species were determined for cheese manufactured during both seasons (average $H_D = 1.7$ and $H_R = 1.5$, respectively). The predominant species in Serro Minas cheese included *Debaryomyces hansenii*, *Kodamaea ohmeri* and *Kluyveromyces marxianus*. *D. hansenii* 28.12 showed low lipolytic and high proteolytic activity. *K. marxianus* 83F and 60P demonstrated lipolytic and β-galactosidase activity, respectively. *K. ohmeri* 88A displayed low lipolytic and β-galactosidase activity. Maximal lipase, β-galactosidase and protease activity was observed at 20 °C and pH 6.0, 30 °C and pH 7.0 and 50 °C and pH 6.0, respectively. Considering that *D. hansenii* 28.12, *K. ohmeri* 88A and *K. marxianus* 60P together showed protease, lipase and β-galactosidase activity in this study, further research on the possibility of including these yeasts as part of a starter culture and research on their effects on the sensory properties of Serro Minas cheese merit more study.

8 Pereira CB, de Oliveira DM, Hughes AF, Kohlhoff M, Vieira MLA, Vaz ABM, Ferreira MC, Carvalho CR, Rosa LH, Rosa CA, Alves TM, Zani CL, Johann S, Cota BB. Endophytic fungal compounds active against *Cryptococcus neoformans* and *C. gattii*. J Antibiot 68:436-444.

Infections with *Cryptococcus* are invasive mycoses associated with significant morbidity and mortality, mainly in immunosuppressed patients. Several drugs have been introduced to combat these opportunistic infections. However, resistance of this organism to antifungal drugs has increased, causing difficulties in the treatment. The goal of this work was to evaluate the antifungal activity of ethanol extracts from endophytic fungi isolated from plants collected from different Brazilian ecosystems and to perform the fractionation of the most promising extract. Four-hundred fungal extracts were investigated by microdilution broth assays against *Cryptococcus neoformans* and *Cryptococcus gattii* at a concentration of 500 µg ml(-1). Among them, the extract of *Mycosphaerella* sp. UFMGCB 2032, an endophytic fungus isolated from the plant *Eugenia bimarginata* DC. (Myrtaceae) exhibited outstanding antifungal activity against *C. neoformans* and *C. gattii*, with MIC values of 31.2 µg ml(-1) and 7.8 µg ml(-1), respectively. The fractionation of this extract using liquid-liquid partitioning and semi-preparative HPLC afforded two eicosanoic acids with antifungal activity, compound 1, (2S,3R,4R)-(E)-2-amino-3,4-dihydroxy-2-(hydroxymethyl)-14-oxoeicos-6,12-dienoic acid with MIC values ranging from 1.3-2.50 µg ml(-1), and compound 2, known as myriocin, with MIC values of 0.5 µg ml(-1) against *C. neoformans* and *C. gattii*. These compounds are reported for the first time in the *Mycosphaerella* genus.

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The following book has been published by the scientific team of Fermentec and is available online.

1 Tailored yeast strains for ethanol production: Process-driven selection

This ebook was launched by Fermentec on October 21, 2015 about selection of tailored yeast strains for ethanol production in Brazil. The Brazilian processes of alcoholic fermentation are characterized by high concentration of yeast cells, short fermentation times, recycling of yeast cells and stressful conditions that can affect yeast cell viability and fermentation yields. On the other hand, there are several differences from one distillery to another, making each one unique. Some strains that ferment well in one distillery does not survive in other processes. Moreover, these Industrial fermentations are subject to contamination by wild yeasts (*Saccharomyces* and non-*Saccharomyces* species) that compete with selected yeast strains and dominate the yeast population causing serious operational difficulties and economic losses. However, tailored yeast strains are more robust, resistant to stressful conditions and compete better with contaminants than traditional strains do. These tailored strains have shown a higher rate of dominance and persistence in industrial fermentations in comparison with traditional strains and baker yeast. This e-book presents the results from the last eight years of monitoring and selection of yeast strains for the Brazilian ethanol industry: a process-driven selection. The e-book can be downloaded freely:

www.fermentec.com.br/capa.asp?pi=ebook.

XII Food and Bioproduct Sciences, College of Agriculture, University of Saskatchewan, c/o 1421 Saturna Drive, Parksville, B.C., Canada V9P2Y1. Communicated by W.M. (Mike) Ingledew <mike.ingledew@usask.ca>.

Although retired from the University of Saskatchewan for eight years and from Ethanol Technology Institute (a division of Lallemand Inc.) after 5 years as Scientific Director organizing The Alcohol School programs in Toulouse and in Montreal and serving as Senior Editor of the 2009, 5th edition of *The Alcohol Textbook*, I continue to lecture twice per year. These Schools are designed to provide the science behind the processes of making alcohol for distilled beverage and fuel alcohol production. I am also serving as a co-editor for the 6th Edition of this book scheduled to be published in 2016. A recent publication (biographical review requested by Editor Dr. C. Bamforth) outlining the work done in my lab from 1970-2007 and its applications to industry is now published.

1 Ingledew WM 2015 Wallowing with the yeasts used to make alcohol. J Amer Soc Brew Chemists 73(3):209-222" - http://dx.doi.org/10.1094/ASBCJ-2015-0614-01 or via email.

XIII Department of Biochemistry and Nutrition; Des Moines University, Des Moines, Iowa, USA. Communicated by Martin Schmidt <Martin.Schmidt@dmu.edu>.

Influence of boric acid on yeast morphogenesis

Over the past five years our laboratory has made steady progress in characterizing the impact of boric acid (BA) on living systems. BA is a small inorganic molecule with very useful properties: At low concentrations it promotes growth, wound healing, bone mineralization and cell wall stability. At higher concentrations it is toxic for all forms of life, making it a reliable agent for the control of a wide spectrum of pests ranging from bacteria to rodents. In medicine BA is used for the control of yeast vaginitis, particularly for the treatment of complicated recurrent infections. Selected aspects of BA action on *in vitro* enzyme activity are known; however, no comprehensive picture of BA stress on living cells has emerged and the root cause of BA toxicity is still elusive. In a groundbreaking study we have furthered the understanding of the antifungal properties that underlie the effectiveness of BA treatments for vaginitis [1]. It was found that in the human pathogenic yeast *Candida albicans* the agent selectively inhibits invasive growth by suppressing the yeast-to-mycelial transition. Much of our following work has been dedicated to explain these effects of BA on yeast cell biology and *C. albicans* polarized growth. Initial fluorescence studies with a pH-sensitive GFP variant (pHluorin) showed that BA does not influence the intracellular pH, ruling out the most obvious explanation that BA acts by acidifying the cytoplasm. The following studies on the model yeast *Saccharomyces cerevisiae* showed that BA impairs cytoskeletal integrity [5]. In *S. cerevisiae* the cytoskeleton prominently features a contractile actomyosin ring (CAR) at the bud neck. In the presence of BA the CAR disintegrates quickly. This forces *S. cerevisiae* to construct bulky default septa and causes a cytokinesis defect. We proceeded to screen comprehensive *S. cerevisiae* deletion collections to identify molecular determinants of BA tolerance [4]. The study failed to identify a specific pathway for BA resistance in *S. cerevisiae.* Instead, BA tolerance in *S. cerevisiae* is dependent on a conserved nonspecific environmental stress response. In a follow-up study we showed that in the dimorphic yeast *C. albicans* the major determinant of BA tolerance is cellular morphology [3]. In *C. albicans,* treatment with BA leads to a rapid reversible disintegration of the hyphal cytoskeleton and to a loss of polarized growth. *C. albicans* mutants with a constitutive hyphal morphology are particularly sensitive to BA since they cannot default to isotropic growth when polarity markers fail. This effect may well explain the efficacy of BA in the treatment of vaginal yeast infections: When polarized growth is impossible, *C. albicans* remains in the commensal yeast form and does not trigger an inflammatory response by invading epithelial cell layers [2]. Finally it should be remarked that no BA-resistant yeast mutant (defined as 2x elevated MIC) has ever been recovered. This suggests that BA is an antimicrobial agent against which no resistance can be developed – a useful property in the age of multidrug resistant microbes.

1 De Seta F, Schmidt M, Vu B, Essmann M, Larsen B. 2009. Antifungal mechanisms supporting boric acid therapy of Candida vaginitis. J Antimicrob Chemother 63: 325-336.

2 Jacobsen ID, Wilson D, Wachtler B, Brunke S, Naglik JR, Hube B. 2012. *Candida albicans* dimorphism as a therapeutic target. Expert Rev Anti Infect Ther 10: 85-93.

3 Pointer BR, Boyer MP, Schmidt M. 2015. Boric acid destabilizes the hyphal cytoskeleton and inhibits invasive growth of *Candida albicans*. Yeast 32: 389-398.

4 Schmidt M, Akasaka K, Messerly JT, Boyer MP. 2012. Role of *Hog1*, *Tps1* and *Sod1* in boric acid tolerance of *Saccharomyces cerevisiae*. Microbiology 158: 2667-2678.

5 Schmidt M, Schaumberg JZ, Steen CM, Boyer MP. 2010. Boric Acid disturbs cell wall synthesis in *Saccharomyces cerevisiae*. Int J Microbiol 2010: 930465.

XIV National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda MD, 20894 USA. Communicated by Conrad Schoch <schoch2@ncbi.nlm.nih.gov> and Barbara Robbertse <robberts@ncbi.nlm.nih.gov>.

Correcting the yeast records at NCBI

At NCBI and GenBank we constantly have to balance the need to archive the research community's sequence data provided and the ability to correct obvious mistakes during submissions. Developments at 2 NCBI databases (that exist as separate entities from GenBank) will improve the ability to apply accurate taxonomic

names for most GenBank users. The first is the annotation of type material in the NCBI Taxonomy database as explained in this blog post: http://www.ncbi.nlm.nih.gov/news/01-21-2014-sequence-by-type.

A more complete description is published (Federhen, 2015). In concert with this we expanded the curated database, RefSeq Targeted Loci (RTL) in order to provide a set of well validated sequences that can act as references for fungal identification (www.ncbi.nlm.nih.gov/refseq/targetedloci/). Our initial focus is on sequences from the region containing the ribosomal internal transcribed spacers (ITS) records extracted from type material. Interaction with expert taxonomists helped us to initially curate a selection of ITS records (Schoch et al. 2014) that has since been expanded to more than 3200. A more complete description of the ideas behind this can be found in this blog post at NCBI: http://ncbiinsights.ncbi.nlm.nih.gov/2015/05/11/accessing-the-hidden-kingdom-fungal-its-reference-sequences-2/#comments

The ITS region was proposed as a universally applicable DNA barcode for Fungi but many mycologists are aware of its limitations, especially for specific groups. In addition to focusing on this marker we currently include RTL projects for the large and small nuclear ribosomal subunits as well. Although these markers do not have the phylogenetic representation of ITS, we recently expanded RTL with more than 500 curated large subunit records containing the variable D1 and D2 regions. We also aim to continue the extension of type material at NCBI Taxonomy so that any sequence record in the NCBI Nucleotide database tied to type material can be identified with a simple query. The dilemma of potential sequence inaccuracies still remains, because these sequences are not as intensively reviewed as those in the individually annotated RTL projects. A final aim is to allow taxonomically accurate full genome comparisons. It will be essential to have accurate strain information from public culture collections available so that genomes can be verified in a similar fashion.

The yeasts, although not a phylogenetically coherent group, represents a compelling target for re annotation and improvement in the public sequence databases. In most cases information on type strains is well known and accessibly through culture collection databases. Additionally, many research practices are shared with other microbes and efforts on improving the accuracy of bacterial and archaeal data can apply to this group as well. This includes current NCBI efforts to update bacterial genome integrity. Scott Federhen, the head of our taxonomy group, recently discussed ways to change and correct bacterial names at the ASM-NGS conference (http://conferences.asm.org/index.php/component/content/article/122-conferences/2015-rapid-ngs-bioinformatic-pipelines-for-enhanced-molecular-epidemiologic-investigation-of-pathogens/251-conference-scope)

This is discussed in a blog commentary from a third party here: http://dna-barcoding.blogspot.ca/2014/11/type-material-on-genbank.html.

As part of this process we want to reach out to the yeast research community for information to improve our yeast taxonomic representation extensively. This will include building up a comprehensive list of type strains and comparing our complete classification with those in MycoBank and Index Fungorum. It should result in easily accessible lists of sequence markers for download and improved options to fine tune BLAST searches. These efforts are still in an early phase and will depend on scientific publications as well as direct interactions with the yeast research community. We hope to engage all interested parties as we proceed.

References:

1 Schoch CL, Robbertse B, Robert V, Vu D, Cardinali G, Irinyi L, et al. 2014. Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. Database: The Journal of Biological Databases and Curation: bau061. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4075928/

2 Federhen S. 2015. Type material in the NCBI Taxonomy Database. Nucleic Acids Research, 43(Database issue): D1086–D1098. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4383940/

XV Food Biotechnology department. Institute of Agrochemistry and Food Technology (IATA, CSIC). P.O. Box 73. E-46100 Burjassot, Valencia, Spain. Communicated by David Peris <david.perisnavarro@wisc.edu>.

Recent publication.

1 Peris D, Pérez-Través L, Belloch C, Querol A 2016 Enological characterization of Spanish *Saccharomyces kudriavzevii* strains, one of the closest relatives to parental strains of winemaking and brewing *S. cerevisiae* × *S. kudriavzevii* hybrids. Food Microbiol 53(B):31–40

Wine fermentation and innovation have focused mostly on *Saccharomyces cerevisiae* strains. However, recent studies have shown that other *Saccharomyces* species can also be involved in wine fermentation or are useful for wine bouquet, such as *S. uvarum* and *S. paradoxus*. Many interspecies hybrids have also been isolated from wine fermentation, such as *S. cerevisiae* × *S. kudriavzevii* hybrids. In this study, we explored the genetic diversity and fermentation performance of Spanish *S. kudriavzevii* strains, which we compared to other *S. kudriavzevii* strains. Fermentations of red and white grape musts were performed, and the phenotypic differences between Spanish *S. kudriavzevii* strains under different temperature conditions were examined. An ANOVA analysis suggested striking similarity between strains for glycerol and ethanol production, although a high diversity of aromatic profiles among fermentations was found. The sources of these phenotypic differences are not well understood and require further investigation. Although the Spanish *S. kudriavzevii* strains showed desirable properties, the quality for must fermentations was no better than those produced with a commercial *S. cerevisiae.* We suggest hybridization or directed evolution as methods to improve and innovate wine.

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The following papers were recently published or accepted for publication.

1 Fátima CO Gomes, Silvana VB Safar, Andrea R Marques, Adriana O Medeiros, Ana Raquel O Santos, Cláudia Carvalho, Marc-André Lachance, José Paulo Sampaio & Carlos A Rosa. 2015 The diversity and extracelular enzymatic activities of yeasts isolated from water tanks of *Vrisea minarum*, na endangered bromeliad species in Brazil, and the description of *Occultifur brasiliense* f.a., sp. nov. Antonie van Leeuwenhoek 107: 597-611.

The diversity of yeast species collected from the bromeliad tanks of *Vriesea minarum*, an endangered bromeliad species, and their ability to produce extracellular enzymes were studied. Water samples were collected from 30 tanks of bromeliads living in a rupestrian field site located at Serrada Piedade, Minas Gerais state, Brazil, during both the dry and rainy seasons. Thirty-six species were isolated, representing 22 basidiomycetous and 14 ascomycetous species. *Occultifur* sp., *Cryptococcus podzolicus* and *Cryptococcus* sp. 1 were the prevalent basidiomycetous species. The yeast-like fungus from the order Myriangiales, *Candida silvae* and *Aureobasidium pullulans* were the most frequent ascomycetous species. The diversity of the yeast communities obtained between seasons was not significantly different, but the yeast composition per bromeliad was different between seasons. These results suggest that there is significant spatial heterogeneity in the composition of populations of the yeast communities within bromeliad tanks, independent of the season. Among the 352 yeast isolates tested, 282 showed at least one enzymatic activity. Protease activity was the most widely expressed extracellular enzymatic activity, followed by xylanase, amylase, pectinase and cellulase activities. These enzymes may increase the carbon and nitrogen availability for the microbial food web in the bromeliad tank of *V. minarum*. Sequence analyses revealed the existence of 10 new species, indicating that bromeliad tanks are important sources of new yeasts. The novel species *Occultifur brasiliensis*, f.a., sp. nov., is proposed to accommodate the most frequently isolated yeast associated with *V. minarum*. The type strain of *O. brasiliensis*, f.a., sp. nov. is UFMG-CM-Y375^T (= CBS 12687^T). The Mycobank number is MB 809816.

2 Virginia de Garcia, Marco A Coelho, Teresa M Maia, Luiz H Rosa, Aline Martins Vaz, Carlos A. Rosa, José Paulo Sampaio, Paula Gonçalves, María van Broock & Diego Libkind. 2015. Sex in the cold: taxonomic reorganization of psychrotolerant yeasts in the order Leucosporidiales. FEMS Yeast Research 15: 4.

Species of Leucosporidiales are a group of psychrotolerant yeasts with biotechnological potential. In the present work, we studied the phenotypic, genetic and sexual characteristics of three species of this genus (*Leucosporidium scottii*, *Leucosporidiella creatinivora* and *Le. yakutica*) to clarify the evolutionary relationship among these closely related taxa. From the results obtained, it becomes clear that these yeasts can interbreed. Although genetic delimitation is possible for the three species, the extent of nucleotide substitutions and phenotypic differences observed between them are lower than that expected for species that have ended the speciation process. Our taxonomic conclusion is to maintain the three taxa until further genomic data are gathered. However the

concept of *Leucosporidium scottii*species-complex is proposed for this group of species. Finally, we transfer all *Leucosporidiella* and*Mastigobasidium* species to *Leucosporidium* (Leucosporidiales), and, in order to end the polyphyly condition of these taxa, we propose the new genus *Pseudoleucosporidium* gen. nov. and the new combination *P. fasciculatum* comb. nov.

3 Marco A Coelho, João MGCF Almeida, Chris Todd Hittinger & Paula Gonçalves. 2015. Draft Genome Sequence of *Sporidiobolus salmonicolor* CBS 6832, a Red-Pigmented Basidiomycetous. Genome Announcements 3:e00444-15.

We report the genome sequencing and annotation of the basidiomycetous red-pigmented yeast *Sporidiobolus salmonicolor* strain CBS 6832. The current assembly contains 395 scaffolds, for a total size of about 20.5 Mb and a G+C content of ~61.3%. The genome annotation predicts 5,147 putative protein-coding genes.

4 Teresa M. Maia, Susana T. Lopes, João MGCF Almeida, Luiz H. Rosa, José Paulo Sampaio, Paula Gonçalves & Marco A Coelho. 2015. Evolution of mating systems in Basidiomycetes and the genetic architecture underlying mating-type determination in the yeast *Leucosporidium scottii*. Genetics 201: 75-89.

In most fungi, sexual reproduction is bipolar; that is, two alternate sets of genes at a single mating-type (*MAT*) locus determine two mating types. However, in the Basidiomycota, a unique (tetrapolar) reproductive system emerged in which sexual identity is governed by two unlinked *MAT* loci, each of which controls independent mechanisms of self/nonself recognition. Tetrapolar-to-bipolar transitions have occurred on multiple occasions in the Basidiomycota, resulting, for example, from linkage of the two *MAT* loci into a single inheritable unit. Nevertheless, owing to the scarcity of molecular data regarding tetrapolar systems in the earliest-branching lineage of the Basidiomycota (subphylum Pucciniomycotina), it is presently unclear if the last common ancestor was tetrapolar or bipolar. Here, we address this question, by investigating the mating system of the Pucciniomycotina yeast *Leucosporidium scottii*. Using whole-genome sequencing and chromoblot analysis, we discovered that sexual reproduction is governed by two physically unlinked gene clusters: a multiallelic homeodomain (*HD*) locus and a pheromone/receptor (*P/R*) locus that is biallelic, thereby dismissing the existence of a third *P/R* allele as proposed earlier. Allele distribution of both *MAT* genes in natural populations showed that the two loci were in strong linkage disequilibrium, but independent assortment of *MAT* alleles was observed in the meiotic progeny of a test cross. The sexual cycle produces fertile progeny with similar proportions of the four mating types, but approximately 2/3 of the progeny was found to be nonhaploid. Our study adds to others in reinforcing tetrapolarity as the ancestral state of all basidiomycetes.

5 Pedro Almeida, Raquel Barbosa, Polona Zalar, Yumi Imanishi, Kiminori Shimizu, Benedetta Turchetti, Jean-Luc Legras, Marta Serra, Sylvie Dequin, Arnaud Couloux, Julie Guy, Douda Bensasson, Paula Gonçalves & José Paulo Sampaio. 2015. A population genomics insight into the Mediterranean origins of wine yeast domestication. Molec Ecol 24:5412-5427.

The domestication of the wine yeast *Saccharomyces cerevisiae* is thought to be contemporary with the development and expansion of viticulture along the Mediterranean basin. Until now, the unavailability of wild lineages prevented the identification of the closest wild relatives of wine yeasts. Here, we enlarge the collection of natural lineages and employ whole-genome data of oak-associated wild isolates to study a balanced number of anthropic and natural S. cerevisiae strains. We identified industrial variants and new geographically delimited populations, including a novel Mediterranean oak population. This population is the closest relative of the wine lineage as shown by a weak population structure and further supported by genomewide population analyses. A coalescent model considering partial isolation with asymmetrical migration, mostly from the wild group into the Wine group, and population growth, was found to be best supported by the data. Importantly, divergence time estimates between the two populations agree with historical evidence for winemaking. We show that three horizontally transmitted regions, previously described to contain genes relevant to wine fermentation, are present in the Wine group but not in the Mediterranean oak group. This represents a major discontinuity between the two populations and is likely to denote a domestication fingerprint in wine yeasts. Taken together, these results indicate that Mediterranean oaks harbour the wild genetic stock of domesticated wine yeasts.

5 Arissa FD Freitas, Raquel Barbosa, José Paulo Sampaio, Marc-André Lachance & Carlos A Rosa. 2015. *Starmera pilosocereana* sp. nov., a yeast isolated from necrotic tissue of cacti in a sandy coastal dune ecosystem. Int J. Syst Evol Microbiol. Epub ahead of print

Two strains of a new cactophilic yeast species were isolated from the columnar cactus *Pilosocereus arrabidae* in a sand dune ecosystem in Rio de Janeiro, Brazil. Phylogenetic analysis of sequences of the large subunit rRNA gene D1/D2 domains showed that the strains represent a sister species to *Starmera caribaea*, from which it differs by 21 nucleotide substitutions and two indels. The new species is heterothallic and the asci are deliquescent with the formation of two to four hat-shaped ascospores. The name *Starmera pilosocereana* sp. nov. is proposed to accommodate the species. The type strain of *S. pilosocereana* sp. nov. is UFMG-CM-Y316T (= CBS 13266T) and the allotype is UFMG-CM-Y346a (=CBS 13265). The Mycobank number is MB 810683. *Candida stellimalicola* belonging to the *Starmera* clade, is reassigned to *Starmera* as a new combination.

7 Carla Gonçalves, Marco A. Coelho, Madalena Salema-Oom & Paula Gonçalves. 2015. Stepwise functional evolution in a fungal sugar transporter family. Molecular Biology and Evolution. Epub ahead of print

Sugar transport is of the utmost importance for most cells and is important to a wide range of applied fields. However, despite the straightforward in silico assignment of many novel transporters, including sugar porters, to existing families, their exact biological role and evolutionary trajectory often remain unclear, mainly because biochemical characterization of membrane proteins is inherently challenging, but also owing to their uncommonly turbulent evolutionary histories. In addition, many important shifts in membrane carrier function are apparently ancient, which further limits our ability to reconstruct evolutionary trajectories in a reliable manner. Here we circumvented some of these obstacles by examining the relatively recent emergence of a unique family of fungal sugar facilitators, related to drug antiporters. The former transporters, named Ffz, were previously shown to be required for fructophilic metabolism in yeasts. We first exploited the wealth of fungal genomic data available to define a comprehensive but well-delimited family of Ffz-like transporters, showing that they are only present in Dikarya. Subsequently, a combination of phylogenetic analyses and in vivo functional characterization was used to retrace important changes in function, while highlighting the evolutionary events that are most likely to have determined extant distribution of the gene, such as horizontal gene transfers (HGTs). One such HGT event is proposed to have set the stage for the onset of fructophilic metabolism in yeasts, a trait that according to our results may be the metabolic hallmark of approximately one hundred yeast species that thrive in sugar rich environments.

8 Andrey Yurkov, Oliver Röhl, Ana Pontes, Cláudia Carvalho, Cristina Maldonado & José Paulo Sampaio. 2015. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. FEMS Yeast Research (accepted).

Soil yeasts represent a poorly known fraction of the soil microbiome due to limited ecological surveys. Here, we provide the first comprehensive inventory of cultivable soil yeasts in a Mediterranean ecosystem, which is the leading biodiversity hotspot for vascular plants and vertebrates in Europe. We isolated and identified soil yeasts from forested sites of Serra da Arrábida Natural Park (Portugal), representing the Mediterranean forests, woodlands, and scrub biome. Both cultivation experiments and the subsequent species richness estimations suggest the highest species richness values reported to date, resulting in a total of 57 and 80 yeast taxa, respectively. These values far exceed those reported for other forest soils in Europe. Furthermore, we assessed the response of yeast diversity to microclimatic environmental factors in biotopes composed of the same plant species but showing a gradual change from humid broadleaf forests to dry maquis. We observed that forest properties constrained by precipitation level had strong impact on yeast diversity and on community structure and lower precipitation resulted in an increased number of rare species and decreased evenness values. In conclusion, the structure of soil yeast communities mirrors the environmental factors that affect aboveground phytocenoses, above ground biomass and plant projective cover.

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Recently published papers.

1 Yurkov AM, Kachalkin AV, Daniel HM, Groenewald M, Libkind D, de Garcia V, Zalar P, Gouliamova DE, Boekhout T, Begerow D. 2015. Two yeast species *Cystobasidium psychroaquaticum f.a.* sp. nov. and *Cystobasidium rietchieii f.a.* sp. nov. isolated from natural environments, and the transfer of *Rhodotorula minuta* clade members to the genus *Cystobasidium*. Antonie Van Leeuwenhoek 107:173-185.

Many species of dimorphic basidiomycetes are known only in their asexual phase and typically those pigmented in different hues of red have been classified in the large polyphyletic genus *Rhodotorula*. These yeasts are ubiquitous and include a few species of some clinical relevance. The phylogenetic distribution of *Rhodotorula* spans three classes: Microbotryomycetes, Cystobasidiomycetes and Exobasidiomycetes. Here, the presented multi-gene analyses resolved phylogenetic relationships between the second largest group of *Rhodotorula* and the mycoparasite *Cystobasidium fimetarium* (Cystobasidiales, Cystobasidiomycetes, Pucciniomycotina). Based on the results, we propose the transfer of nine species belonging to the *Rhodotorula minuta* clade into the genus *Cystobasidium*. As a result, the clinically relevant species *R. minuta* will be renamed *Cystobasidium minutum*. This proposal follows ongoing reassessments of the anamorphic genus *Rhodotorula* reducing the polyphyly of this genus. The delimitation of the *R. minuta* clade from *Rhodotorula* species comprised in Sporidiobolales including the type species *Rhodotorula glutinis* is an important step to overcome obsolete generic placements of asexual basidiomycetous yeasts. Our proposal will also help to distinguish most common red yeasts from clinical samples such as members of Sporidiobolales and Cystobasidiales. The diagnosis of the genus *Cystobasidium* is amended by including additional characteristics known for the related group of species. The taxonomic change enables us to classify two novel species with the phylogenetically related members of the *R. minuta* clade in *Cystobasidium*. The recently from natural environments isolated species are described here as *Cystobasidium psychroaquaticum f.a.* sp. nov. (K-833^T = KBP 3881^T = VKPM Y-3653^T = CBS 11769^T = MUCL 52875^T = DSM 27713^T) and *Cystobasidium rietchiei f.a.* sp. nov. (K-780^T = KBP 4220^T = VKPM Y-3658^T = CBS 12324^T = MUCL 53589^T = DSM 27155^T). The new species were registered in MycoBank under MB 809336 and MB 809337, respectively.

2 Solis MJL, Yurkov A, dela Cruz TE, Unterseher M. 2015. Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three *Ficus* species from botanical garden greenhouses in Germany. Mycological Progress 14:1019.

Yeasts of both Ascomycota and Basidiomycota occur in various ecological zones of many geographic regions and climatic conditions, but environmental yeast research has often been conducted in either extreme habitats or the phyllosphere. Here, we report on the occurrence of foliar endophytic yeasts of three tropical *Ficus* species from two German greenhouses in Greifswald and Berlin. Living leaves were collected and subjected to dilution-to-extinction cultivation. Fungal colonies were used for morphological analyses, microsatellite-primed fingerprinting, sequencing and phylogeny of the internal transcribed spacer (ITS) DNA. Fifteen percent (~200 colonies) of all fungal isolates belonged to the genera *Cryptococcus* (Filobasidiales) and *Rhodotorula* (Sporidiobolales and Cystobasidiales) that split into 23 species / operational taxonomic units. No other yeast-forming taxa were isolated. Both side-specific and host-specific variations in species composition and abundance were observed; however, statistics did not support significant associations. Further evidence exists that gardening practices, such as moving potted plants, could influence fungal endophytic communities.

3 Yurkov A, Inácio J, Chernov IYu, Fonseca Á. 2015. Yeast biogeography and the effects of species recognition approaches: the case study of widespread basidiomycetous species from birch forests in Russia. Current Microbiology 70: 587-601.

Understanding diversity and distribution patterns of fungi, including yeasts, ultimately depends on accuracy of species recognition. However, different approaches to yeast species recognition often result in different entities or

operational taxonomic units. We studied the effects of using different yeast species recognition approaches, namely morphological species recognition (MSR) and phylogenetic species recognition (PSR), on the distribution patterns of widespread basidiomycetous yeasts. Hence, we have revised a collection of yeast fungi isolated from spatially remote birch forests in the Moscow Region and Western Siberia with molecular typing and identification tools. PCR fingerprinting and rDNA sequencing analyses of strains of nine species previously identified on the basis of morphological and physiological tests (MSR) yielded 21 phylogenetic species (PSR), including three currently undescribed taxa. The number of distinct phylogenetic species comprised within a single morphospecies ranged from one to seven. A total of ten species were found in both regions, whereas the distribution of 11 yeasts was restricted to a single region only. Both geographical region and type of substrate (plant or soil) influence yeast distribution. *Cryptococcus wieringae*, *C. victoriae*, *C. magnus*, and *Leucosporidium scottii* were frequently found on plant substrates, whereas *C. terricola* and *C. podzolicus* were associated to soil substrates. Occurrence of *C. magnus*, *C. albidus* and *Sporobolomyces roseus* was found to depend on the geographical region. Microsatellite-PCR fingerprinting, MSP-PCR, applied to studying yeast intraspecific variability revealed three different types of distribution: (a) variability that depends on geographical factors (*Curvibasidium cygneicollum*, *C. podzolicus*, *C. victoriae*), (b) genetic identity irrespectively of the region of isolation (*Rhodotorula pinicola*, *C. terricola*), and (c) high degree of genetic variability that did not correlate with region of sampling (*C. albidus* and *C. magnus*).

4 Mittelbach M, Yurkov AM, Nocentini D, Nepi M, Weigend M, Begerow D. 2015. Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. BMC Ecology 2015 15:2.

Background. Studies on the diversity of yeasts in floral nectar were first carried out in the late 19th century. A narrow group of fermenting, osmophilous ascomycetes were regarded as exclusive specialists able to populate this unique and species poor environment. More recently, it became apparent that microorganisms might play an important role in the process of plant pollination. Despite the importance of these nectar dwelling yeasts, knowledge of the factors that drive their diversity and species composition is scarce. Results. In this study, we linked the frequencies of yeast species in floral nectars from various host plants on the Canary Islands to nectar traits and flower visitors. We estimated the structuring impact of pollination syndromes (nectar volume, sugar concentration and sugar composition) on yeast diversity. The observed total yeast diversity was consistent with former studies, however, the present survey yielded additional basidiomycetous yeasts in unexpectedly high numbers. Our results show these basidiomycetes are significantly associated with ornithophilous flowers. Specialized ascomycetes inhabit sucrose-dominant nectars, but are surprisingly rare in nectar dominated by monosaccharides. Conclusions. There are two conclusions from this study: (i) a shift of floral visitors towards ornithophily alters the likelihood of yeast inoculation in flowers, and (ii) low concentrated hexose-dominant nectar promotes colonization of flowers by basidiomycetes. In the studied floral system, basidiomycete yeasts are acknowledged as regular members of nectar. This challenges the current understanding that nectar is an ecological niche solely occupied by ascomycetous yeasts.

5 Yurkov A, Guerreiro MA, Sharma L, Carvalho C, Fonseca Á. 2015. Multigene assessment of the species boundaries and sexual status of the basidiomycetous yeasts *Cryptococcus flavescens* and *C. terrestris* (Tremellales). PLoS ONE 10: e0120400. Correction: Multigene assessment of the species boundaries and sexual status of the basidiomycetous yeasts *Cryptococcus flavescens* and *C. terrestris* (Tremellales). PLoS ONE 10: e0126996.

Cryptococcus flavescens and *C. terrestris* are phenotypically indistinguishable sister species that belong to the order Tremellales (Tremellomycetes, Basidiomycota) and which may be mistaken for *C. laurentii* based on phenotype. Phylogenetic separation between *C. flavescens* and *C. terrestris* was based on rDNA sequence analyses, but very little is known on their intraspecific genetic variability or propensity for sexual reproduction. We studied 59 strains from different substrates and geographic locations, and used a multilocus sequencing (MLS) approach complemented with the sequencing of mating type (*MAT*) genes to assess genetic variation and reexamine the boundaries of the two species, as well as their sexual status. The following five loci were chosen for MLS: the rDNA ITS-LSU region, the rDNA IGS1 spacer, and fragments of the genes encoding the largest subunit of RNA polymerase II (*RPB1*), the translation elongation factor 1 alpha (*TEF1*) and the p21-activated protein kinase (*STE20*). Phylogenetic network analyses confirmed the genetic separation of the two species and revealed two

additional cryptic species, for which the names *Cryptococcus baii* and *C. ruineniae* are proposed. Further analyses of the data revealed a high degree of genetic heterogeneity within *C. flavescens* as well as evidence for recombination between lineages detected for this species. Strains of *C. terrestris* displayed higher levels of similarity in all analysed genes and appear to make up a single recombining group. The two *MAT* genes (*STE3* and *SXI1*/*SXI2*) sequenced for *C. flavescens* strains confirmed the potential for sexual reproduction and suggest the presence of a tetrapolar mating system with a biallelic pheromone/receptor locus and a multiallelic HD locus. In *C. terrestris* we could only sequence *STE3*, which revealed a biallelic P/R locus. In spite of the strong evidence for sexual recombination in the two species, attempts at mating compatible strains of both species on culture media were unsuccessful.

6 Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, … Yurkov A, Begerow D, Roehl O, Guerreiro M, Fonseca Á, … Robert V. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia – Molecular Phylogeny and Evolution of Fungi 35: 242-263.

The aim of this study was to assess potential candidate gene regions and corresponding universal primer pairs as secondary DNA barcodes for the fungal kingdom, additional to ITS rDNA as primary barcode. Amplification efficiencies of 14 (partially) universal primer pairs targeting eight genetic markers were tested across > 1 500 species (1931 strains or specimens) and the outcomes of almost twenty thousand (19577) polymerase chain reactions were evaluated. We tested several well-known primer pairs that amplify: i) sections of the nuclear ribosomal RNA gene large subunit (D1–D2 domains of 26/28S); ii) the complete internal transcribed spacer region (ITS1/2); iii) partial β-tubulin II (*TUB2*); iv) γ-actin (*ACT*); v) translation elongation factor 1-α (*TEF1α*); and vi) the second largest subunit of RNA-polymerase II (partial *RPB2*, section 5–6). Their PCR efficiencies were compared with novel candidate primers corresponding to: i) the fungal-specific translation elongation factor 3 (*TEF3*); ii) a small ribosomal protein necessary for t-RNA docking; iii) the *60S L10* (*L1*) RP; iv) DNA topoisomerase I (*TOPI*); v) phosphoglycerate kinase (*PGK*); vi) hypothetical protein *LNS2*; and vii) alternative sections of *TEF1α*. Results showed that several gene sections are accessible to universal primers (or primers universal for phyla) yielding a single PCR-product. Barcode gap and multi-dimensional scaling analyses revealed that some of the tested candidate markers have universal properties providing adequate infra- and inter-specific variation that make them attractive barcodes for species identification. Among these gene sections, a novel high fidelity primer pair for *TEF1α*, already widely used as a phylogenetic marker in mycology, has potential as a supplementary DNA barcode with superior resolution to ITS. Both *TOPI* and *PGK* show promise for the Ascomycota, while *TOPI* and *LNS2* are attractive for the *Pucciniomycotina*, for which universal primers for ribosomal subunits often fail.

Recently accepted paper.

7 Yurkov A, Röhl O, Pontes A, Carvalho C, Maldonado C, Sampaio JP. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands, and scrub biome. FEMS Yeast Res.

Recent presentations.

8 Yurkov AM. 2015. Yeasts in Pucciniomycotina. The Second International Workshop on Ascomycete Systematics, Amsterdam, The Netherlands.

9 Yurkov AM, Sampaio JP. 2015. Yeast biodiversity in culture collections: old sources and new challenges. 32nd International Specialized Symposium on Yeasts, ISSY32, Perugia, Italy

Environmental studies established the foundation of our knowledge of the microbial biodiversity. Furthermore, cultures originating from these assays remain often the only source of microbial resources available to the society through open culture collections. Collections are expected to (a) provide safe preservation of cultures, (b) ensure identity of microbial resources, (c) enable easy distribution of strains, (d) offer isolates of different origin and (e) perform research. These tasks represent also the major challenges for a collection of microorganisms, including yeast culture collections. (a) Since yeasts are phylogenetically heterogeneous, a combination of different preservation techniques should be used. (b) Methods for yeast taxonomy and identification have rapidly evolved in the last decades, and consequently yeast classification and nomenclature had to be updated. Among methods

facilitating routine identification and quality control in collections, rDNA sequencing and MALDI-TOF are the most promising tools. (c) Unlike plants and animals microorganisms require labor-intensive handling. Therefore, yeasts are distributed mainly through public repositories in accordance with the Convention on Biological Diversity and the recently ratified by EU Nagoya protocol. (d) Recent changes to data availability polices driven through major microbiological journals force, among other requirements, free access to cultures through a national culture collection. Despite limited funding, culture collections may still suit well for biodiversity assessments of different scales, however they were not conceived to cover large population studies involving tens or hundreds of strains of the same species. (e) Apart from performing pure taxonomic studies, culture collections can be successfully linked to scientific projects to provide necessary taxonomic expertise to the scientific community. With a few ongoing projects we exemplify how biodiversity assessments enlarge our knowledge on yeast diversity. This work was partly supported by Fundação para a Ciência e a Tecnologia (Portugal), projects PTDC/BIA- MIC/113051/2009, PTDC/BIA-BIC/4585/2012.

XVIII Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by MA Lachance <lachance@uwo.ca>.

Recently accepted papers.

1 Santos ARO, Perri AM, Andrietta MdGS, Rosa CA, Lachance MA 2015 The expanding large-spored *Metschnikowia* clade: *Metschnikowia matae* sp. nov., a yeast species with two varieties from the Brazilian Atlantic Forest. Antonie van Leeuwenhoek 108:753-763.

Fifty-two yeast isolates from flowers and associated nitidulid beetles of the Brazilian Atlantic Forest (Mata Atlântica) region were found to represent a new species in the large-spored Metschnikowia clade. The species is heterothallic, haploid, and allogamous, and produces asci with two aciculate ascospores that can reach 80 µm in length, as is typical in the clade. Analysis of sequences of the ribosomal RNA gene cluster indicates that the new species is closely related to *Metschnikowia lochheadii*, which ranges across Central America to northern Brazil, occurs as an adventive species in Hawaii, but is rarely found in central Brazil. The species is not readily distinguishable from relatives based on morphology or growth responses, but is well delineated from *M. lochheadii* on reproductive isolation. Based on an intron splice site PCR screen, we selected 26 isolates for further study. The sequence of the region that includes the complete internal transcribed spacer/5.8S rRNA gene segment as well as the D1/D2 domains of the large subunit rRNA gene contained three polymorphic segments and 14 haplotypes were identified. Of these, a single divergent isolate from the southernmost of four sampled localities exhibited diminished mating success when crossed with others. We describe two varieties, *Metschnikowia matae* var. *matae* sp. nov. var. nov. (type UFMG-CM-Y395^{T}, CBS 13986^{T}, NRRL Y-63736^{T}; allotype UFMG-CM-Y391^{A}, CBS 13987^{A}, NRRL Y-63735^{A}) and *Metschnikowia matae* var. *maris* sp. nov. var. nov. (type UFMG-CM-Y397^{T}, CBS 13985^{T}, NRRL Y-63737^{T}). We also report on the discovery of the h^{+} mating type of *Candida ipomoeae* and transfer of the species to *Metschnikowia ipomoeae* comb. nov. (allotype UWOPS 12-660.1^{A}, CBS 13988^{A}, NRRL Y-63738^{A}).

2 Khunnamwong P, Lertwattanasakul N, Jindamorakot S, Limtong S, Lachance MA 2015 The genus *Diutina*, description of *Diutina siamensis*, f.a. sp. nov., and reassignment of *Candida catenulata*, *C. mesorugosa*, *C. neorugosa*, *C. pseudorugosa*, *C. ranongensis*, *C. rugosa* and *C. scorzettiae* to the genus *Diutina*. Int J Syst Evol Microbiol. doi: 10.1099/ijsem.0.000634.

Three strains (DMKU-RE28, DMKU-RE43T and DMKU-RE123) of a novel anamorphic yeast species were isolated from rice leaf tissue collected in Thailand. DNA sequence analysis demonstrated that the species forms a sister pair with *Candida ranogenesis* CBS 10861T but differs by 24-30 substitutions in the LSU rRNA gene D1/D2 domains and 30-35 substitutions in the ITS region. A phylogenetic analysis based on both the small and the large rRNA gene subunits confirmed this connection and demonstrated the presence of a clade that also includes *Candida catenulata*, *C. mesorugosa*, *C. neorugosa*, *C. pseudorugosa*, *C. rugosa* and *C. scorzettiae*. The clade is not closely affiliated to any known teleomorphic genus and forms a well-separated lineage from currently recognized genera of the Saccharomycetales. Hence, the genus *Diutina* gen. nov. is proposed to accommodate members of the clade, including *Diutina siamensis* f.a. sp. nov. and the preceding seven *Candida* species. The type strain is DMKU-RE43^{T} (CBS 13388^{T} =BCC 61183^{T} =NBRC 109695^{T}).

3 Freitas LFD, Barbosa R, Sampaio JP, Lachance MA, Rosa CA 2015 *Starmera pilosocereana* sp. nov., a yeast isolated from necrotic tissue of cacti in a sandy coastal dune ecosystem. Int J Syst Evol Microbiol doi: 10.1099/ijsem.0.000596.

See abstract under Dr. Rosa's communication.

4 Lopes MR, Ferreira MC, Carvalho TFC, Pagnocca FC, Chagas RA, Morais PB, Rosa LH, Lachance MA, Rosa CA *Yamadazyma riverae* sp. nov., a yeast species isolated from plant materials. Int J Syst Evol Microbiol doi: 10.1099/ijsem.0.000597.

See abstract under Dr. Rosa's communication.

5 Hittinger CT, Rokas A, Bai FY, Boekhout T, Gonçalves P, Jeffries TW, Kominek J, Lachance MA, Libkind D, Rosa CA, Sampaio JP, Kurtzman CP 2015 Genomics and the Making of Yeast Biodiversity. Curr Opin Genet Devel (Special issue on Genomes and Evolution)

See abstract under Dr. Hittinger's communication.

The following abstract is of the opening lecture presented at ISSY32.

6 Lachance MA. 2015. Some big questions of yeast ecology. 32nd International Specialized Symposium on Yeasts, Perugia, Italy, September 2015.

Yeasts, in particular a handful of model species, have played a foundational role in the development of modern biochemistry and genetics. Yeasts as a whole have blazed the trail in the systematics of eukaryotic microbes, as exemplified by a fully functional DNA barcode by the turn of the 21st century and an enviable tradition known as "The Yeasts, a Taxonomic Study". But what about ecology? In spite of a number of inspiring studies of a small array of species, a unifying view of the place of yeasts in nature would appear to remain elusive. For those concerned with yeast ecology, there seems to be an epistemic divide that opposes Bass Becking's "*alles is overal:* maar *het milieu selecteert*"[1] to the biogeographer's 'Everything is endemic',[2] and more recently to an ultra-neutral model[3] that would deny, at least for baker's yeast, both a biogeography and a niche. The fact that the search for a habitat for baker's yeast remains inconclusive may be due to the in part in the highly typological mindset that pervades yeast ecology, which seeks to identify in nature the equivalent of selective agar plates where yeast populations boom and crash. A more flexible, probabilistic approach may be preferable. Having discussed these theoretical and historical considerations, I shall review some of the "big" questions facing yeast ecologists today. I shall also provide highlights of some of my own work on yeasts associated with floricolous beetles, their phylogenetics, their distribution at various spatial scales, and some attempts to identify the adaptive basis for their ecological specificity.

References

[1] de Wit R & Bouvier T (2006) 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Environmental Microbiology 8:755–758

[2] Williams DM (2011) Historical biogeography, microbial endemism and the role of classification: everything is endemic. In Fontaneto D "Biogeography of microscopic organisms. Cambridge pp. 11-31.

[3] Goddard MR & Duncan G (2015) *Saccharomyces cerevisiae*: a nomadic yeast with no niche? FEMS Yeast Res 15:1-6.

The 100th anniversary of the birth of Anna Kocková-Kratochvílová (1915-1992)

Anna Kocková-Kratochvílová was born in Tuzla, Bosnia and Herzegovina. She graduated in plant physiology from the Faculty of Natural Sciences, at Charles University, in Prague, in 1938. Kocková-Kratochvílová defended her PhD thesis "The significance of fermentation types" in 1962. She received her Dr.Sc. degree in 1968 from the Faculty of Natural Sciences, at Masaryk University, in Brno.

Kocková-Kratochvílová obtained her first research position at the laboratory of Vitamin and Hormone Chemistry in Prague (1942 to 1945). It allowed her to work with all types of microorganisms, study their industrially important properties and participate in medical research supervised by doctors. In 1946 she entered the brewing industry and became head of the microbial laboratory at the Research Institute of Brewing and Malting in Prague. She dealt with the industrially important properties of brewer's yeasts with the intention of using them as a nourishing supplement in both human diets and microbial cultivation media.

When the activities of the institute were terminated, Kocková-Kratochvílová joined the Department of Technical Microbiology and Biochemistry at the Faculty of Chemistry Technical University, in Bratislava, where she trained students in technical microbiology (she became Associate Professor and Head of department from 1953 to 1959).

In 1959 Kocková-Kratochvílová was invited to establish the microbial laboratory at the Institute of Chemistry at the Slovak Academy of Science, where she worked for the rest of her life. Her main research activities took place at this institution. Kocková-Kratochvílová investigated brewer's yeasts, selected the strains with optimal industrial properties and published the Catalogue of Brewery Yeasts. In 1963 the team moved to the new building of the Institute of Chemistry. Kocková-Kratochvílová continued with her work on *Saccharomyces* strains, isolated other strains from different niches (also in collaboration with another laboratories), verified the phenotype of strains by 68 tests and evaluated the relationships between strains and species. She applied numerical methods to classifing the species of the genera *Saccharomyces* and *Candida* and was one of the pioneers who applied this method to yeast taxonomy. Her collaboration with H.H. Harman, an author of a highly cited book, Modern Factor Analysis, was the peak of her achievements in this area.

Kocková-Kratochvílová also focused her interest in long-term storage of yeasts (mainly in liquid nitrogen), various enzymatic activities with colleagues from neighbouring laboratories, and diversity of yeasts. She isolated yeast cultures from insects, wild plants, water and man-made habitats. From the latter habitat she discovered the species *Candida ethanolica*. However, mushrooms may well have been her favoured source of yeast cultures which she studied for 20 years. Her first study focused on *Candida humicola* (present name *Cryptococcus humicolus*) which causes atypical deformation of a fruiting body of *Agaricus xanthodermus* (yellow-staining mushroom) and here last was on the yeasts inhabiting mushrooms grown in the locality Záhorská Nížina. Kocková-Kratochvílová discovered two new species related to mushrooms: *Torulopsis inconspicua* (present name *Candida inconspicua*) and *Torulopsis schatavii* (present name *Candida schatavii*). She was also a passionate mushroom picker and together with her team offered an advisory service on the edibility of mushrooms throughout many seasons.

Kocková-Kratochvílová was also a founder of the Culture Collection of Yeasts (former name Czechoslovak Collection of Yeasts). Kocková-Kratochvílová started to built up the collection of bacteria, yeasts and filamentous fungi at her first research position. She purchased microbial cultures from other collections (mainly form CBS) and also by her own isolation. After the war the collection was divided into two parts. The first one consisting of bacteria and filamentous fungi, was moved to the Institute of Microbiology, at the Czechoslovak Academy of Sciences and yeasts went together with Kocková-Kratochvílová to the Research Institute of Brewing and Malting in Prague (1946). Over the years Kocková-Kratochvílová enlarged the collection with brewer's yeasts obtained from many parts of the world including from the Czech breweries. In 1953 Kocková-Kratochvílová moved the collection to Bratislava and from 1959 the Collection became a part of the Institute of Chemistry at the SAS. In this period, she enriched the collection with endemic species originating from natural and human-made sources. Kocková-Kratochvílová also contributed significantly to the establishment of the International Symposia on Yeasts.

In 1964 Kocková-Kratochvílová, as chairperson, and E. Minárik, as secretary, organized the First International Symposium on Yeasts in Smolenice as a meeting of friends who cooperated in various fields of yeast research. About 60 researchers from Czechoslovakia and neighbouring countries attended. Theoretical lectures, lectures aimed at the application of yeasts in industry and lectures on

pathogenic yeasts were presented. The symposium was very succesful and therefore, it was decided to organize subsequent conference. The second symposium was organized in Bratislava in 1966. About 140 participants from 27 countries attended the conference. At this conference, the Resolution of the Second International Symposium On Yeasts was adopted by the participants:

"We have all observed that the importance of yeasts have been increasing more and more in the last years. The great importance of yeasts and yeast-like organisms according to their purpose is seen in various spheres, e.g. in the classical fermentation of beer, wine, sake, baker's yeast production, etc, in the preparation of fine biochemical compounds and medical preparations, e.g. enzymes, nucleic acids, vitamins, antigens, etc., in agricultural production of the fodder basis for animals, in the scientific research as models for investigations of the biological processes, in sanitary matters as causes of human and animal diseases, etc."

It was also decided to organize conferences on regular basis. In 1967 the Council for Yeast Research, composed of prominent specialists in the field of yeasts, was established in Bratislava. In 1971 the Coucil was included in the International Association of Microbiological Societies (IAMS). The "News-letter", edited by H. Phaff of the University of California started to serve as a communication tool for the decisions of the Council. H. Phaff edited the News-letter until 1987 when he was replaced by M.A. Lachance. Over the years, the name International Symposium on Yeasts changed to International Yeast Congress, the Yeast Newsletter changed its look and the Council for Yeast Research was renamed as International Commission on Yeasts. However, the main idea of organizing symposia on yeasts on a regular basis remained unchanged.

To sumarize, the profesional life of Dr. Anna Kocková-Kratochvílová can be characterized by: 300 research publications, 9 books, 20 diploma students, 11 PhD. students. Dr. Kocková- Kratochvílová received awards from numerous Czechoslovak organizations for her scientific work. She received the Prezident's award, the highest award possible in Slovakia, for her contribution to scientific research.

Dr. Kocková-Kratochvílová was also recognized by Japanese researchers when the species *Rhodosporidium kratochvilovae* and the genus *Kockovaella* were named after her.

Although Dr. Kocková-Kratochvílová formally retired in 1986, she stayed on at the SAS as a consultant. Her indomitable soul enabled her to finish her last book "The taxonomy of yeasts and yeast-like species" at a time where she was almost blind.

The world of yeasts was her destiny, passion and love.

Renáta Vadkertiová and Emília Breierová
Yeast Culture Collection, Institute of Chemistry, Slovak Academy of Sciences

A Mournful Note
Professor Graham Harold Fleet
June 20 1946 - October 23 2015

On October 23 2015, renowned Australian microbiologist Prof. Graham Harold Fleet passed away at 69 years old. His life was dedicated to the study of microorganisms, especially those involved in food production. As a result of his productive scientific career he published more than one hundred scientific papers and twenty scientific reviews; he coauthored several books and also contributed tens of chapters to scientific books. Graham was a simple but very talented man. He got his doctorate at the University of California, Davis, where he was a prominent pupil of the well- known researcher Herman J. Phaff. During his scientific career, he received a number of awards and occupied important administrative and management positions in academic and governmental institutions. He belonged to the editorial board of highly reputed journals both in Australia and abroad. In 1996 he attained the rank of Professor (Level E) at the University of New South Wales Sydney. He was a wise man, with a charming and adventurous spirit. Till the end of his life he was involved in

Prof. Graham Fleet in Mindo, Ecuador, with craft chocolate producers in October 2014

the academic and scientific environment, interacting with researchers worldwide, including several Latin Americans, to whom he served an inspiration and a role model. The last year of his life he travelled to South America, especially to Brazil and Ecuador, in the search of yeasts involved in cocoa fermentations. He was especially enthusiastic about creating links among South-American yeast researchers. For the Latin American community of yeast researchers, his memory will remain written in his scientific contributions which will continue to be cited by the younger researchers who in the future will stand on his shoulders to achieve new discoveries and scientific developments.

Farewell, Professor Graham Fleet!

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Sandra Regina Ceccato Antonini, Universidade Federal de São Carlos, Brazil
Sandra Denise Camargo Mendes, Empresa de Pesquisa Agropecuária, Brazil
Victoria Mestre, Universidad Nacional de San Juan, Argentina
Rubén Peña Bernales, Universidad de Santiago de Chile
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Obituary

Ivan Yurjevich Chernov (December 12 1959 - November 16 2015)

Ivan Yur'evich Chernov devoted his scientific career to the study of yeasts in natural habitats. He started working with yeasts as a student at the soil yeasts laboratory at the Soil Science Faculty of Lomonosov Moscow State University (MSU), when he joined the best know yeast research group in the Soviet Union, headed by Professor Inna Pavlovna Babjeva. He was a member of that group until his untimely death last month.

After graduating Ivan Chernow pursued PhD studies on yeasts of polar regions in the Taimir Peninsula. This resulted in the discovery and description of *Cryptococcus gilvescens*, a species which is now known to inhabit various cold habitats. While keeping his heart focused on yeasts, he dedicated the next few years to the teaching of food microbiology to students at the Moscow Institute of Food Production. The next step in his scientific career was to join the Institute of Geography of the USSR Academy of Sciences. In the period from 1989 to 1996, Chernov studied yeasts in desert soils of Middle Asia and the Middle East. He participated in the last Yeast Course organized at the Gulbenkian institute in Oieras, Portugal. In 1996, he returned to Moscow State University as a research associate at the Institute of Soil Sciences. During this time he was conceived of bringing together the available knowledge on yeast populations. Accodingly, he designed and created a database that brought together the data generated during 50 years of studies by Babjeva and co-workers. This work made it possible to perform statistical analyses of older data that allowed the visualization of trends in distribution of yeasts across different substrates and many terrestrial biomes. He obtained his habilitation at the Moscow State University in 2000 and continued research and teaching after Inna Babjeva's retirement. Chernov developed further an approach known as spatially-successional arrays, where yeast communities in a biotope are studied on a subset of most typical substrates, for instance living plant material, litter and soils. The reliability of this method in the study yeast population was tested in different biotopes. After 2000, Chernov dedicated his research to phylloplane yeasts, their diversity, and their seasonal dynamics. He was also inspired to investigate the influence of invasive plants species on yeast communities above and below ground. He became a corresponding member of the Russian Academy of Science in 2006 and after 2009 served as Head of the department of Soil Biology at the Soil Science Faculty of the Lomonosov Moscow State University.

Ivan Chernov passed away during an expedition in Vietnam on 16 November 2015.

Ivan Chernov was an extraordinary microbiologist. He was true field scientist, who participated in numerous expeditions. He knew the yeasts, knew their habitats, and knew the methods needed to isolate them. He was also an inspiring teacher, who was able to instill upon student a passion to yeasts. He published over 200 publications during his career but probably the most important are his books on yeasts, in which he reviewed their ecology, physiology, and systematics. He also authored a laboratory handbook describing the methods for the study this amazing group of fungi.

I had the great pleasure of meeting Ivan when I started my education at Moscow State University and came to the soil yeast laboratory in 1998. He supervised me during my graduate work through the PhD. I owe much my knowledge about yeasts to him. I learned how to plan experiments, how to sample, and how to isolate yeasts. Most of all, I learned from him the passion for research and the importance of careful scientific work.

Much too early in his life, we have lost a scientist, a colleague, a teacher, and a dear friend.

Andrey Yurkov

Forthcoming Meetings

9th International Fission Yeast Meeting, May 14-19, 2017, in Banff, Alberta, Canada

The 9th International Fission Yeast Meeting is coming to Canada and will be held May 14-19, 2017, in Banff, Alberta, at the Banff Centre Conference Facility. This is particularly exciting because the first Fission Yeast Workshop was held in conjunction with the Thirteenth International Congress on Yeast Genetics and Molecular Biology at the Banff Centre in 1986. In 2017 we are expecting 350-450 guests representing Pombe labs from around the globe. The organizing committee is comprised of Paul Young, Queen's, Gordon Chua, Calgary, and Dallan Young, Calgary. For further information, contact:

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43rd Annual Conference on Yeasts, Czech and Slovak Commission on Yeasts Smolenice Castle, Slovakia, May 10-13 2016

The 43rd Annual Conference on Yeasts is being planned for 10-13 May 2016 at Smolenice Castel, Slovakia. On-line registration will be opened on http://yeastconference.sk/ in December, 2015.

14th International Congress on Yeasts (ICY14) Awaji Yumebutai International Conference Center Awaji Island, Japan, September 11-15, 2016

ICY2016

On behalf of the Organization Committee, I'm great honored to host the 14th International Congress on Yeasts (ICY14) at Awaji Yumebutai International Conference Center, Awaji Island, Japan, in September 11-15, 2016.

General topic of ICY14 is "Yeasts for Global Happiness". It means that yeast science & technology will contribute to the world in terms of food & beverage, health & medicine, energy & environment. In addition, this congress will be the first time held in Japan since ISSY2 in back to 1972, when I was a junior high school student. We'd like to send valuable message and information from Japan to the world. More importantly, I believe that this will be a great opportunity for young scientists as they can attend and present their research. In contrast, we senior must tell the fun and importance of yeast research to the next generation.

ICY14 is sponsored by the International Commission on Yeasts as part of the International Union of Microbiological Societies (IUMS). The ICY has been held once every four years since 1955. It provides an opportunity for presenting the latest research progress in yeast metabolism, physiology, genetics, genomics, regulation, ecology, systematics, phylogeny, food and beverage applications, biofuel production and clinical applications.

I am very much looking forward to seeing you on middle September in 2016 at Awaji Island, Japan!

Hiroshi Takagi, Head of the Organizing Committee of ICY14 http://icy2016.com/index.html

Brief News Item

OPATHY - A new European training network focused on diagnostics and research on pathogenic yeasts

OPATHY is an innovative translational research training network that will explore the potential of next-generation high-throughput technologies, including genomics, transcriptomics and proteomics, to study the interactions of yeasts that cause disease to humans (e.g. *Candida* and *Cryptococcus* spp.) with their host, and to develop new diagnostic tools to monitor yeast infections in the clinic. For more information: www.opathy.eu

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50 Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

November 1965 Volume XIV, Number 2

Editor
Herman J. Phaff, University of California, Davis, California

Associate Editor
Leslie R. Hedrick, Illinois Institute of Technology, Chicago, Illinois

Associate Editor
F. M. Clark, University of Illinois, Urbana, Illinois

Associate Editor
Cecil G. Dunn, Massachusetts Institute of Technology, Cambridge, Massachusetts

Miss W. Slooff reported that type strains of seven new yeast species were received by the CBS, including three *Hansenula* species: *H. bimundalis, H. bimundalis* var. *americana* and *H. fabiani.*

Dr. Kenkichi Kodama of Kodama Brewing Co., Ltd., Japan presented a key to the classification of yeasts isolated from rice Koji, based on assimilation in Wickerham's liquid media and other tests.

Dr. R. Storck, Department of Microbiology, University of Texas described a preliminary survey on the base composition of RNA and DNA in fungi, including several yeast species. % GC values for 12 yeast species were calculated from buoyant density measurements in CsCl gradients. The ascosporogenous yeasts "have significantly lower GC content than the others." "*These observations, as a whole, suggest to me that DNA GC content could perhaps become a tool for systematic and phylogenetic studies*."

Dr. N. Loprieno, Istituto de Genetica della Universita, Pisa, Italy listed six publications on chemical mutagenesis of $\underline{ad}_6$ and $\underline{ad}_7$ loci (purple mutants) in *Schizosaccharomyces pombe*.

Professor C. A. Tobias, Lawrence Radiation Laboratory, University of California Berkeley communicated the abstract of the doctoral thesis of Dr. John T. Lyman, who completed his dissertation in biophysics titled, "Dark Recovery of Yeast Following Ionizing Radiations".

Dr. J. R. Johnston, University of Strathelyde, Glasgow C1, Scotland listed three publications regarding breeding of yeasts for brewing and reproductive capacity of yeast cells. A summary of the publication on reproductive capacity was included, and described micromanipulation of budding yeast cells to determine the number of generations and bud scars one brewing yeast strain and one hexaploid hybrid could produce. The brewing strain had an average reproductive capacity of 34 daughter cells, while the hexaploid strain had an average of 17 daughter cells.

Professor F. M. Clark, University of Illinois, USA shared the abstract of a paper presented at the Illinois Society of Microbiology, regarding the capsular material produced by *Lipomyces starkeyii.*

Dr. Akira Yuasa, University of Tokyo, presented the abstract of a paper published in the Japanese Journal of Botany, which described cytological studies of *Saccharomyces cerevisiae* and related species. Both light and electron microscopy revealed details of nuclear and other cellular structures, during mitotic and meiotic divisions. Four chromosomes were observed in *S. cerevisiae* strains, though two were expected.

Dr. Henri Saez, Parc Zoologique, Paris, France presented a list of five publications, some relating to yeasts and other fungi isolated from animals in the zoological garden, and three publications on *Geotrichum* from various sources.

T. A. LaRue and J. F. T. Spencer, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada tested approximately 60 yeasts for ability to utilize eighteen D-amino acids as a nitrogen source. Over 80% of strains tested could utilize at least one of these amino acids, leading the authors to the conclusion that this property is not likely to be useful in identification and taxonomy.

S. P. Meyers, University of Miami, Florida, USA reported isolation of 450 fungi from the Mediterranean and adjacent water masses. Meyers also visited with several other prominent scientists including Dr. N. van Uden, of the Institute of Botany at Lisbon, Portugal. Van Uden showed Meyers the impressive facilities of the Gulbenkian Foundation, under construction.

Prof. H. Iizuka, University of Tokyo, Japan shared the abstract of a paper recently published in the Journal Gen. Appl. Microbiol. on monosaccharide components of a hydrocarbon-assimilating yeasts isolated from soil samples of oil fields and gas fields.

Dr. L. D. Haley of the Yale-New Haven Medical Center, who received training in zymology from Dr. Lynferd J. Wickerham of Peoria, Illinois and Dr. N. J. W. Kreger-van Rij in Delft, Netherlands, published a book titled, "Diagnostic Medical Mycology", directed at bacteriologists and physicians.

The Second **International Symposium on Yeast** will take place in Slowakia in 1966, with emphasis on yeast taxonomy from several points of view: cytological, genetical, serological, biochemical, etc. Chairman of the organizing committee was Dr. A. Kocková-Kratochvílová.

The editor (**Dr. H. J. Phaff**) attended the International Symposium on Yeast Protoplasts, September 21-24, in Jena, D.D.R. A summary of papers presented was listed in the Yeast News Letter.

Dr. L. J. Wickerham of USDA, Peoria, Illinois, USA reported publication of four papers, three dealing with heterothallic species.

Dr. Thomas D. Brock of Indiana University, USA listed four recent publications dealing with yeast biochemistry.

Dr. Carl C. Lindegren of Southern Illinois University, USA published two articles, including one on genetical mutants of *Saccharomyces* induced by ethyl methanesulfonate.

Kyria Boundy-Mills, Phaff Yeast Culture Collection, University of California Davis