

Yeast

A Newsletter for Persons Interested in Yeast

ISSN 0513-5222

Official Publication of the International Commission on Yeasts
of the International Union of Microbiological Societies (IUMS)

DECEMBER 2013

Volume LXII, Number II

Marc-André Lachance, Editor
University of Western Ontario, London, Ontario, Canada N6A 5B7
<lachance@uwo.ca>

<http://www.uwo.ca/biology/YeastNewsletter/Index.html>

Associate Editors

Peter Biely
Institute of Chemistry
Slovak Academy of Sciences
Dúbravská cesta 9, 842 3
8 Bratislava, Slovakia

Patrizia Romano
Dipartimento di Biologia,
Difesa e Biotecnologie
Agro-Forestali
Università della Basilicata,
Via Nazario Sauro, 85, 85100
Potenza, Italy
Kyria Boundy-Mills

Herman J. Phaff Culture
Collection
Department of Food Science
and
Technology
University of California,
Davis
Davis California 95616-5224

WI Golubev, Puschino, Russia	32	E Breierová, Bratislava, Slovakia	45
M Kopecká, Brno, Czech Republic	32	P Buzzini, Perugia, Italy	48
GI Naumov and E.S. Naumova, Moscow, Russia	33	CA Rosa, Belo Horizonte, Minas Gerais, Brazil	49
D Kregiel, Lodz, Poland	34	G Miloshev, Sofia, Bulgaria	51
K Boundy-Mills, Davis, California, USA	35	M Sipicski, Debrecen, Hungary	52
B Gibson, VTT, Finland	36	A Speers, Edinburgh, Scotland	53
T Boekhout & M Groenewald, Utrecht, The Netherlands	41	SO Suh, Manassas, Virginia, USA	54
H Prillinger, Großmeisdorf, Austria	42	MA Lachance, London, Ontario, Canada	55
Á Fonseca & JP Sampaio, Caparica, Portugal	42	Forthcoming Meetings	56
NA Khan, New York, New York, USA	44	Brief News Item	58
A Bakalinsky, Corvallis, Oregon, USA	45	New book	59
		Fifty Years Ago	60

Editorial

Jane Margaret Bowles (1952-2013)



I regret to announce the death of Jane Bowles, my wife of 33 years and an avid yeast hunter. Several readers may have had the opportunity to interact with Jane at various ICYs and ISSYs or at international workshops, where she joined me as an accompanying participant. What many may not know is that Jane was a respected biologist in her own right. In addition to her involvement in 30 publications having to do with yeasts, she authored or co-authored 130-some papers or technical reports dealing with areas of ecological importance or species at risk in Canada. A botanist by training, she served as an ecological consultant, as Curator of the University of Western Ontario Herbarium, and as Director of the Sherwood Fox Arboretum (UWO). She was Adjunct Professor in the Departments of Biology and Geography and frequently taught field courses in Desert Ecology in Arizona and Mexico, and occasionally in Argentina. She served on the Committee on the Status of Endangered Wildlife in Canada and on Committee on the Status of Species at Risk in Ontario. Her efforts in conservation included being the Chair of the Property Management Committee of the Thames Talbot Land Trust, a task that occupied a significant proportion of her time in the last few years. She was the recipient of a number of awards for conservation, teaching, and other contributions. People who knew Jane enjoyed her artistic talents, her special sense of humor, her ability to recite poetry endlessly, and her endurance in the field. Unexpectedly, Jane was diagnosed with stomach cancer in the spring and died July 27th. I miss her terribly.

I wish all readers a happy and scientifically prosperous New Year!

M.A. Lachance, Editor

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

Recent publications.

- 1 Golubev WI. 2013 Diversity of *Wickerhamomyces anomalus* strains in activity against pathogenic *Candida* species. *Probl Med Mycol* 15:49-51.

Among 55 strains of *Wickerhamomyces anomalus*, the cultures were revealed that inhibit a growth of pathogenic *Candida* species. These 38 strains fall into six groups by their action spectra. Only three strains secreted were active against all

seven of *Candida* species tested. 25 strains inhibited growth of *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. tropicalis* and *C. viswanathii*. The remaining strains were few in number and active against some species only.

- 2 Golubev W I. 2013 Mycocinogeny in *Hanseniaspora* species. *Mykologia Phytopathologia* (accepted).

Five mycocinogenic and nine sensitive strains were found in *Hanseniaspora guilliermondii*, *H. occidentalis*, *H. osmophila*, *H. uvarum* and *H. vineae*. The other 24 strains of these species, together with *H. valbyensis* and *Kloeckera lindneri*, have neutral phenotype. The optimum pH for killer

activities was 4.5 in the presence of glycerol or NaCl into medium. Action spectra of all five mycocins were identical, they were restricted to some strains and species of 13 ascomycetous genera. Mycocinogenic strains differ from sensitive and neutral ones by some physiological characteristics.

II Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic. Communicated by Prof. MUDr. Marie Kopecká, Csc.
<mkopecka@med.muni.cz> <http://www.med.muni.cz/~mkopecka/>

Recent lecture.

- 1 Kopecká M. 2013 Yeast β -(1 \rightarrow 3)-glucan microfibrils, cell-walls and actin cytoskeleton. 30th ISSY Cell Surface & Organelles in Yeasts. Stará Lesná, Slovakia, June 18-22, 2013. The abstract can be found in Proceedings of 30th ISSY - Cell Surface and Organelles in Yeasts: from Basics to Applications, p.42.

Recent research articles.

- 2 Kopecká, M 2013 Title: Yeast and fungal cell-wall polysaccharides can self-assemble in vitro into an ultrastructure resembling in vivo yeast cell walls. *Microscopy* 62:327-339
DOI: 10.1093/jmicro/dfs076

Polysaccharides account for more than 90% of the content of fungal cell walls, but the mechanism underlying the formation of the architecture of the cell walls, which consist of microfibrils embedded in an amorphous wall matrix, remains unknown. We used electron microscopy to investigate ten different fungal cell-wall polysaccharides to determine whether they could self-assemble into the fibrillar or amorphous component of fungal cell walls in a test tube without enzymes. The ultrastructures formed by precipitating β -1,3-glucan and β -1,6-glucan are different depending on the existence of branching in the molecule. Linear

β -1,3-glucan and linear β -1,6-glucan precipitate into a fibrillar ultrastructure. Branched β -1,6-glucan, mannan and glycogen precipitates are amorphous. Branched β -1,3-glucan forms a fibrillar plus amorphous ultrastructure. Self-assembly among combinations of different linear and branched cell-wall polysaccharides results in an ultrastructure that resembles that of a yeast cell wall, which suggests that self-assembly of polysaccharides may participate in the development of the three-dimensional architecture of the yeast cell wall.

- 3 Marie Kopecká, Susumu Kawamoto and Masashi Yamaguchi. 2012 A new F-actin structure in fungi: actin ring formation around the cell nucleus of *Cryptococcus neoformans*. J Electron Microsc (Tokyo) Doi: [10.1093/jmicro/dfs074](https://doi.org/10.1093/jmicro/dfs074) [PDF] - [Epub ahead of print].

The F-actin cytoskeleton of *Cryptococcus neoformans* is known to comprise actin cables, cortical patches and cytokinetic ring. Here, we describe a new F-actin structure in fungi, a perinuclear F-actin collar ring around the cell nucleus, by fluorescent microscopic imaging of rhodamine phalloidin-stained F-actin. Perinuclear F-actin rings form in *Cryptococcus neoformans* treated with the microtubule inhibitor Nocodazole or with the drug

solvent dimethyl sulfoxide (DMSO) or grown in yeast extract peptone dextrose (YEPD) medium, but they are absent in cells treated with Latrunculin A. Perinuclear F-actin rings may function as 'funicular cabin' for the cell nucleus, and actin cables as intracellular 'funicular' suspending nucleus in the central position in the cell and moving nucleus along the polarity axis along actin cables.

III 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 are grateful to Dr. Duncan Greig for visiting his laboratory at Max Planck Institute for Evolutionary Biology (Plön, Germany) in August 2013 and fruitful discussions. We were glad to welcome in our lab Taiwanese colleagues Lee Ching-Fu, Yang Shu-Sen (National Hsinchu University of Education) and Wang Pin-Han (Tonghai University) during a short visit in June 2013.

The following are papers published in 2013 or submitted.

- 1 Naumov GI, Naumova ES, Tjurin OV, Kozlov DG. 2013. *Komagataella kurtzmanii* sp. nov., a new sibling species of *Komagataella (Pichia) pastoris* in accordance with multigene sequence analysis. Antonie van Leeuwenhoek. 104:339-347.

A novel methanol assimilating yeast species *Komagataella kurtzmanii* is described using the type strain VKPM Y-727 (= KBP Y-2878 = UCD 76-20 = Starmer #75-208.2 = CBS 12817 = NRRL Y-63667) isolated by W.T. Starmer from a fir flux in the Catalina Mountains, Southern AZ, USA. The new species is registered in MycoBank under MB 803919. The species was differentiated by divergence in gene

sequences for D1/D2 LSU rRNA, ITS1-5.8S-ITS2, RNA polymerase subunit I, translation elongation factor-1 α and mitochondrial small subunit rRNA. *K. kurtzmanii* differs from its phenotypically similar sibling species *K. pastoris*, *K. pseudopastoris*, *K. phaffii*, *K. populi* and *K. ulmi* by absence of growth at 35°C and inability to assimilate trehalose.

- 2 Naumov GI. 2013 Ecological and biogeographical features of *Saccharomyces paradoxus* Batschinskaya yeast and related species: I. The early studies. Microbiology (Moscow). 82:397-403. © Pleiades Publishing, Ltd.
- 3 Naumova ES, Dmytruk KV, Kshanovska BV, Sibirny AA, Naumov GI. 2013 Molecular identification of industrially important strain *Ogataea parapolyomorpha*. Microbiology (Moscow). 82 (4): 453-458. © Pleiades Publishing, Ltd.
- 4 Naumov GI., Naumova ES. 2013 Polymeric genes of lactose fermentations in the yeast *Kluyveromyces lactis*: a new locus *LAC3*. Doklady Biological Sciences (submitted).
- 5 Naumov GI, Naumova ES. 2013 Structure and function of complex lactose polymeric loci *LAC1*, *LAC2* and *LAC3* in the yeast *Kluyveromyces lactis* var. *lactis*. 26th Int. Conf. on Yeast Genetics and Molecular Biology, 29 August – 3 September 2013, Frankfurt/Main, Germany. (Book of abstracts). Yeast. 30 (S1): S220.

Great attention is paid to basic and applied studies of the cluster-regulon containing β -galactosidase gene *LAC4* and lactose permease gene *LAC12* of the yeast *Kluyveromyces lactis*. However, the relationship between this cluster and polymeric determinants *LAC1* and *LAC2*, which were discovered by A. Herman and H. Halvorson (1963), remains obscure. The attempt to determine a biochemical function of the *LAC1* and *LAC2* genes has failed (Boze et al. 1987 a, b). Using tetrad analysis, Southern hybridization and gene sequencing, we found that the *LAC1*, *LAC2* and a new locus *LAC3* have a complex

structure, each consisting of *LAC4-LAC12* cluster. According to molecular karyotyping, the *LAC1*, *LAC2* (known earlier as the *LAC4-LAC12*) and *LAC3* clusters are located on chromosomes III, II and IV, respectively. Taking into account the data obtained, we propose the following nomenclature of the *LAC* genes: (*LAC4-LAC12*)₁, (*LAC4-LAC12*)₂, (*LAC4-LAC12*)₃, (*LAC4*)₁, (*LAC4*)₂, (*LAC4*)₃, (*LAC12*)₁, (*LAC12*)₂ and (*LAC12*)₃. Discovery of yeast polymeric *LAC* genes opens new possibilities for studying their intra- and interspecific evolution. The report is dedicated to the memory of A. Herman.

- 6 Naumov GI, Sadykova AZh, Naumova ES. 2013 Chromosomal polymorphism of *LAC* genes for lactose fermentation in dairy probiotic yeasts *Kluyveromyces*. Abstracts of 38th FEBS Congress, St. Petersburg, Russia, 6-11 July 2013. FEBS Journal. 280 (S1), SW01.S1-39, P. 14-15.
- 7 Sadykova AZh., Naumova ES., Martynenko NN, Naumov GI. 2013 Intra- and interspecies evolution of beta-fructosidase *SUC* genes in the yeast *Saccharomyces*. Abstracts of 38th FEBS Congress, St. Petersburg, Russia, 6-11 July 2013. FEBS Journal. 280 (S1), SW01.S1-39, p. 14.
- 8 Tyurin O, Gubaidullin I, Cheperegin S, Efremov B, Naumova E, Naumov G, Kozlov D. 2013 The new expression system based on a novel yeast species of the genus *Komagataella*. Abstracts of 38th FEBS Congress, St. Petersburg, Russia, 6-11 July 2013. FEBS Journal. 280 (S1), SW06. W33-39, p. 602.
- 9 Naumov GI, Naumova ES. 2013 α and β -galactosidase probiotic yeasts. Advances in Medical Mycology. Proceedings of Conference on Medical Mycology. Moscow: All-Russian National Academy of Mycology, Vol. 5: 356-357 (in Russian).

IV Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Wolczanska 171/173, 90-924 Lodz, Poland. Communicated by Dorota Kregiel <dorota.kregiel@p.lodz.pl>.

The following papers were published in journals.

- 1 Berlowska Joanna, Kręgiel Dorota, Ambroziak Wojciech. 2013 Physiological tests for yeast brewery cells immobilized on modified chamotte carrier. Antonie van Leeuwenhoek, Int J Gen Molec Microbiol 104:703–714.
- 2 Kręgiel Dorota, Berlowska Joanna, Ambroziak Wojciech. 2013 Growth and metabolic activity of conventional and non-conventional yeasts immobilized in foamed alginate. Enz Microbial Tech 53:229-234.
- 3 Kręgiel Dorota, Berlowska Joanna. 2013 Effect of quaternary ammonium silane coating on adhesive immobilization of industrial yeasts. Chemical Papers - DOI: 10.2478/s11696-013-0462-1.
- 4 Balcerek Maria, Pielech-Przybylska Katarzyna. 2013 Effect of simultaneous saccharification and fermentation conditions of native triticale starch on the dynamics and efficiency of process and composition of the obtained distillates. J Chemical Technol Biotechnol 88: 615-622.

- 5 Sapinska Ewelina, Balcerek Maria, Pielech-Przybylska Katarzyna. 2013 Alcoholic fermentation of high-gravity corn mashes with the addition of supportive enzymes. *J Chemical Technol Biotechnol* 88:2152–2158.
- 6 Dziugan Piotr, Balcerek Maria, Pielech-Przybylska Katarzyna, Patelski Piotr. 2013 Evaluation of fermentation of high gravity thick sugar beet juice worts for efficient bioethanol production. *Biotechnol Biofuels* - DOI: doi:10.1186/1754-6834-6-158.
- 7 Kordialik-Bogacka Edyta, Diowksz Anna. 2013 Physiological state of reused brewing yeast. *Czech J Food Sci* 3:264-269.
- 8 Kordialik-Bogacka Edyta, Diowksz Anna. 2013 Metal uptake capacity of modified *Saccharomyces pastorianus* biomass from different types of solution. *Environ Sci Pollution Res* - DOI: 10.1007/s11356-013-2144-5.

The following posters were presented.

- 9 Kręgiel Dorota, Berłowska Joanna, Ambroziak Wojciech. 2013 Monitoring of pyruvate decarboxylase activity in industrial yeasts. 4th Workshop COST Bioflavour Action, Freising, Germany.
- 10 Berłowska Joanna, Kręgiel Dorota. 2013 Adhesion of yeast strains isolated from biofilms in water distribution systems on PVC surface. The 3th Workshop on Microbiology in Health and Environmental Protection - Mikrobiot 2013, Lodz, Poland, II-P4.
- 11 Patelski Piotr, Dziekonska Urszula, Pielech-Przybylska Katarzyna, Balcerek Maria. 2013 Effect of furfural on the fermentation activity of distillery yeast. Conference QUAERE 2013, Prague 2013. Reviewed Proceedings of the Interdisciplinary Scientific International Conference for PhD students and assistants, QUAERE 2013, vol. III, pp. 2877-2881.

V Food Science and Technology, University of California Davis, Davis, California, USA 95616. Communicated by Kyria Boundy-Mills <klbmills@ucdavis.edu>.

New URL: Please update your bookmarks in your web browser: The URL for the Phaff Yeast Culture Collection website has changed to <http://phaffcollection.ucdavis.edu>. The website has an online catalog, where you can browse over 7,000 strains of yeasts belonging to over 800 different species. Yeasts can be ordered and used for research in a variety of areas including taxonomy, ecology, physiology, genomics, and biotechnology.

Radio interview.

Radio host Simon Morton of the Radio New Zealand show “This Way Up” interviewed Phaff collection curator Kyria Boundy-Mills. The 13-minute story covers the nature of yeasts, where they live in the environment, what functions they perform, and how to maintain a yeast collection. The URL for the podcast is <http://www.radionz.co.nz/national/programmes/thiswayup/audio/2570012/yeast-museum>.

Recent publications.

- 1 Sitepu IR, Sestric R, Ignatia L, Levin D, Bruce German J, Gillies LA, Almada LA, Boundy-Mills KL. 2013 Manipulation of culture conditions alters lipid content and fatty acid profiles of a wide variety of known and new oleaginous yeasts species. *Bioresource Technol* 144:360-369.

Oleaginous yeasts have been studied for few species have been studied intensely. To expand oleochemical production for over 80 years. Only a the diversity of oleaginous yeasts available for lipid

research, we surveyed a broad diversity of yeasts with indicators of oleaginicinity including known oleaginous clades, and buoyancy. Sixty-nine strains representing 17 genera and 50 species were screened for lipid production. Yeasts belonged to Ascomycota families, Basidiomycota orders, and the yeast-like algal genus *Prototheca*. Total intracellular lipids and fatty acid composition were determined under different incubation times and nitrogen availability. Thirteen

new oleaginous yeast species were discovered, representing multiple ascomycete and basidiomycete clades. Nitrogen starvation generally increased intracellular lipid content. The fatty acid profiles varied with the growth conditions regardless of taxonomic affiliation. The dominant fatty acids were oleic acid, palmitic acid, linoleic acid, and stearic acid. Yeasts and culture conditions that produced fatty acids appropriate for biodiesel were identified.

- 2 Kanti A, Sukara E, Latifah K, Sukarno N, Boundy-Mills K. 2013 Indonesian oleaginous yeasts isolated from *Piper betle* and *P. nigrum*. *Mycosphere* 4:1015-1026.

In this study, 71 strains of yeast were isolated from *Piper betle* and *P. nigrum*. Isolates were identified using sequence analysis of the D1/D2 region of large 26S ribosomal subunit rDNA. They belong to 25 species in 11 genera. Strains *Cryptococcus luteolus* InaCC Y-265, *Candida orthopsilosis* InaCC Y-302, and *Candida oleophila* InaCC Y-306 could accumulate more than 40% of lipid per g of cell

biomass on a dry weight basis. The fatty acids observed were primarily palmitic acid (C18:1), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linolenic acid (C18:2). The fatty acid profiles suggest that these yeasts may be good candidates for biodiesel production, as they are similar to the fatty acids of plant oils currently used for biodiesel.

VI 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 Gibson BR, Londesborough J, Rautio J, Mattinen L & Vidgren V. 2013 Transcription of α -glucoside transport and metabolism genes in the hybrid brewing yeast *Saccharomyces pastorianus* with respect to gene provenance and fermentation temperature. *J Inst Brewing* 119:23-31.

The hybrid lager yeast *Saccharomyces pastorianus* (*S. cerevisiae* \times *S. eubayanus*) contains several genes encoding proteins responsible for the uptake and metabolism of maltose and maltotriose. In many cases the genes occur as orthologues, that is, the *S. cerevisiae* gene exists along with the *S. eubayanus* gene. Prior to formation of the hybrid, these genes existed in organisms, which had been separated for tens of millions of years and were expected to show some level of genetic and functional differentiation. In this study, oligonucleotide probes were designed for TRAC analysis of transcription of the *S. cerevisiae* and *S. eubayanus* orthologues of *AGT1*, *MALx1*, *MALx2* and *MALx3* as well as the *S. cerevisiae*-

derived *MPH2/3* genes within the *S. pastorianus* genome. Specificity of probes was validated using mRNA from *S. cerevisiae* and from *S. eubayanus*. Probes were used to analyse gene expression during 15 $^{\circ}$ P wort fermentations conducted at different temperatures (10–20 $^{\circ}$ C). As well as differential expression of different genes, differential expression of orthologues was also observed during fermentation. The differences suggest that, where two forms of the gene exist, either one will dominate (as with *AGT1*) or expression will be staggered (*MALx2*), possibly to maximize transport and for efficient degradation of sugars.

- 2 Gibson BR, Storgårds E, Krogerus K & Vidgren V. 2013 Comparative physiology and fermentation performance of Saaz and Froberg lager yeast strains and the parental species *Saccharomyces eubayanus*. *Yeast* 30:255-266.

Two distinct genetic groups (Saaz and Froberg) exist within the hybrid *Saccharomyces pastorianus* (*S. cerevisiae* \times *S. eubayanus*) taxon.

However, physiological/technological differences that exist between the two groups are not known. Fermentative capability of the parental *S. eubayanus*

has likewise never been studied. Here, 58 lager strains were screened to determine which hybrid group they belonged to, and selected strains were characterized to determine salient characteristics. In 15 °P all-malt wort fermentations at 22 °C, Frohberg strains showed greater growth and superior fermentation (80% apparent attenuation, 6.5% alcohol by volume in 3–4 days) compared to all other strains and maintained highest viability values (>93%). Fermentation with *S. eubayanus* was poor at the same temperature (33% apparent attenuation, 2.7% alcohol by volume at 6 days and viability reduced to 75%). Saaz strains and *S. eubayanus* were the least sensitive to cold (10 °C),

though this did not translate to greater fermentation performance. Fermentation with *S. eubayanus* was poor at 10 °C but equal to or greater than that of the Saaz strains. Performance of Saaz yeast/*S. eubayanus* was limited by an inability to use wort maltotriose. [¹⁴C]-Maltotriose transport assays also showed negligible activity in these strains ($\leq 0.5 \mu\text{mol min}^{-1} \text{g}^{-1}$ dry yeast). Beers from Saaz fermentations were characterized by two- to sixfold lower production of the flavour compounds methyl butanol, ethyl acetate and 3-methylbutyl acetate compared to Frohberg strains. Higher alcohol and ester production by *S. eubayanus* was similar to that of Frohberg strains.

- 3 Ilmén M, Koivuranta K, Ruohonen L, Rajgarhia V, Suominen P, Penttilä M. 2013 Production of L-lactic acid by the yeast *Candida sonorensis* expressing heterologous bacterial and fungal lactate dehydrogenases. *Microb Cell Fact.* May 25;12(1):53. [Epub ahead of print].

Poly-lactic acid is a renewable raw material that is increasingly used in the manufacture of bioplastics, which offers a more sustainable alternative to materials derived from fossil resources. Both lactic acid bacteria and genetically engineered yeast have been implemented in commercial scale in biotechnological production of lactic acid. In the present work, genes encoding l-lactate dehydrogenase (LDH) of *Lactobacillus helveticus*, *Bacillus megaterium* and *Rhizopus oryzae* were expressed in a new host organism, the non-conventional yeast *Candida sonorensis*, with or without the competing ethanol fermentation pathway. Each LDH strain produced substantial amounts of lactate, but the properties of the heterologous LDH affected the distribution of carbon between lactate and by-products significantly, which was reflected in extra- and intracellular metabolite concentrations. Under neutralizing conditions *C. sonorensis* expressing *L. helveticus* LDH accumulated lactate up to 92 g/l at a yield of 0.94 g/g glucose, free of ethanol, in minimal medium containing 5 g/l dry cell weight. In rich medium with a final pH of 3.8, 49 g/l lactate was

produced. The fermentation pathway was modified in some of the strains studied by deleting either one or both of the pyruvate decarboxylase encoding genes, *PDC1* and *PDC2*. The deletion of both *PDC* genes together abolished ethanol production and did not result in significantly reduced growth characteristic to *Saccharomyces cerevisiae* deleted of *PDC1* and *PDC5*. We developed an organism without previous record of genetic engineering to produce L-lactic acid to a high concentration, introducing a novel host for the production of an industrially important metabolite, and opening the way for exploiting *C. sonorensis* in additional biotechnological applications. Comparison of metabolite production, growth, and enzyme activities in a representative set of transformed strains expressing different *LDH* genes in the presence and absence of a functional ethanol pathway, at neutral and low pH, generated a comprehensive picture of lactic acid production in this yeast. The findings are applicable in generation other lactic acid producing yeast, thus providing a significant contribution to the field of biotechnical production of lactic acid.

- 4 Koivistoinen O Catabolism of biomass-derived sugars in fungi and metabolic engineering as a tool for organic acid production. PhD Thesis (2013), University of Helsinki.

The use of metabolic engineering as a tool for production of biochemicals and biofuels requires profound understanding of cell metabolism. The pathways for the most abundant and most important hexoses have already been studied quite extensively but it is also important to get a more complete picture of sugar catabolism. In this thesis, catabolic pathways of L-rhamnose and D-galactose were studied in fungi.

Both of these hexoses are present in plant biomass, such as in hemicellulose and pectin. Galactoglucomannan, a type of hemicellulose that is especially rich in softwood, is an abundant source of D-galactose. As biotechnology is moving from the usage of edible and easily metabolisable carbon sources towards the increased use of lignocellulosic biomass, it is important to understand how the

different sugars can be efficiently turned into valuable biobased products. Identification of the first fungal L-rhamnose 1-dehydrogenase gene, which codes for the first enzyme of the fungal catabolic L-rhamnose pathway, showed that the protein belongs to a protein family of short-chain alcohol dehydrogenases. Sugar dehydrogenases oxidising a sugar to a sugar acid are not very common in fungi and thus the identification of the L-rhamnose dehydrogenase gene provides more understanding of oxidative sugar catabolism in eukaryotic microbes. Further studies characterising the L-rhamnose cluster in the yeast *Scheffersomyces stipitis* including the expression of the L-rhamnonate dehydratase in *Saccharomyces cerevisiae* finalised the biochemical characterisation of the enzymes acting on the pathway. In addition, more understanding of the regulation and evolution of the pathway was gained. D-Galactose catabolism was studied in the filamentous fungus *Aspergillus niger*. Two genes coding for the enzymes of the oxido-reductive pathway were identified. Galactitol dehydrogenase is the second enzyme of the pathway converting galactitol to L-xylo-3-hexulose. The galactitol dehydrogenase encoding gene *ladB* was identified and the deletion of the gene resulted in growth arrest on galactitol indicating that the enzyme is an essential part of the oxido-reductive galactose pathway in fungi. The last step of this pathway converts D-sorbitol to D-fructose by sorbitol dehydrogenase encoded by *sdhA* gene. Sorbitol dehydrogenase was found to be a medium chain dehydrogenase and transcription analysis suggested that the enzyme is involved in

D-galactose and D-sorbitol catabolism. The thesis also demonstrates how the understanding of cell metabolism can be used to engineer yeast to produce glycolic acid. Glycolic acid is a chemical, which can be used for example in the cosmetic industry and as a precursor for biopolymers. Currently, glycolic acid is produced by chemical synthesis in a process requiring toxic formaldehyde and fossil fuels. Thus, a biochemical production route would be preferable from a sustainability point of view. Yeasts do not produce glycolic acid under normal conditions but it is a desired production host for acid production because of its natural tolerance to low pH conditions. As a proof of concept, pure model substrates, e.g. D-xylose and ethanol, were used as starting materials for glycolic acid production but the knowledge can be further applied to an expanded substrate range such as biomass derived sugars. Already the introduction of a heterologous glyoxylate reductase gene resulted in glycolic acid production in the yeasts *S. cerevisiae* and *Kluyveromyces lactis*. Further modifications of the glyoxylate cycle increased the production of glycolic acid and it was successfully produced in bioreactor cultivation. The challenge of biotechnology is to produce high value products from cheap raw materials in an economically feasible way. This thesis gives more basic understanding to the topic in the form of new information regarding L-rhamnose and D-galactose metabolism in eukaryotic microbes as well as provides an example on how cell metabolism can be engineered in order to turn the cell into a cell factory that is able to produce a useful chemical.

- 5 Koivistoinen OM, Kuivanen J, Barth D, Turkia H, Pitkänen JP, Penttilä M, Richard P. 2013 Glycolic acid production in the engineered yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. *Microb Cell Fact* 12:82 [Epub ahead of print].

Glycolic acid is a C2 hydroxy acid that is a widely used chemical compound. It can be polymerised to produce biodegradable polymers with excellent gas barrier properties. Currently, glycolic acid is produced in a chemical process using fossil resources and toxic chemicals. Biotechnological production of glycolic acid using renewable resources is a desirable alternative. The yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* are suitable organisms for glycolic acid production since they are acid tolerant and can grow in the presence of up to 50 g l⁻¹ glycolic acid. We engineered *S. cerevisiae* and *K. lactis* for glycolic acid production using the reactions of the glyoxylate cycle to produce glyoxylic acid and then reducing it to glycolic acid. The

expression of a high affinity glyoxylate reductase alone already led to glycolic acid production. The production was further improved by deleting genes encoding malate synthase and the cytosolic form of isocitrate dehydrogenase. The engineered *S. cerevisiae* strain produced up to about 1 g l⁻¹ of glycolic acid in a medium containing d-xylose and ethanol. Similar modifications in *K. lactis* resulted in a much higher glycolic acid titer. In a bioreactor cultivation with d-xylose and ethanol up to 15 g l⁻¹ of glycolic acid was obtained. This is the first demonstration of engineering yeast to produce glycolic acid. Prior this work glycolic acid production through glyoxylate cycle has only been reported in bacteria. The benefit of yeast host is the possibility

for glycolic acid production also in low pH which was demonstrated in flask cultivations. Production of glycolic acid was first shown in *S. cerevisiae*. To test whether a Crabtree negative yeast would be better

suited for glycolic acid production we engineered *K. lactis* in the same way and demonstrated it to be a better host for glycolic acid production.

- 6 Krogerus K & Gibson BR. 2013 Diacetyl and its control during brewing fermentation: 125th Anniversary Review. *J Inst Brewing* 119:86-97.

Diacetyl is a butter-tasting vicinal diketone produced as a by-product of yeast valine metabolism during fermentation. Concentration is dependent on a number of factors including rate of formation of the precursor α -acetolactate by yeast, spontaneous decarboxylation of this acetohydroxy acid to diacetyl and removal of diacetyl by yeast via the action of various reductase enzymes. Lowering concentrations of diacetyl in green beer represents an expensive and time-consuming part of the brewing process and strategies to minimize diacetyl formation or hasten its reduction have potential for improving overall efficiency of the lager brewing system. Here we review the processes that determine diacetyl levels in

green beer as well as the various ways in which diacetyl levels can be controlled. The amount of diacetyl produced during fermentation can be affected by modifying process conditions, wort composition or fermentation technique, or by yeast strain development through genetic engineering or adaptive evolution. The process of diacetyl reduction by yeast is not as well understood as the process of formation, but is dependent on factors such as physiological condition, cell membrane composition, temperature and pH. The process of diacetyl removal is typically rate-limited by the reaction rate for the spontaneous decarboxylation of α -acetolactate to diacetyl.

- 7 Ogata T, Kobayashi M & Gibson BR. 2013 Pilot scale brewing using self-cloning bottom-fermenting yeast with high *SSUI* expression. *J Inst Brewing* 119:17-22.

A pilot-scale fermentation was performed using *SSUI*-overexpressing bottom-fermenting yeast strains constructed by 'self-cloning'. In these strains, the gene *SSUI*, encoding a plasma membrane protein that excretes sulphite, was highly expressed. The rate of fermentation of the two *SSUI*-overexpressing strains tested showed some reduction during the mid-fermentation phase as compared with the parental strain. These differences, however, did not affect overall fermentation and the final apparent extracts had decreased to a level normally obtained during brewing. The concentration of hydrogen sulphide in the wort remained low during fermentation in the case of the two self-cloning strains compared with the parent. The concentration of 2-mercapto-3-methyl-1-

butanol, a sulphur compound that causes an 'onion-like' off-flavour, was also reduced in the case of the self-cloning strains, a result confirmed by sensory evaluation of the beer immediately after bottling. Furthermore, with these strains the anti-oxidation potential of bottled beer, as measured by electron spin resonance, was improved and the concentration of trans-2-nonenal in bottled beer after 7 days of accelerated aging at 37°C was decreased. These observations, together with the lower stale flavour score determined by sensory evaluation of bottled beer after a month of aging at 25°C, indicated that the flavour stability of the beer had been successfully improved.

- 8 Salusjärvi L, Kaunisto S, Holmström S, Vehkomäki ML, Koivuranta K, Pitkänen JP, Ruohonen L. 2013 Overexpression of NADH-dependent fumarate reductase improves D-xylose fermentation in recombinant *Saccharomyces cerevisiae*. *J Ind Microbiol Biotechnol* Oct 10 - Epub ahead of print.

Deviation from optimal levels and ratios of redox cofactors NAD(H) and NADP(H) is common when microbes are metabolically engineered. The resulting redox imbalance often reduces the rate of substrate utilization as well as biomass and product formation. An example is the metabolism of d-xylose by recombinant *Saccharomyces cerevisiae* strains

expressing xylose reductase and xylitol dehydrogenase encoding genes from *Scheffersomyces stipitis*. This pathway requires both NADPH and NAD⁺. The effect of overexpressing the glycosomal NADH-dependent fumarate reductase (FRD) of *Trypanosoma brucei* in d-xylose-utilizing *S. cerevisiae* alone and together with an endogenous, cytosol directed NADH-kinase

(POS5Δ17) was studied as one possible solution to overcome this imbalance. Expression of FRD and FRD + POS5Δ17 resulted in 60 and 23 % increase in ethanol yield, respectively, on d-xylose under anaerobic conditions. At the same time, xylitol yield decreased in the FRD strain suggesting an improvement in redox balance. We show that fumarate

reductase of *T. brucei* can provide an important source of NAD⁺ in yeast under anaerobic conditions, and can be useful for metabolic engineering strategies where the redox cofactors need to be balanced. The effects of FRD and NADH-kinase on aerobic and anaerobic d-xylose and d-glucose metabolism are discussed.

- 9 Turkia H, Holmström S, Paasikallio T, Sirén H, Penttilä M, & Pitkänen JP. 2013 Online capillary electrophoresis for monitoring carboxylic acid production by yeast during bioreactor cultivations. *Anal Chem* 85:9705–9712.

Bioprocess monitoring can improve the understanding and control of biotechnological processes. When analyses are carried out as automated online measurements, manual steps of the analysis procedures are avoided, thus decreasing both the time required for analyses and systematic errors. In this study, an online capillary electrophoresis (CE) system with flow-through sample vial made in-house and action control programming was assembled to monitor carboxylic acid production by *Kluyveromyces lactis*

and *Saccharomyces cerevisiae* during two different bioreactor cultivations. The relative standard deviations were less than 0.6% for intraday migration times and the total analysis time was less than 20 min. The system operated continuously and automatically up to 6 days and produced data concerning carboxylic acid production during the cultivations. The successful test runs demonstrated that this system has potential for the monitoring of biotechnological processes.

- 10 Nordlund E, Katina K, Aura AM, Poutanen K. 2013 Changes in bran structure by bioprocessing with enzymes and yeast modifies the *in vitro* digestibility and fermentability of bran protein and dietary fibre complex. *J Cereal Sci* 58:200–208.

Bran is a good source of dietary fibre, phytochemicals, and also protein, but highly insoluble and recalcitrant structure of bran hinders accessibility of these components for gastrointestinal digestion. In the present work, influence of bioprocessing on the microstructure and chemical properties of rye bran and wheat bread fortified with the rye bran were studied. *In vitro* protein digestibility, and release of short chain fatty acids (SCFA) and ferulic acid in a gut model were studied. Bioprocessing of rye bran was performed with subsequent treatments with cell-wall hydrolysing enzymes (40 °C, 4 h) and yeast fermentation (20 °C, 20 h). Bioprocessing of rye bran

resulted in reduced total dietary fibre content, caused mainly by degradation of fructan and β-glucan, and increased soluble fibre content, caused mainly by solubilisation of arabinoxylans. Microscopic analysis revealed degradation of aleurone cell wall structure of the bioprocessed rye bran. Bioprocessing caused release of protein from aleurone cells, assessed as a larger content of soluble protein in bran and a higher hydrolysis rate *in vitro*. Bioprocessed bran had also faster SCFA formation and ferulic acid release in the colon fermentation *in vitro* as compared to native bran.

- 11 Zdraljevic S, Wagner D, Cheng K, Ruohonen L, Jäntti J, Penttilä M, Resenekov O, Pesce CG. 2013 Single cell measurements of enzyme level as a predictive tool for cellular fates during organic acid production. *Appl Environ Microbiol* 13 - Epub ahead of print.

Organic acids derived from engineered microbes can replace fossil-derived chemicals in many applications. Fungal hosts are preferred for organic acid production because they tolerate lignocellulosic hydrolysates and low pH, allowing economic production and recovery of the free acid. However, cell death caused by cytosolic acidification constrains productivity. Cytosolic acidification affects cells

asynchronously, suggesting that there is an underlying cell-to-cell heterogeneity in acid productivity and/or in resistance to toxicity. We used fluorescence microscopy to investigate the relationship between enzyme concentration, cytosolic pH and viability at the single cell level in *S. cerevisiae* engineered to synthesize xylonic acid. We found that cultures producing xylonic acid accumulate cells with

cytosolic pH below 5 (referred to here as “acidified”). Using live-cell time courses we found that the probability of acidification was related to the initial levels of xylose dehydrogenase, and sharply increased from 0.2 to 0.8 with just a 60% increase in enzyme abundance (Hill coefficient >6). This “switch-like” relationship likely results from an enzyme level threshold above which the produced acid overwhelms the cell's pH buffering capacity. Consistent with this hypothesis, we showed that expression of xylose dehydrogenase from a chromosomal locus yields ~20

times less acidified cells and ~2-fold more xylonic acid, relative to expression of the enzyme from a plasmid with variable copy number. These results suggest that strategies that further reduce cell-to-cell heterogeneity in enzyme levels could result in additional gains in xylonic acid productivity. Our results demonstrate a generalizable approach that takes advantage of the cell-to-cell variation of a clonal population to uncover causal relationships in the toxicity of engineered pathways.

VII CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, the Netherlands.
Communicated by Teun Boekhout and Marizeth Groenewald <m.groenewald@cbs.knaw.nl>.

Recent publications.

- 1 Arendrup MC, Boekhout T, Akova M, Cornely OA, Lorthlaro O & ESCMID/EFISG study group. In press. ESCMID/ECMM Guideline diagnosis and management of emerging yeast infections. Clin Microbiol Infect (accepted).
- 2 Cornely OA Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, Lanternier F, Pagano L, Skiada A, Akova M, Arendrup MC, Boekhout T, Chowdhary A, Cuenca-Estrella M, Freiburger T, Guinea J, Guarro J, de Hoog S, Hope W, Johnson E, Kathuria S, Lackner M, Lass-Flörl C, Lortholary O, Meis J, Meletiadiis J, Munoz P, Richardson M, Roilides E, Tortorano AM, Ullmann AJ, van Diepeningen A, Verweij P, Petrikkos G. In press. ESCMID* and ECMM Joint clinical guidelines for the diagnosis and management of mucormycosis. Clin Microbiol Infect (accepted).
- 3 Hagen F, Ceresini PC, Polacheck I, Ma H, van Nieuwerburgh F, Gabaldón T, Kagan S, Pursall ER, Sionov E, Falk R, Hoogveld HL, van Iersel LJJ, Klau GW, Kelk SM, Stougie L, Bartlett KH, Castañeda E, Lazera M, Meyer W, Deforce D, Meis JF, May RC, Klaassen CHW & Boekhout T. 2012 Ancient dispersal of the human fungal pathogen *Cryptococcus gattii* from the Amazon rainforest. PLoS ONE 8: e71148 - doi:10.1371/journal.pone.0071148.
[blog <http://latinamericanscience.org/2013/08/human-fungal-outbreaks-traced-to-brazil-rainforest/>]
- 4 Iatta R, Cafarchia C, Cuna T, Montagna O, Laforgia N, Gentile O, Rizzo A, Boekhout T, Otranto D & Montagna MT. 2013 Bloodstream infections by *Malassezia* and *Candida* species in critical care patients. Med. Mycol. (in press).
- 5 Khayhan K, Hagen F, Pan W-H, Simwami S, Fisher M, Wahyuningsih R, Chakrabarti A, Chowdhary A, Ikeda R, Taj-Aldeen SJ, Khan Z, Ip M, Imran D, Sjam R, Sriburee P, Liao W-W, Chaicumpar K, Vuddhakul van Iersel LJJ, Meis JF, Klaassen CHW. & Boekhout T. 2013 Geographically structured populations of *Cryptococcus neoformans* variety *grubii* in Asia correlate with HIV status and show a clonal population structure. PLoS ONE 8: e72222 - doi:10.1371/journal.pone.0072222
- 6 Kolecka A, Khayhan K, Arabatzis M, Velegraki A, Kostrzewa M, Andersson A, Scheynius A, Cafarchia C, Iatta R, Montagna MT, Youngchim S, Cabañes FJ, Hoopman, P, Kraak B, Groenewald M, Boekhout T. 2013 Efficient identification of *Malassezia* yeasts by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Br. J. Dermatol. (in press).
- 7 López-Quintero CA, Atanasova L, Franco-Molano AE, Gams W, Komon-Zelazowska M, Theelen B, Müller WH, Boekhout T & Druzhinina I. 2013 Amazonian rainforest reveals *Trichoderma strigosellum* sp.nov. and other species. Antonie van Leeuwenhoek 104:657-674 - doi 10.1007/s10482-013-9975-4.

- 8 Manohar CS, Boekhout T, Müller WH, Stoeck T. 2013 *Tritirachium candoliense* sp. nov, a novel basidiomycetous fungus isolated from the anoxic zone of the Arabian sea. *Fung Biol* (in press).
- 9 Nyanga LK, Nout MJR, Smid EJ, Boekhout T & Zwietering MH. 2012 Fermentation characteristics of yeasts isolated from traditionally fermented *masau* (*Ziziphus mauritiana*) fruits. *Int J Food Microbiol* (in press).
- 10 Smith MTh, Groenewald M. 2012. The treasure trove of yeast genera and species described by Johannes van der Walt (1925–2011). *IMA Fungus* 3:179-187.
- 11 Sun S, Hagen F, Xu J, Dawson T, Heitman J, Kronstad J, Saunders C & Boekhout T. 2013. Ecogenomics of Human and Animal Basidiomycetous Yeast Pathogens. In : *The Ecological Genomics of Fungi* (Ed. F. Martin). Wiley-Blackwell, Oxford, UK, pp. 215-242.

VIII Ebersbrunn 70 A-3711 Großmeiseldorf, Austria. Communicated by Hansjörg Prillinger
 <Hansjoerg.Prillinger@gmx.at>.

The following manuscript has been submitted.

- 1 Hansjörg Prillinger, Ksenija Lopandic & Masako Takashima. Yeast-types of the Basidiomycota using cell wall sugars. *J Gen Appl Microbiol*.

Within the Basidiomycota three yeast-types occur using cell wall sugars the *Microbotryum*-type, the *Ustilago*-type and the *Tremella*-type. The *Microbotryum*-type corresponds with the subphylum Pucciniomycotina, the *Ustilago*-type with the Ustilaginomycotinia and the *Tremella*-type with the Agaricomycotina. The following species were found in the *Microbotryum*-type: *Erythrobasidium hasegawianum*, *Occultifur externus*, *Rhodotorula minuta* (Cystobasidiomycetes), *Bensingtonia yuccicola*, *Kurtzmanomyces nectairei*, *K. tardus*, *Sporobolomyces ruber*, *Sp. xanthus*, *Sterigmatomyces elviae*, *St. halophilus* (Agaricostilbomycetes), *Mastigobasidium intermedium*, *Microbotryum salviae*, *M. succisae*, *Rhodotorula auriculariae*, *R. glutinis*, *R. yarrowii*, *Kriegeria eriophori* (Micro-

botryomycetes), *Platyglaea disciformis* (Pucciniomycetes), *Mixia osmundae* (Mixiomycetes); the members of the *Ustilago*-type are *Nannfeldtiomyces sparganii*, *Rhamphospora nymphaeae*, *Malassezia furfur* (Exobasidiomycetes), *Schizonella caricis-atratae*, *S. cocconii*, *Sporisorium ophiuri*, *Sp. reilianum*, *Ustilago avenae*, *U. bullata*, *U. hordei* (Ustilaginomycetes); *Tremella*-type: *Asterotremella albida*, *A. humicola*, *Christiansenia pallida*, *Filobasidiella neoformans* (Tremellamycetes). Yeast isolates of *Cyphomyrmex minutes* and *C. salvini* (Agaricomycetes, Agaricaceae tribe Leucocoprineae) exhibit a fourth yeast-type, the glucose-mannose-type. It seems that the glucose-mannose cell wall sugar pattern appears at the beginning and at the end of evolution of ascomycetous and basidiomycetous yeast.

IX CREM – Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.
 Communicated by Á Fonseca <amrf@fct.unl.pt> and J.P. Sampaio <jss@fct.unl.pt>.

The following papers were recently published or accepted for publication.

- 1 Nicola Francesca, Cláudia Carvalho, Pedro Miguel Almeida, Ciro Sannino, Luca Settanni, José Paulo Sampaio & Giancarlo Moschetti. 2013 *Wickerhamomyces sylviae* f.a., sp. nov., na ascomycetous yeast species isolated from migratory birds. *International Journal of Systematic and Evolutionary Microbiology* 63 - DOI 10.1099/ijs.0.056382-0

In the present work, we investigate the phylogenetic position and phenotypic characteristic of eight isolates collected from migratory birds on the island of Ustica, Italy. A phylogenetic analysis based on the D1/D2 region of the large-subunit rRNA gene

showed that all isolates clustered as a single separate lineage within the *Wickerhamomyces* clade. They exhibited distinct morphological and physiological characteristics and were clearly separated from their closest relatives, *Wickerhamomyces lynfersii*,

Wickerhamomyces anomalus and *Wickerhamomyces subpelliculosus*, in BLASTN searches. On the basis of the isolation source, physiological features and molecular strain typing carried out with randomly amplified polymorphic DNA (RAPD-PCR) and minisatellite-primed (MSP)-PCR analysis, the isolated

were identified as strains of the same species. The name *Wickerhamomyces sylviae* f.a., sp. Nov. Is proposed to accommodate these novel strains; the type strain is U88A2^T (=PYCC 6345^T =CBS 12888^T). The MycoBank number is MB 804762.

- 2 OH Cissé, JM GCF Almeida, Á Fonseca, AA Kumar, J Salojärvi, K Overmyer, PM Hauser & M Pagni. 2013 Genome sequencing of the plant pathogen *Taphrina deformans*, the causal agent of Peach Leaf Curl. *mBio* 4(3) - doi: 10.1128/mBio.00055-13

Taphrina deformans is a fungus responsible for peach leaf curl, an important plant disease. It is phylogenetically assigned to the Taphrinomycotina subphylum, which includes the fission yeast and the mammalian pathogens of the genus *Pneumocystis*. We describe here the genome of *T. deformans* in the light of its dual plant-saprophytic/plant-parasitic lifestyle. The 13.3-Mb genome contains few identifiable repeated elements (ca. 1.5%) and a relatively high GC content (49.5%). A total of 5,735 protein-coding genes were identified, among which 83% share similarities with other fungi. Adaptation to the plant host seems reflected in the genome, since the genome carries genes involved in plant cell wall degradation (e.g.,

cellulases and cutinases), secondary metabolism, the hallmark glyoxylate cycle, detoxification, and sterol biosynthesis, as well as genes involved in the biosynthesis of plant hormones. Genes involved in lipid metabolism may play a role in its virulence. Several locus candidates for putative *MAT* cassettes and sex-related genes akin to those of *Schizosaccharomyces pombe* were identified. A mating-type switching mechanism similar to that found in ascomycetous yeasts could be in effect. Taken together, the findings are consistent with the alternate saprophytic and parasitic-pathogenic lifestyles of *T. deformans*.

- 3 Anjos J, de Sousa HR, Roca C, Cássio F, Luttk M, Pronk JT, Salema-Oom M, Gonçalves P. 2013 Fsy1, the sole hexose-proton transporter characterized in *Saccharomyces* yeasts, exhibits a variable fructose:H(+) stoichiometry. *Biochim Biophys Acta* 1828:201–207.

In the model yeast *Saccharomyces cerevisiae*, hexose uptake is mediated exclusively by a family of facilitators (Hxt, hexose transporters). Some other *Saccharomyces* species (e.g. *Saccharomyces bayanus* and *Saccharomyces pastorianus*) possess, in addition, a specific fructose transporter (Fsy1, fructose symporter) that has been previously described to function as a proton symporter. In the present work, we compared growth of a yeast strain in which FSY1 occurs naturally in anaerobic, fructose- and glucose-limited chemostat cultures. Especially at low specific growth rates, fructose-proton symport was shown to have a strong impact on the biomass yield on sugar. We subsequently employed energized hybrid plasma membrane vesicles to confirm previous observations concerning the mode of operation and specificity of

Fsy1 mediated transport. Surprisingly, these experiments suggested that the carrier exhibits an unusual fructose:H⁺ stoichiometry of 1:2. This energetically expensive mode of operation was also found consistently in vivo, in shake flask and in chemostat cultures, and both when Fsy1 is the sole transporter and when the Hxt carriers are present. However, it is observed only when Fsy1 is operating at higher glycolytic fluxes, a situation that is normally prevented by downregulation of the gene. Taken together, our results suggest the possibility that fructose symport with more than one proton may constitute an energetically unfavorable mode of operation of the Fsy1 transporter that, in growing cultures, is prevented by transcriptional regulation.

- 4 Coelho MA, Gonçalves C, Sampaio JP, Gonçalves P. 2013 Extensive intra-kingdom horizontal gene transfer converging on a fungal fructose transporter gene. *PLoS Genetics* 9(6): e1003587 (abstract given in the last YNL issue).

Oral presentations.

- 5 José Sampaio. 2013 Surveying wild lineages of *Saccharomyces* to understand patterns of domestication. 5th Conference on Physiology of Yeast and Filamentous Fungi. June 4-7, Montpellier, France.

- 6 Carla Gonçalves. 2013 Fructophily in yeasts a special role for specific fructose transporters. 30th International Specialised Symposium on Yeast “Cell Surface and Organelles in Yeasts: from Basics to Applications”. Stará Lesná. June 18-22.
- 7 Pedro Miguel Almeida, Douda Bensasson and José Paulo Sampaio. 2013 Tracing the origins of domestication in the wine yeast *Saccharomyces cerevisiae*. Congress of the European Society for Evolutionary Biology. 19-24 August. 2013. Lisbon. Portugal.

The following posters were recently presented.

- 8 C Gonçalves, MA Coelho JP Sampaio & P Gonçalves. 2013 Multiple horizontal gene transfer events involving a fungal transporter gene. EMBO Conference: Comparative Genomics of Eukaryotic Microorganisms, Sant Feliu de Guixols, Spain.
- 9 M David-Palma, MA Coelho, D Libkind, JP Sampaio & P Gonçalves. 2013 First insights into the molecular determinants of the homothallic sexual behaviour of the yeast *Phaffia rhodozyma*. EMBO Conference: Comparative Genomics of Eukaryotic Microorganisms, Sant Feliu de Guixols, Spain.
- 10 MA Guerreiro, A Yurkov, AJL Phillips & Á Fonseca. 2013 Genetic variation and species boundaries in the basidiomycetous yeasts *Cryptococcus victoriae* and *C. carnescens* (Tremellales) – a multigene approach. MICROBIOTEC’13. Aveiro, Portugal.

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

Letter to the Editor

The work of Yamamoto et. al. (2004) clearly showed that the two major α -glucosidases (maltase and isomaltase) in *Saccharomyces cerevisiae* are structurally related proteins. The most significant part of their paper is that they found a critical amino acid residue the Val216 in the catalytic site of isomaltase which can discriminate the α -1,4- and α -1,6-glucosidic linkages of substrates. These findings are consistent with the earlier studies that maltase is specific for the hydrolysis of maltose and isomaltase is specific for isomaltose and alpha-methylglucoside (Khan and Eaton, 1967, Khan and Haynes 1972). A recent paper by Teste et. al. (2010), however, has raised the possibility that there exists an alpha-glucosidase with a broader substrate specificity, which can hydrolyze both maltose and isomaltose. These authors have identified 5 genes (IMA1-IMA5) encoding four distinct isomaltases in yeast. These genes have been studied in detail at the molecular level, and three of the 5 genes (IMA3, IMA4, and IMA5) produce an α -glucosidase with a broader substrate specificity. These results suggest the existence of an α -glucosidase in the natural population of yeast strains (species) which can

hydrolyze both maltose and isomaltose as a substrate. This would be analogous to an enzyme such as a chimeric enzyme constructed by the Yamamoto group which can act both on maltose and isomaltose as substrates.

References:

- 1 Yamamoto k, Nakayama A, Yamamoto Y & Tabata S. 2004 Val216 decides the substrate specificity of alpha-glucosidase in *Saccharomyces cerevisiae*. Eur J Biochem 271:3414-4320.
- 2 Khan NA & Eaton NR. 1967 Purification and characterization of maltase and α -methylglucosidase from yeast. Biochim Biophys Acta 146:173-178
- 3 Khan NA & Haynes, RH. 1972 Genetic redundancy in yeast: non-identical products in a polymeric gene system. Molec gen Genet 118:279-285
- 4 Teste MA, Francois JM & 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

Recent publication.

- 1 Ding J, Bierma J, Smith MR, Poliner E, Wolfe C, Hadduck AN, Zara S, Jirikovic M, van Zee K, Penner MH, Patton-Vogt J, Bakalinsky AT. 2013 Acetic acid inhibits nutrient uptake in *Saccharomyces cerevisiae*: auxotrophy confounds the use of yeast deletion libraries for strain improvement. *Appl Micro Biotech.* 97:7405-7416.

Acetic acid inhibition of yeast fermentation has a negative impact in several industrial processes. As an initial step in the construction of a *Saccharomyces cerevisiae* strain with increased tolerance for acetic acid, mutations conferring resistance were identified by screening a library of deletion mutants in a multiply auxotrophic genetic background. Of the 23 identified mutations, 11 were then introduced into a prototrophic laboratory strain for further evaluation. Because none of the 11 mutations was found to increase resistance in the prototrophic strain, potential interference by the auxotrophic mutations themselves was investigated. Mutants carrying single auxotrophic mutations were constructed and found to be more sensitive to growth inhibition by acetic acid than an otherwise isogenic prototrophic strain. At a concentration of 80 mM acetic acid at pH 4.8, the

initial uptake of uracil, leucine, lysine, histidine, tryptophan, phosphate, and glucose was lower in the prototrophic strain than in a non-acetic acid-treated control. These findings are consistent with two mechanisms by which nutrient uptake may be inhibited. Intracellular adenosine triphosphate (ATP) levels were severely decreased upon acetic acid treatment, which likely slowed ATP-dependent proton symport, the major form of transport in yeast for nutrients other than glucose. In addition, the expression of genes encoding some nutrient transporters was repressed by acetic acid, including HXT1 and HXT3 that encode glucose transporters that operate by facilitated diffusion. These results illustrate how commonly used genetic markers in yeast deletion libraries complicate the effort to isolate strains with increased acetic acid resistance.

Recent publication.

- 1 Lucia Paulovičová, Ema Paulovičová, Alexander A. Karelin, Yury E. Tsvetkov, Nikolay E. Nifantiev, Slavomír Bystrický. 2013 Effect of branched α -oligomannoside structures on induction of anti-*Candida* humoral immune response. *Scand J Immunol* 77:431–441.

Several studies have established the potential efficacy of humoral immunity, primarily mannan specific antibodies, in host protection against major fungal pathogen *Candida albicans*. In this study, we analysed humoral immune response induced by immunization with BSA-based conjugates bearing synthetic α -1,6-branched α -oligomannosides (pentamannosides (M5) or hexamannosides (M6)) mimicking antigenic sequences of *Candida* cell wall mannan. We analysed the ability of antibodies prepared by immunization to recognize relevant antigenic determinants in mannan polysaccharide structure and in *C. albicans* yeast and hyphal morphoforms. M6-BSA conjugate induced markedly higher levels of mannan specific IgG compared to M5-BSA conjugate. In contrast to M5-BSA conjugate,

M6-BSA conjugate induced immunoglobulin isotype class switch from IgM to IgG, as revealed also from ELISpot analysis. Immunization induced antibodies showed higher reactivity with hyphal form of *C. albicans* cells. The reduced immunogenicity of M5-BSA conjugate seems to be related to branching point location at terminal non-reducing end in comparison with M6-BSA oligomannoside with branching point at non-terminal location. Candidacidal activity assay revealed different capacity of sera prepared by immunization with M5-BSA and M6-BSA conjugates to improve candidacidal activity of polymorphonuclear leukocytes. Limited capacity of α -1,6-branched oligomannoside - BSA conjugates to induce antibodies significantly enhancing candidacidal activity of polymorphonuclear leukocytes was

presumably related to absence of antibodies with strong reactivity to corresponding antigenic determinants in natural cell wall mannan and with reduced ability to activate complement. The study

documented markedly structure dependent immunogenicity and limited capacity of branched α -mannooligosides conjugates to induce production of potentially protective antibodies.

- 2 Ema Paulovičová, Lucia Paulovičová, Ruzena Pilisiová, Slavomir Bystrický, Dmitri V. Yashunsky, Alexander A. Karelin, Yury E. Tsvetkov and Nikolay E. Nifantiev. 2013 Synthetically prepared glycooligosaccharides mimicking *Candida albicans* cell wall glycan antigens – novel tools to study host–pathogen interactions. FEMS Yeast Res 13:659–673.

The immunobiological efficacy of synthetically prepared mannoooligosaccharides and a glucooligosaccharide mimicking the structure of *Candida albicans* cell wall glycans was assessed in vivo and in vitro to exploit immune responses. The exposure of mice splenocytes to BSA-based conjugates of synthetic oligomannosides and oligoglucoside revealed intense influence on T-cell subset polarization. The conjugates biased the immune responses towards Th1 and Th17 with respect to the prevalence of interferon-gamma (IFN- γ) and interleukin (IL)-17 (IL-17) over IL-4 and IL-10 levels. The inflammatory activity of the conjugates has been evaluated based on the induction of pro-inflammatory cytokines. Postvaccination, antimannoooligosaccharide and antiglucooligosaccharide antisera were subjected to an evaluation of the

structure–immunomodulation activity relationship. Clinical isolates of *C. albicans* CCY 29-3-32 and *C. albicans* CCY 29-3-164 were applied to study interactions between *Candida* cells and anti-oligosaccharide antibodies. In situ recognition of parietal oligomannosyl and oligoglucosyl sequences in *C. albicans* cell wall by the antisera raised against BSA-based conjugates of synthetic oligomannosides and oligoglucoside revealed the effective recognition of specific distribution of natural oligosaccharide sequences in the cell wall of *C. albicans* serotype A. With respect to these results, it can be concluded that new, synthetically prepared oligosaccharides mimicking *Candida* cell wall structures represent prospective immunobiologically effective components for further immuno-pharmacologically relevant *Candida* vaccine design.

- 3 Milan Čertík, Emília Breierová, Monika Oláhová, Ján Šajbidor, and Ivana Márová. 2013 Effect of selenium on lipid alternations in pigment-forming yeasts Food Sci. Biotechnol. 22(S):45-51.

The work deals with lipid modifications of pigment-forming yeasts *Rhodotorula* and *Sporobolomyces* growing under presence of selenium. This metal in the medium significantly prolonged lag-phase of all cultures and enlarged yeast cells. Total, neutral, and membrane yeast lipids (phosphatidylcholine, phosphatidyl-ethanolamine, phosphatidylserine, and phosphatidylinositol) consisted of predominantly palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids. Selenium activated fatty acid unsaturation mainly in phosphatidylcholine due to elevated levels of linoleic and linolenic acids. Because biosynthesis of C18

unsaturated fatty acids in *Rhodotorula* and *Sporobolomyces* species may be associated with phosphatidylcholine moieties, selenium might be involved to the induction of membranebound fatty acid Δ 12 and Δ 15 desaturases in red yeasts. Oppositely, neutral lipids (primarily triacylglycerols) did not show such intensive changes in fatty acid composition as their polar counterparts. These observations could be applied for preparation of selenized red yeasts containing carotenoid pigments with enhanced accumulation of linoleic and linolenic acids.

- 4 Ivana Marová, Andrea Haronikova, Sinisa Petrik, Terezie Dvořaková, Emilia Breierová. 2012 Production of enriched biomass by red yeasts of *Sporobolomyces* sp. grown on waste substrates. J Microbiol Biotechnol Food Sci 1(4):534-551.

Carotenoids and ergosterol are industrially significant metabolites probably involved in yeast stress response mechanisms. Thus, controlled physiological and nutrition stress including use of

waste substrates can be used for their enhanced production. In this work two red yeast strains of the genus *Sporobolomyces* (*Sporobolomyces roseus*, *Sporobolomyces shibatanus*) were studied. To

increase the yield of metabolites at improved biomass production, several types of exogenous as well as nutrition stress were tested. Each strain was cultivated at optimal growth conditions and in medium with modified carbon and nitrogen sources. Synthetic media with addition of complex substrates (e.g. yeast extract) and vitamin mixtures as well as some waste materials (whey, apple fibre, wheat, crushed pasta) were used as nutrient sources. Peroxide and salt stress were applied too, cells were exposed to oxidative stress (2-10 mM H₂O₂) and osmotic stress (2-10 % NaCl). During the experiment, growth characteristics and the production of biomass, carotenoids and ergosterol were evaluated. In optimal conditions tested

strains substantially differed in biomass as well as metabolite production. *S.roseus* produced about 50 % of biomass produced by *S.shibatanus* (8 g/L). Oppositely, production of pigments and ergosterol by *S.roseus* was 3-4 times higher than in *S.shibatanus*. *S.roseus* was able to use most of waste substrates, the best production of ergosterol (8.9 mg/g d.w.) and beta-carotene (4.33 mg/g d.w.) was obtained in medium with crushed pasta hydrolyzed by mixed enzyme from *Phanerochaetae chrysosporium*. Regardless very high production of carotenes and ergosterol, *S.roseus* is probably not suitable for industrial use because of relatively low biomass production.

- 5 Renáta Vadkertiová, Jana Molnárová, Dana Vránová, Elena Sláviková. 2012 Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. Can J Microbiol 58:1344-1352.

Yeasts are common inhabitants of the phyllosphere, but our knowledge of their diversity in various plant organs is still limited. This study was focused on the diversity of yeasts and yeast-like organisms associated with matured fruits and fully open blossoms of apple, plum, and pear trees, during two consecutive years at three localities in southwest Slovakia. The occurrence of yeasts and yeast-like organisms in fruit samples was two and half times higher and the yeast community more diverse than that in blossom samples. Only two species (*Aureobasidium pullulans* and *Metschnikowia pulcherrima*) occurred regularly in the blossom samples, whereas *Galactomyces candidus*, *Hanseniaspora guilliermondii*, *Hanseniaspora*

uvarum, *M. pulcherrima*, *Pichia kluyveri*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae* were the most frequently isolated species from the fruit samples. The ratio of the number of samples where only individual species were present, to the number of samples where two or more species were found (consortium), was counted. The occurrence of individual species in comparison to consortia was much higher in blossom samples than in fruit samples. In the latter, consortia predominated. *Aureobasidium pullulans*, *M. pulcherrima*, and *S. cerevisiae*, isolated from both the fruits and blossoms, can be considered as resident yeast species of various fruit tree species cultivated in southwest Slovakia localities.

- 6 Jana Molnárová, Renáta Vadkertiová and Eva Stratilová. 2013 Extracellular enzymatic activities and physiological profiles of yeasts colonizing fruit trees. J Basic Microbiol 53: (Early view).

Yeasts form a significant and diverse part of the phyllosphere microbiota. Some yeasts that inhabit plants have been found to exhibit extracellular enzymatic activities. The aim of the present study was to investigate the ability of yeasts isolated from leaves, fruits, and blossoms of fruit trees cultivated in Southwest Slovakia to produce extracellular enzymes, and to discover whether the yeasts originating from these plant organs differ from each other in their physiological properties. In total, 92 strains belonging to 29 different species were tested for: extracellular protease, β -glucosidase, lipase, and polygalacturonase activities; fermentation abilities; the assimilation of xylose, saccharose and alcohols (methanol, ethanol, glycerol); and for growth in a medium with 33% glucose. The black yeast *Aureobasidium pullulans* showed the largest spectrum of activities of all the

species tested. Almost 70% of the strains tested demonstrated some enzymatic activity, and more than 90% utilized one of the carbon compounds tested. Intraspecies variations were found for the species of the genera *Cryptococcus* and *Pseudozyma*. Interspecies differences of strains exhibiting some enzymatic activities and utilizing alcohols were also noted. The largest proportion of the yeasts exhibited β -glucosidase activity and assimilated alcohols independently of their origin. The highest number of strains positive for all activities tested was found among the yeasts associated with leaves. Yeasts isolated from blossoms assimilated saccharose and D xylose the most frequently of all the yeasts tested. The majority of the fruit-inhabiting yeasts grew in the medium with higher osmotic pressure.

- 7 Milan Čertík, Marta Brlejšová, Emília Breierová, Lucia Guothová, Zuzana Adamechová, Tatiana Klemková. 2012 Biotechnological production of useful compounds by carotenogenic microorganisms. 8th International Symposium on Biocatalysis and Agricultural Biotechnology DoubleTree Hotel - Sonoma Wine Country, California USA October 28-31, 2012.
- 8 Nemcová Kornélia, Paulovičová Ema, Breierová Emília. 2013 The influence of Cu²⁺ residues on autochthonous yeast and yeast-like grape species. June 2013, Stará Lesná, Slovakia.
- 9 Ivana Márová, Andrea Haroniková, Sinisa Petrik, Milan Čertík, Emilia Breierová. 2013 Biotechnological potential of red yeasts cultivated on selected agroindustrial waste substrates. 9th International Symposium on Biocatalysis and Agricultural Biotechnology Piešťany, Slovak Republic, October 13 – 16, 2013.

XII Department of Agricultural, Food and Environmental Sciences & Industrial Yeasts Collection DBVPG, Borgo XX Giugno 74, 06121, University of Perugia (Italy). Communicated by Pietro Buzzini <pietro.buzzini@unipg.it>.

Recent publications.

- 1 Buzzini P, Margesin R. 2013 Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance, Springer-Verlag, Berlin, Germany.
- 2 Buzzini P, Margesin R. 2013 Cold-adapted yeasts: a lesson from the cold and a challenge for the XXI century. In: Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance (Buzzini P & Margesin R eds), Springer-Verlag, Berlin, pp. 3-22.
- 3 Turchetti B, Goretti M, Buzzini P, Margesin R. 2013 Cold-adapted yeasts in Alpine and Apennine glaciers. In: Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance (Buzzini P & Margesin R eds), Springer-Verlag, Berlin, pp. 99-122.
- 4 Gunde-Cimerman N, Plemenitaš A, Buzzini P. 2013 Changes in Lipids Composition and Fluidity of Yeast Plasma Membrane as Response to Cold. In: Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance (Buzzini P., Margesin R. eds.), Springer-Verlag, Berlin, Germany, pp. 225-242.
- 5 Turchetti B, Goretti M, Branda E, Diolaiuti G, D'Agata C, Smiraglia C, Onofri A, Buzzini P. 2013 Influence of abiotic variables on culturable yeast diversity in two distinct Alpine glaciers. FEMS Microbiol Ecol 86:327-340.
- 6 Goretti M, Turchetti B, Cramarossa MR, Forti L, Buzzini P. 2013 Production of flavours and fragrances via bioreduction of (4R)-(-)-carvone and (1R)-(-)-myrtenal by non-conventional yeast whole-cells. Molecules 8:5736-5748.
- 7 Ricchi M, De Cicco C, Buzzini P, Cammi G, Arrigoni N, Cammi M, Garbarino C. 2013 First outbreak of bovine mastitis caused by *Prototheca blaschkeae*. Vet Microbiol 162:997-999.
- 8 Cadez N, Raspor P, Turchetti B, Cardinali G, Ciafardini G, Veneziani G, Peter G. 2012 *Candida adriatica* sp. nov. and *Candida molendinolei* sp. nov., two novel yeast species isolated from olive oil and its by-products. Int J Syst Evol Microbiol 62:2296-2302.
- 9 Perretti G, Floridi S, Turchetti B, Marconi O, Fantozzi P. 2011 Quality control of malt - turbidity problems of standard worts given by the presence of microbial cells. J Inst Brewing 117:212–216.

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

- 1 Godinho VM, Furbino LE, Santiago IR, Pellizzari FM, Yokoya NS, Pupo D, Alves TMA, Junior PAS, Romanha AJ, Zani CL, Cantrell CL, Rosa CA, Rosa LH. 2013 Diversity and bioprospecting of fungal communities associated with endemic and cold adapted macroalgae in Antarctica. *ISME J* 7:1434-1451.

We surveyed the distribution and diversity of fungi associated with eight macroalgae from Antarctica and their capability to produce bioactive compounds. The collections yielded 148 fungal isolates, which were identified using molecular methods as belonging to 21 genera and 50 taxa. The most frequent taxa were *Geomyces* species (sp.), *Penicillium* sp. and *Metschnikowia australis*. Seven fungal isolates associated with the endemic Antarctic macroalgae *Monostroma hariatii* (Chlorophyte) displayed high internal transcribed spacer sequences similarities with the psychrophilic pathogenic fungus *Geomyces destructans*. Thirty-three fungal singletons (66%) were identified, representing rare components of the fungal communities. The fungal communities displayed high diversity, richness and dominance indices; however, rarefaction curves indicated that not all of the fungal diversity present was recovered.

Penicillium sp. UFMGCB 6034 and *Penicillium* sp. UFMGCB 6120, recovered from the endemic species *Palmaria decipiens* (Rhodophyte) and *M. hariatii*, respectively, yielded extracts with high and selective antifungal and/or trypanocidal activities, in which a preliminary spectral analysis using proton nuclear magnetic resonance spectroscopy indicated the presence of highly functionalised aromatic compounds. These results suggest that the endemic and cold-adapted macroalgae of Antarctica shelter a rich, diversity and complex fungal communities consisting of a few dominant indigenous or mesophilic cold-adapted species, and a large number of rare and/or endemic taxa, which may provide an interesting model of algal–fungal interactions under extreme conditions as well as a potential source of bioactive compounds.

- 2 Morais CG, Cadete RM, Uetanabaro APT, Rosa LH, Lachance MA, Rosa CA 2013 D-xylose-fermenting and xylanase-producing yeast species from rotting wood of two Atlantic Rainforest habitats in Brazil. *Fungal Gen Biol* 60:19-28 - doi.org/10.1016/j.fgb.2013.07.003.

This study investigated the yeast species associated with rotting wood in Brazilian Atlantic Rainforest ecosystems focusing on the identification of D-xylose-fermenting and/or xylanase-producing species. A total of 321 yeast strains were isolated from rotting wood samples collected in two Atlantic Rainforest areas. These samples were cultured in yeast nitrogen base (YNB)-D-xylose or YNB-xylan media. *Schwanniomyces polymorphus*, *Scheffersomyces queiroziae*, *Barnettozyma californica*, and *Candida (Ogataea) boidinii* were the most frequently isolated yeasts. The rarefaction curves for the yeast communities isolated in YNB-D-xylose and YNB-xylan from both areas continued to rise and did not reach an asymptote, indicating that not all yeast diversity had been recovered. Additionally, the yeast composition was variable among the samples

and areas, which was confirmed by the values of the Sorensen index. Among the 69 species identified, only 12 were found in both areas sampled. Fifteen possible new species were obtained. Among them, two species (*Sugiyamaella* sp. 1 and *Sugiyamaella xylanicola*) showed the ability to ferment D-xylose into ethanol, and three species (*Spencermartinsiella* sp. 1, *Su. xylanicola* and *Tremella* sp.) were able to produce extracellular xylanases. Indeed, most of the xylanase-producing isolates belong to the new species *Su. xylanicola*, which was also positive for D-xylose fermentation. *S. queiroziae* and *S. stipitis* were the main D-xylose-fermenting yeasts identified. The results of this work showed that rotting wood collected from the Atlantic Rainforests is a huge source of yeasts, including new species, with promising biotechnological properties.

- 3 Chandel AK, Antunes FFA, Anjos V, Bell MJV, Rodrigues LN, Singh OV, Rosa CA, Pagnocca FC, Silva SS. 2013 Ultra-structural mapping of sugarcane bagasse after oxalic acid fiber expansion (OAFEX) and ethanol production by *Candida shehatae* and *Saccharomyces cerevisiae*. *Biotechnol Biofuels* 6:4.

Background: Diminishing supplies of fossil fuels and oil spills are rousing to explore the alternative sources of energy that can be produced from non-food/feed-based substrates. Due to its abundance, sugarcane bagasse (SB) could be a model substrate for the second-generation biofuel cellulosic ethanol. However, the efficient bioconversion of SB remains a challenge for the commercial production of cellulosic ethanol. We hypothesized that oxalic-acid-mediated thermochemical pretreatment (OAFEX) would overcome the native recalcitrance of SB by enhancing the cellulase amenability toward the embedded cellulosic microfibrils. Results: OAFEX treatment revealed the solubilization of hemicellulose releasing sugars (12.56 g/l xylose and 1.85 g/l glucose), leaving cellulignin in an accessible form for enzymatic hydrolysis. The highest hydrolytic efficiency (66.51%) of cellulignin was achieved by enzymatic hydrolysis (Celluclast 1.5 L and Novozym

188). The ultrastructure characterization of SB using scanning electron microscopy (SEM), atomic force microscopy (AFM), Raman spectroscopy, Fourier transform– near infrared spectroscopy (FT-NIR), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) revealed structural differences before and after OAFEX treatment with enzymatic hydrolysis. Furthermore, fermentation mediated by *C. shehatae* UFMG HM52.2 and *S. cerevisiae* 174 showed fuel ethanol production from detoxified acid (3.2 g/l, yield 0.353 g/g; 0.52 g/l, yield, 0.246 g/g) and enzymatic hydrolysates (4.83 g/l, yield, 0.28 g/g; 6.6 g/l, yield 0.46 g/g). Conclusions: OAFEX treatment revealed marked hemicellulose degradation, improving the cellulases' ability to access the cellulignin and release fermentable sugars from the pretreated substrate. The ultrastructure of SB after OAFEX and enzymatic hydrolysis of cellulignin established thorough insights at the molecular level.

- 4 Badotti F, Vilaça ST, Arias A, Rosa CA, Barrio E. 2013 Two interbreeding populations of *Saccharomyces cerevisiae* strains coexist in cachaça fermentations from Brazil. *FEMS Yeast Res* - doi: 10.1111/1567-1364.12108.

In this study, the phylogenetic relationships between cachaça strains of *Saccharomyces cerevisiae* isolated from different geographical areas in Brazil were obtained on the basis of sequences of one mitochondrial (*COX2*) and three nuclear (*EGT2*, *CAT8*, and *BRE5*) genes. This analysis allowed us to demonstrate that different types of strains coexist in cachaça fermentations: wine strains, exhibiting alleles related or identical to those present in European wine strains; native strains, containing alleles similar to those found in strains isolated from traditional fermentations from Latin America, North America, Malaysian, Japan, or West Africa; and their intraspecific hybrids or 'mestizo' strains, heterozygous

for both types of alleles. Wine strains and hybrids with high proportions of wine-type alleles predominate in southern and southeastern Brazil, where cachaça production coexists with winemaking. The high frequency of 'wine-type' alleles in these regions is probably due to the arrival of wine immigrant strains introduced from Europe in the nearby wineries due to the winemaking practices. However, in north and northeastern states, regions less suited or not suited for vine growing and winemaking, wine-type alleles are much less frequent because 'mestizo' strains with intermediate or higher proportions of 'native-type' alleles are predominant.

- 5 Morais CG, Lara CA, Marques S, Fonseca C, Lachance MA, Rosa CA. 2013 *Sugiyamaella xylanicola* sp. nov., a xylan-degrading yeast species isolated from rotting wood. *Int J Syst Evol Microbiol*. 63:2356-2360.
- 6 de Lima JR, Gonçalves, LRB, Brandão LR, Rosa CA, Viana FMP. 2013 Isolation, identification, and activity in vitro of killer yeasts against *Colletotrichum gloeosporioides* isolated from tropical fruits. *J Basic Microbiol* 53:590-599.

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

The following are abstracts of conference attendance and recently published papers of the group.

- 1 Uzunova K, Georgieva M & Miloshev G. 2013 *Saccharomyces cerevisiae* linker histone - Hho1p maintains chromatin loop organization during ageing. *Oxidative Med Cellular Longevity* - Article ID 437146 - <http://dx.doi.org/10.1155/2013/437146>.

Intricate, dynamic, and absolutely unavoidable ageing affects cells and organisms through their entire lifetime. Driven by diverse mechanisms all leading to compromised cellular functions and finally to death, this process is a challenge for researchers. The molecular mechanisms, the general rules that it follows, and the complex interplay at a molecular and cellular level are yet little understood. Here, we present our results showing a connection between the linker histones, the higher-order chromatin structures, and the process of chronological lifespan of yeast cells. By deleting the gene for the linker histone in *Saccharomyces cerevisiae* we have created a model

for studying the role of chromatin structures mainly at its most elusive and so far barely understood higher-order levels of compaction in the processes of yeast chronological lifespan. The mutant cells demonstrated controversial features showing slower growth than the wild type combined with better survival during the whole process. The analysis of the global chromatin organization during different time points demonstrated certain loss of the upper levels of chromatin compaction in the cells without linker histone. The results underlay the importance of this histone for the maintenance of the chromatin loop structures during ageing.

- 2 Staneva D, Georgieva M, Peycheva E & Miloshev G. 2013 Histone H1 influences cell growth and morphology in yeast *Kluyveromyces lactis*. XXIII^d International Scientific Conference, June, 6th - 7th 2013, Stara Zagora, Bulgaria.

Linker histones are conservative, highly basic proteins with homologs from bacteria to human. H1 histones contribute to the establishment and maintenance of the higher-order chromatin structures and to the fine regulation of gene expression. Seven somatic H1 subtypes are identified in mammals. It has been demonstrated that deletion of one or two of the linker histone encoding genes had no discernible effect. However, a triple H1 knockout was crucial – resulting in embryonic death. The H1 subtypes redundancy and interchangeability in higher eukaryotes hamper the investigation of linker histone functions. Yeasts are unicellular eukaryotes harboring a single gene coding for histone H1 and thus represent an expedient model system to elucidate the cellular role of linker histones. In the *Kluyveromyces lactis* genome a gene, designated *KIH1*, was identified and

its product was predicted to belong to the histone H1/5 protein family. In silico analysis revealed that the putative KIH1 protein has the canonical tri-domain structure characteristic of mammalian linker histones i.e., N- and C- terminal domains and a central globular (G) domain. In order to examine the importance of KIH1p for cell survival we constructed a *K. lactis* H1 knockout strain, KIH1KO. We will present data concerning the phenotypic outcomes from the lack of the functional KIH1 gene and respectively protein on the growth, viability and morphology of yeast cells. Our results demonstrate that although non-essential, KIH1 affects a number of cellular processes which are discussed in terms of their influence on the cell survival. Acknowledgments: This work was partially supported by the Bulgarian Science Fund, Grant number DMU 02/8 (given to M.G.).

XV Department of Genetics and Applied Microbiology, University of Debrecen, Egyetem-ter 1, H-4032 Debrecen, Hungary. Communicated by Matthias Sipiczki <gecela@post.sk>.

Recent publications.

- 1 Pfliegler WP, Horvath E, Kallai Z, Sipiczki M. 2013 Diversity of *Candida zeylanoides* isolates inferred from RAPD, micro/mini-satellite and physiological analysis. *Microbiol Res.* 2013 Oct 28. doi:pii: S0944-5013(13)00147-X. 10.1016/j.micres.2013.09.006. [Epub ahead of print].

Among non-*Saccharomyces* wine yeasts, *Candida zemplinina* is one of the frequently isolated and oenologically important species. It is mostly known from European winemaking areas and it has become one of the key species of non-*Saccharomyces* wine yeasts to study. Investigating the diversity of *C. zemplinina* isolates is important for a deeper understanding of the non-*Saccharomyces* wine yeasts and for the yeast starter industry, as numerous researches have pointed to the potential use of this species in winemaking. For assessing the biodiversity of a larger number of strains, RAPD and micro/minisatellite PCR is often the method of choice, however, this technique is often unstandardized. Whereas some laboratories use these methods for species identifications, others apply RAPD primers for

determining intraspecies diversity. In this study, we have tested 5 different RAPD and micro/minisatellite primers on strains of *C. zemplinina* isolated from different locations. We show that after a rigorous PCR-optimization aimed at reproducibility and comparability of band patterns with these PCR-reactions, diversity of different strains from a wide range of geographic locations is relatively low. The analysis of several oenologically important physiological traits of the strains showed a relatively low level of diversity as well. We also demonstrate that the intraspecific diversity of *C. zemplinina* observable with different techniques (RAPD, micro/minisatellite or physiological analysis) may be fairly different and not necessarily comparable.

- Muñoz J, Cortés JC, Sipiczki M, Ramos M, Clemente-Ramos JA, Moreno MB, Martins IM, Pérez P, Ribas JC. 2013 Extracellular cell wall $\beta(1,3)$ glucan is required to couple septation to actomyosin ring contraction. *J Cell Biol.* 203:265-82.

Cytokinesis has been extensively studied in different models, but the role of the extracellular cell wall is less understood. Here we studied this process in fission yeast. The essential protein Bgs4 synthesizes the main cell wall $\beta(1,3)$ glucan. We show that Bgs4-derived $\beta(1,3)$ glucan is required for correct and stable actomyosin ring positioning in the cell middle, before the start of septum formation and anchorage to the cell wall. Consequently, $\beta(1,3)$ glucan loss generated ring sliding, oblique positioned rings and septa, misdirected septum synthesis indicative of relaxed rings, and uncoupling

between a fast ring and membrane ingression and slow septum synthesis, suggesting that cytokinesis can progress with defective septum pushing and/or ring pulling forces. Moreover, Bgs4-derived $\beta(1,3)$ glucan is essential for secondary septum formation and correct primary septum completion. Therefore, our results show that extracellular $\beta(1,3)$ glucan is required for cytokinesis to connect the cell wall with the plasma membrane and for contractile ring function, as proposed for the equivalent extracellular matrix in animal cells.

- Hegedusova E, Brejova B, Tomaska L, Sipiczki M, Nosek J. 2013 Mitochondrial genome of the basidiomycetous yeast *Jaminalia angkorensis*. *Curr Genet.* 2013 Sep 27. [Epub ahead of print].

Jaminalia angkorensis is an anamorphic basidiomycetous yeast species originally isolated from decaying leaves in Cambodia. Taxonomically, *J. angkorensis* is affiliated with Microstromatales (Exobasidiomycetes, Ustilaginomycotina, Basidiomycota) and represents a basal phylogenetic lineage of this fungal order. To perform a comparative analysis of *J. angkorensis* with other basidiomycetes, we determined and analyzed its complete mitochondrial DNA sequence. The mitochondrial genome is represented by 29,999 base pairs long, circular DNA containing 32 % guanine and cytosine residues. Its genetic organization is relatively compact and comprises typical genes for 15 conserved proteins

involved in oxidative phosphorylation (*atp6*, 8, and 9; *cob*; *cox1*, 2, and 3; and *nad1*, 2, 3, 4, 4L, 5, and 6) and translation (*rps3*), two ribosomal RNAs (*rml* and *rns*) and twenty-two transfer RNAs (*trnA-Y*). Although the gene content is similar to other basidiomycetes, the gene orders in the examined species exhibit only a limited synteny, reflecting their phylogenetic distances and extensive genome rearrangements. In addition, a comparative analysis of basidiomycete mitochondrial genomes indicates that stop-to-tryptophan reassignment of the UGA codon was accompanied by structural alterations of tRNA-Trp(CCA). These results provide an insight into the evolution of the genetic code in fungal mitochondria.

- 4 Sipiczki M, Pfliegler WP, Holb IJ. 2013 *Metschnikowia* species share a pool of diverse rRNA genes differing in regions that determine hairpin-loop structures and evolve by reticulation. PLoS One. 21:e67384.

Modern taxonomy of yeasts is mainly based on phylogenetic analysis of conserved DNA and protein sequences. By far the most frequently used sequences are those of the repeats of the chromosomal rDNA array. It is generally accepted that the rDNA repeats of a genome have identical sequences due to the phenomenon of sequence homogenisation and can thus be used for identification and barcoding of species. Here we show that the rDNA arrays of the type strains of *Metschnikowia andauensis* and *M. fructicola* are not homogenised. Both have arrays consisting of diverse repeats that differ from each other in the D1/D2 domains by up to 18 and 25 substitutions. The variable sites are concentrated in two regions that correspond to back-folding stretches

of hairpin loops in the predicted secondary structure of the RNA molecules. The substitutions do not alter significantly the overall hairpin-loop structure due to wobble base pairing at sites of C-T transitions and compensatory mutations in the complementary strand of the hairpin stem. The phylogenetic and network analyses of the cloned sequences revealed that the repeats had not evolved in a vertical tree-like way but reticulation might have shaped the rDNA arrays of both strains. The neighbour-net analysis of all cloned sequences of the type strains and the database sequences of different strains further showed that these species share a continuous pool of diverse repeats that appear to evolve by reticulate evolution.

- 5 Sipiczki M. 2013 *Starmerella caucasica* sp. nov., a novel anamorphic yeast species isolated from flowers in the Caucasus. J Gen Appl Microbiol. 59:67-73.

Taxonomic analysis of budding yeast strains isolated from flowers of *Wisteria sinensis* (Fabales, Fabaceae) abundantly visited by flying insects, mainly bees in city parks of Baku is described. The isolates forming slightly pink colonies and propagating by budding represent a hitherto unknown yeast species for which the name *Starmerella caucasica* is proposed. The sequences of the D1/D2 domains of the large subunit rRNA genes and the ITS1-5.8S-ITS2 regions were highly similar in the isolates and indicated a close relationship with *Candida kuoi* and *Starmerella bombycolina* in the phylogenetic analysis. *S. caucasica* can be separated from these species by its growth on glucosamine and D-tryptophan, in vitamin-

free medium and at 37°C, and its inability to grow on citrate, ethylamine, cadaverine and in media supplemented with 0.01% of cycloheximide. The type strain is 11-1071.1(T). It has been deposited in Centralbureau voor Schimmelcultures (Utrecht, the Netherlands) as CBS 12650(T), the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary) as NCAIM Y.02030(T) and the Culture Collection of Yeasts (Bratislava, Slovakia) as CCY 90-1-1(T). The GenBank accession numbers for nucleotide sequences of *S. caucasica* are JX112043 (D1/D2 domain of the 26S rRNA gene) and JX112044 (ITS1-5.8S-ITS2). Mycobank: MB 800536.

XVI International Centre of Brewing and Distilling, Heriot-Watt University Edinburgh, Scotland EH14 4AS. Communicated by A. Speers <A.Speers@HW.AC.UK>.

Peer-reviewed publications.

- 1 Stewart GG, Hill AE & Russell I. 2013 125th Anniversary Review – Developments in brewing and distilling yeast strains. J Inst Brew 119(4):In press.
- 2 Potter G, Speers RA & Budge S. 2013 3-OH oxylipins in *Saccharomyces cerevisiae*. Letter J. Inst. Brew. 119:85.
- 3 MacIntosh AJ, MacLeod A, Beattie AD, Eck E, Edney M, Rossnagel B & Speers, RA. 2013 Assessing fermentability of fungally infected malts. J. ASBC. In press.

- 4 Kaur M, Bowman JP, Stewart DC, Sheehy M, Janusz A, Speers RA, Koutoulis A & Evans DE. 2012 TRFLP analysis reveals that fungi rather than bacteria are associated with premature yeast flocculation in brewing. *Journal of Industrial Microbiology & Biotechnology* 39: 1821-1832
- 5 ASBC. 2012 Miniature Fermentation Assay. ASBC Methods of Analysis, Yeast-14. American Society of Brewing Chemists, St. Paul, MN.

Presentations.

- 6 Speers RA. 2013 Barley and brewing; a matter of taste. Scotland Food and Drink, Dundee, SCT. Oct 2.
- 7 Russell I, Stewart GG & Speers RA. 2013 Yeast and fermentation: building better beer and spirits through science and nature. Invited Presentation, International Craft Brewing and Distilling Convention. Dublin. IRE. July, 19-20.
- 8 MacIntosh AJ & Speers RA. 2013 Modeling the attenuation of extract during brewing operations: tracing the black box. EBC Meeting, Luxemburg City, LUX. May 26-30.
- 9 Adler J & Speers RA. 2013 Determining the premature yeast flocculation potential of malt by using the miniature fermentation assay with synthetic wort and a malt washing technique. ASBC Annual Meeting, Tucson, AZ June 11-15.
- 10 Speers RA & MacIntosh AJ. 2013 Modelling brewing fermentations: The shape of the black box! MBAA District Ontario, Toronto, ON. Jan. 25.
- 11 MacIntosh AJ & Speers RA. 2013 Modelling brewing fermentations: Drawing the black box! MBAA District Ontario, Toronto, ON. Jan. 25 .

XVII Mycology Collection, American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA. Communicated by Sung-Oui Suh <ssuh@atcc.org>.

Recent publications.

- 1 Suh S-O, Houseknecht JL, Gujjari P & Zhou JJ. 2013 *Scheffersomyces parashehatae* f.a., sp. nov., *Scheffersomyces xylosifermentans* f.a., sp. nov., *Candida broadrunensis* sp. nov., and *Candida manassasensis* sp. nov., four novel yeasts associated with wood-ingesting insects and their ecological and biofuel implications. *Int J Syst Evol Microbiol* 63:4330-4339.

During a survey of yeasts associated with wood-ingesting insects, 69 strains in the *Scheffersomyces* clade and related taxa were isolated from passalid and tenebrionid beetles and the decayed wood inhabited by them. The majority of these yeasts was found to be capable of fermenting xylose, and was recognized as *Scheffersomyces stipitis* or its close relative *Scheffersomyces illinoisensis*, which are known to be associated with wood-decaying beetles and rotten wood. Yeasts in '*Scheffersomyces*' (= *Candida*) *ergatensis* and '*Scheffersomyces*' (= *Candida*) *coipomoensis* were also frequently isolated. The remaining six strains were identified as representing four novel species in the genera *Scheffersomyces* and *Candida* based on multilocus sequence analyses of nuclear rRNA genes and four protein-coding genes, as

well as other taxonomic characteristics. Two xylose-fermenting species, *Scheffersomyces parashehatae* f.a., sp. nov. (type strain ATCC MYA-4653^T = CBS 12535^T = EH045^T) and *Scheffersomyces xylosifermentans* f.a., sp. nov. (type strain ATCC MYA-4859^T = CBS 12540^T = MY10-052^T), formed a clade with *Scheffersomyces shehatae* and related *Scheffersomyces* species. Interestingly, *S. xylosifermentans* can survive at 40 °C, which is a rare property among xylose-fermenting yeasts. *Candida broadrunensis* sp. nov. (type strain ATCC MYA-4650^T = CBS 11838^T = EH019^T) is a sister taxon of *C. ergatensis*, while *Candida manassasensis* sp. nov. (type strain ATCC MYA-4652^T = CBS 12534^T = EH030^T) is closely related to *Candida palmioleophila* in the *Candida glabosa* clade. The

multilocus DNA sequence comparisons in this study suggest that the genus *Scheffersomyces* needs to be circumscribed to the species near *S. stipitis* (type

species) and *S. shehatae* that can be characterized by the ability to ferment xylose.

- 2 Suh SO, Gujjari P, Beres C, Beck B & Zhou J. 2013 Proposal of *Zygosaccharomyces parabailii* sp. nov. and *Zygosaccharomyces pseudobailii* sp. nov., two new species closely related to *Zygosaccharomyces bailii*. *Int J Syst Evol Microbiol* 63:1922-1929.

Twenty-three yeast strains traditionally identified as *Zygosaccharomyces bailii* were studied in order to clarify their taxonomy and phylogenetic relationships. The molecular phylogeny from rRNA gene sequences showed that these yeasts were well divided into three major groups, and two of the groups could be clearly distinguished from the type strain of *Z. bailii* at the species level. Therefore, we propose *Zygosaccharomyces parabailii* sp. nov. (type strain ATCC 56075^T = NBRC 1047^T = NCYC 128^T = CBS 12809^T) and *Zygosaccharomyces pseudobailii* sp. nov.

(type strain ATCC 56074^T = NBRC 0488^T = CBS 2856^T) to accommodate the yeasts belonging to the two groups. By conventional physiological tests, *Z. bailii* and the two novel species are not clearly distinguished from one another, as variations exist more frequently between individual strains and are not species-specific. However, the conclusions from rRNA gene sequence analyses are well supported by genome fingerprinting patterns as well as other protein-coding gene sequence comparisons.

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

Recently accepted papers.

- 1 Morais CG, Cadete RM, Uetanabaro APP, Rosa LH, Lachance MA. 2013 D-xylose-fermenting and xylanase-producing yeast species from rotting wood of two Atlantic Rainforest habitats in Brazil. *Fungal Genet Biol* 60:19-28. See abstract under Dr Rosa's communication, page 49.
- 2 Lachance MA, Perri A, Farahbakhsh A & Starmer WT 2013 Genetic structure of *Kurtzmaniella cleridarum*, a cactus flower beetle yeast of the Sonoran and Mojave Deserts: speciation by distance? *FEMS Yeast Research* 13:674-681.

We studied 95 isolates of the yeast species *Kurtzmaniella cleridarum* recovered from nitidulid beetles collected in flowers of cacti of the Sonoran Desert of southern Arizona and the Mojave Desert of California. They were characterized on the basis of mating type and ten polymorphic DNA markers in relation to their geographic distribution. Although all loci appeared to be free of strong linkage, the recovered haplotypes represented but a small fraction of possible combinations, indicating that abundant asexual reproduction of local genotypes accounts for much of population growth, even though the yeast is capable of sexual recombination in nature. Much of

the genetic differentiation took place at the local level, indicating that gene flow across the various localities is limited. However, a relationship exists between overall genetic differentiation and geography over long distances. We estimated that populations separated by c. 1300 km would share no alleles in common and that such a separation might be enough to favor the onset of speciation.

In the memory of Jane M. Bowles (1952-2013), biophile and co-discoverer of *Kurtzmaniella cleridarum*.

- 3 Daniel HM, Rosa CA, Thiago-Calaça PSS, Antonini Y, Bastos EMAF, Evrard P, Huret S, Fidalgo-Jiménez A, Lachance MA 2013 *Starmerella neotropicalis* f.a., sp. nov., a new yeast species found in bees and pollen from Brazil and Cuba. *Int J Syst Evol Microbiol* 63:3896-3903.

A novel yeast species was found repeatedly and in high cell densities in underground-nesting stingless

bees of the species *Melipona quinquefasciata* and their provisions in northern Minas Gerais (Brazil).

One additional strain was isolated from bee-collected pollen in Cuba. Phylogenetic analyses based on rRNA gene sequences (D1/D2 large subunit gene and internal transcribed spacer) indicated that the novel species belongs to the *Starmerella* clade and is most closely related to *Candida* (iter. nom. *Starmerella*) *apicola*. Growth reactions on carbon and nitrogen sources were typical of those observed in related species of the *Starmerella* clade. PCR-fingerprinting with mini- and microsatellite specific primers allowed

the distinction of the novel species from *Candida apicola*, *Candida bombi* and a yet undescribed species represented by strain CBS 4353. On the basis of phylogenetic relationships, the novel species is assigned to the genus *Starmerella* despite the failure to observe sexual reproduction after extensive mating tests. We propose the name *Starmerella neotropicalis* f. a., sp. nov. (Mycobank MB 804285) and designate UFMG PST 09(T) (= MUCL 53320^T = CBS 12811^T) as the type strain.

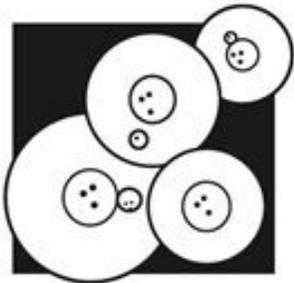
- 4 Freitas LFD, Carvajal Barriga EJ, Portero Barahona P, Lachance MA, Rosa CA 2013 *Kodamaea transpacific* f.a., sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galápagos Islands and Malaysia: further evidence for ancient human transpacific contacts. Int J Syst Evol Microbiol 63:4324–4329.

Twenty-four yeast strains were isolated from ephemeral flowers of *Ipomoea* spp. and *Datura* sp. and their associated insects in the Galápagos Archipelago, Ecuador, and from *Ipomoea* spp. and associated insects in the Cameron Highlands, Malaysia. Sequences of the D1/D2 domains of the large subunit rRNA gene indicated that these strains belong to a novel yeast species of the *Kodamaea*

clade, although the formation of ascospores was not observed. The closest relative is *Candida restingae*. The human-mediated dispersion of this species by transpacific contacts in ancient times is suggested. The name *Kodamaea transpacific* f.a., sp. nov. is proposed to accommodate these isolates. The type strain is CLQCA-24i-070^T (= CBS 12823^T= NCYC 3852^T); MycoBank number MB 803609.

Forthcoming Meetings

The 9th International Conference on *Cryptococcus* and Cryptococcosis (ICCC9) Amsterdam, May15-19 2014



Welcome to the 9th International Conference on *Cryptococcus* and Cryptococcosis (ICCC-9), to be held in Amsterdam, 15 – 19 May 2014. Be our guest at the century old Museum of the Tropics in Amsterdam! It is our pleasure to invite you to visit Amsterdam in the spring of 2014 for the 9th International Conference on *Cryptococcus* and Cryptococcosis which will be held in the historic and beautiful ‘Tropenmuseum’. We look forward to an exciting meeting focusing on new insights into this important killer yeast. Topics will include clinical perspectives in the developed and developing world, disease management, treatment, diagnostics, epidemiology, taxonomy, immunology,

pathophysiology, molecular biology, and much more. For further details on the program see

http://www.iccc2014.org/en/Home_10_6_12.html

The medieval city of Amsterdam is on the UNESCO heritage list and is home to people from all over the globe giving it a truly international flavour. In the spring, the canals and neighbourhoods are perfect for a boat trip or for just strolling around taking in the sights and sounds of this unique city. The Van Gogh Museum and the recently renovated Rijksmuseum house magnificent art collections. Amsterdam is also renowned for its open character and its hotels, restaurants and bars ranging from the cheap and cheerful to the chic and exclusive and expensive. Mark 15 – 19 May 2014 in your agenda and join us for this unique scientific event in Amsterdam, or Mokum as the locals call it.

Venue: ICC9-2014 will be held at the Royal Tropical Institute ([KIT](#)), in Amsterdam. Built in 1926, the imposing, unique KIT building has a large and small conference hall and theatre, a magnificent reception area and seven atmospheric meeting rooms.

Next to the conference center, the KIT features a library, the Tropenmuseum and a restaurant. Amsterdam has excellent connections: [Airport Amsterdam Schiphol](#) (international destinations) can be reached within 30 minutes by public transport and

approximately 25 minutes by taxi. Moreover Amsterdam is well linked to both (high speed) train and motorway networks. For more information on Amsterdam, please visit www.iamsterdam.com.

41st Annual Conference on Yeasts Smolenice Castle, Slovakia, May 20-23 2014

The 41st Annual Conference on Yeasts is still being planned for 20 -23 May 2014 at Smolenice Castle, Slovakia. On-line registration will be opened in December.

XIVth International Congress of Mycology & Eukaryotic Microbiology Montréal, Québec, Canada, July 27th - August 1st 2014

The Congresses of the International Union of Microbiological Societies (IUMS 2014) will take place from July 27th to August 1st, 2014 at the Palais des Congrès de Montréal (Montréal's Convention Centre), in Montréal, Canada.

The three congresses [XIVth International Congress of Mycology; XIVth International Congress of Bacteriology and Applied Microbiology; XVIth International Congress of Virology] will be held simultaneously within one week to stimulate cross talk.

The Mycology Division of IUMS is in charge of the International Congress of Mycology and we foresee to expand its scope beyond the fungi. Thus the congress will cover Mycology and other Eukaryotic microorganisms. The 'Congress of Mycology' in Montreal to also include important aspects of the biology non-fungal eukaryotic microbes.

The congresses will have a key note and bridging sessions across all divisions, shared sessions between de Divisions Bacteriology and 'Mycology', and of course 'Mycology and other Eukaryotic Microbe-oriented' sessions.

The congress will be of interest to all involved or interested in research and training in eukaryotic microbiology, and will cover basic research leading to advancement of knowledge, clinical research bringing new knowledge from the bench to patients, and applied research dealing with the development and use of innovative approaches to prevent and treat microbe-related health problems or to the use of microbes for the benefit of mankind.

Call for Abstracts is now open. [... more](#)

Registration is now open. [... more](#)

Opening Scientific Lecture

We are pleased to announce that the Opening Scientific Lecture, scheduled on Sunday, July 27, 2014, will be delivered by Prof. Julian Davies from the Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia.

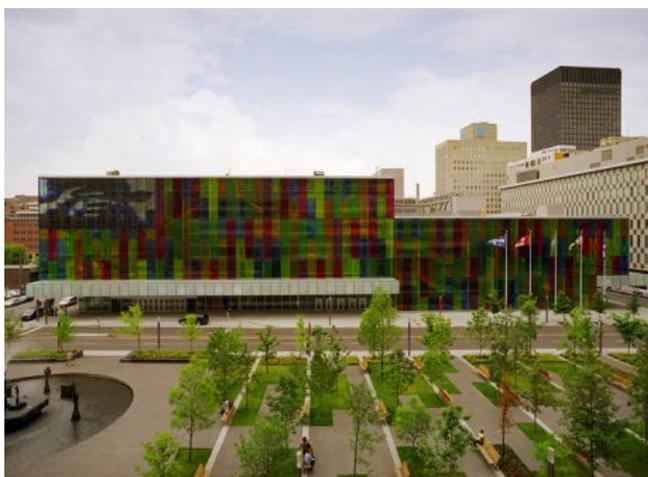
Confirmed Keynote and Workshop Speakers (September 2013):

[Bridging Sessions](#)

[Bacteriology & Applied Microbiology](#)

[Mycology & Eukaryotic Microbiology](#)

[Virology](#)



Travel Support

We are happy to announce that Travel Support will be available for Graduate Students & Postdoctoral Fellows. Applications will be done with abstract

submission, starting in October 2013 and by December 2, 2013

Further information at <http://www.montrealiums2014.org/>

Teun Boekhout (Chair of the Mycology congress, vice-chair Mycology Division)

Pierre Belhumeur (Vice-chair of the Mycology congress)

Scott Baker (Former chair Mycology congress 2011, Chair Mycology Division)

10th International Mycological Congress, August 3-8, 2014, Bangkok, Thailand

The 10th International Mycological Congress will be held in Bangkok, Thailand from August 3 to 8, 2014. Although a predominance of hyphal-growth enthusiasts may be counted on, many facets of yeast research will integrate well into the program, for example stress metabolism, insect-fungal symbioses, endophytes, biocontrol, extreme environments, diversity assessments, traditional fermented Asian foods, metabolites, biotechnology and genomes. A session dedicated to “Diversity and molecular

taxonomy of yeasts” will be organised and abstract submission is open till March 31, 2014. It is hoped that a Special Interest Group (SIG) on yeast nomenclatural issues and the future of “The Yeasts: A taxonomic study” will be met with interest and participation by the yeast community. For further information on program and deadlines please refer to www.imc10.com.

We look forward seeing you in Bangkok.

Heide-Marie Daniel, Masako Takashima, and Teun Boekhout

ISSY 31 Yeast Fermentation: From Genes to Application Aspects Vipava, Slovenia 9-12th October 2014

The conference is organized in the renovated Lanthieri Palace by Lund University, University of Nova Gorica, EU FP7 Cornucopia and Jubilekinase

ApS. ISSY 31 is organized under auspices of International Commission on Yeasts (ICY). For further information, contact:

Jure Piskur <Jure.Piskur@biol.lu.se>

http://www.yeast-cornucopia.se/index.php?option=com_content&view=article&id=12&Itemid=24

Brief News Item

New coordinates: Alex Speers

In the spring of 2013, I became the Director of the ICBD focusing on both applied and hypothesis driven research while overseeing the activities of the Centre.

A. Speers
International Centre of Brewing and Distilling
Heriot-Watt University
Edinburgh EH14 4AS
Scotland

<A.Speers@HW.AC.UK>

New book

Cold-adapted Yeasts.

Biodiversity, Adaptation Strategies and Biotechnological Significance

Pietro Buzzini and Rosa Margesin (Eds)



This book presents our current understanding of the diversity and ecology of cold-adapted yeasts in worldwide cold ecosystems, their adaptation strategies, and their biotechnological significance. Special emphasis is placed on the exploitation of cold-adapted yeasts as a source of cold-active enzymes and biopolymers, as well as their benefits for food microbiology, bioremediation and biocontrol. Further, aspects of food biodeterioration are considered. Cold-adapted yeasts inhabit numerous low-temperature environments where they are subjected to seasonal or permanent cold conditions. Hence, they have evolved a number of adaptation strategies with regard to growth and reproduction, metabolic activities, survival and protection. Due to their distinctive ability to thrive successfully at low and even subzero temperatures, cold-adapted yeasts are increasingly attracting attention in basic science and industry for their enormous biotechnological potential. This book provides an overview of the biology and potential biotechnological applications. All Chapters were written by internationally recognized experts.

Table of contents

www.springer.com/978-3-642-39680-9

<http://link.springer.com/book/10.1007/978-3-642-39681-6>

Cold-Adapted Yeasts: a Lesson from the Cold and a Challenge for the XXI Century.

Methods for the Isolation and Investigation of the Diversity of Cold-Adapted Yeasts and their ex situ Preservation in Worldwide Collections.

Cold-Adapted Yeasts in Arctic Habitats.

Cold-Adapted Yeasts in Antarctic Deserts.

Cold-adapted yeasts in Alpine and Apennine glaciers.

Cold-Adapted Yeasts in Patagonian Habitats.

Cold-Adapted Yeasts in Deep-Sea Environments .

Black Yeasts in Cold Habitats.

Production of Pigments and Photo-Protective Compounds by Cold-Adapted Yeasts.

Changes in Lipids Composition and Fluidity of Yeast Plasma Membrane as Response to Cold.

Cold-Shock Response and Adaptation to Near-Freezing Temperature in Cold-Adapted Yeasts.

Production of Antifreeze Proteins by Cold-Adapted Yeasts.

Role of Sterol Metabolism and Endoplasmic-Reticulum-Associated Degradation (ERAD) of Proteins in Cold Adaptation of Yeasts.

Subzero Activity of Cold-Adapted Yeasts.

Fundamentals of Cold-Active Enzymes.

Cold-Active Yeast Lipases: Recent Issues and Future Prospects.

Miscellaneous Cold-Active Yeast Enzymes of Industrial Importance.

Production of Polymers and other Compounds of Industrial Importance by Cold-Adapted Yeasts.

Low-Temperature Production of Wine, Beer and Distillates using Cold-Adapted Yeasts.

Cold-Adapted Yeasts as Biocontrol Agents: Biodiversity, Adaptation Strategies and Biocontrol Potential.

Bioremediation and Biodegradation of Hydrocarbons by Cold-Adapted Yeasts.

Heterologous Expression of Proteins from Cold-Adapted Yeasts in Suitable Hosts: Methods and Applications.

Food Spoilage by Cold-adapted Yeasts.

50 Years Ago

[Yeast Newsletter Vol XII No 2 November 1963](#)

Y E A S T

A News Letter for Persons Interested in Yeast

November 1963

Volume XII, Number 2

Editor

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

Mrs. N. J. W. Kreger-van Rij of the Centraalbureau voor Schimmelcultures, Delft, Holland announced receipt of type cultures of two new yeast species and publication of a manuscript describing ascospores of *Endomycopsis selenospora*.

Dr. J. Lodder of CBS announced plans for a new and thoroughly revised edition of "The Yeasts". Updates to the 1952 edition include many new genera and species. The planned list of contributing authors included Mrs. N.J.W. Kreger-van Rij, Dr. H. J. Phaff, Miss W. Ch. Slooff, Dr. N. van Uden, Dr. L. J. Wickerham and Dr. J. P. van der Walt. Readers were asked to submit yeast cultures and reprints of their papers.

Dr. J. Santa Maria, Madrid, Spain, listed one paper in press on *Saccharomyces oleaginosus*, and two papers accepted for publication regarding novel species of yeasts found in "alpechin" and taxonomy of *Torulaspora* (Lindner).

Dr. R. C. Artagaveytia-Allende, Montevideo, Uruguay, published an examination of the methods used to classify species within the genus *Rhodotorula* (Harrison).

O. Verona, Universita di Pisa, Italy, reported six recent publications, many of them proposing taxonomic revisions based on physiological, morphological and environmental characters of yeasts. Revisions of the genus *Taphrina* were proposed, with asporogenous forms of *Taphrina* and *Symbiotaphrina* placed in new genus *Saprotaphrina*.

Dr. Shoji Goto, Yamanishi University, Kofu, Japan published the proposal of new genus *Naganishia*. One strain of species *N. globosus* was isolated from Bleu cheese. This species may be the perfect form of *Cryptococcus diffluens*.

A summary of the Ph.D. thesis of **Dr. Sheena S. Ross** was communicated by **Prof. E. O. Morris**, The Royal College of Science and Technology, Glasgow, Scotland. Titled "A Study of Yeasts of Marine Origin", the work involved analysis of 235 yeast cultures from marine sources (fish and sea water), 200 isolated as part of this study. Fish skin and slime yielded the most yeast strains; faeces yielded the fewest. The characteristics of most isolates agreed closely with those of species listed in the 1952 edition of "The Yeasts". Complex nitrogen sources stimulated halotolerance in many marine strains. At high NaCl concentrations, the lag-phase was prolonged, in some cases up to three weeks, after which exponential phase began suddenly.

Dr. J. F. T. Spencer, Prairie Regional Laboratory, Saskatoon, Canada listed work in progress including a survey of yeasts in surface waters in Saskatchewan, the structure of tri- and tetrasaccharides formed by *Sporobolomyces singularis*, and metabolic pools in *Candida utilis*.

Dr. Anna Kockova-Kratochvilova, Chemical Institute of the Slovak Academy of Sciences, Czechoslovakia described the work of the Department of Microbiology, and listed 10 publications since 1962. Publications dealt with taxonomy and pathogenicity of *Candida albicans*, and the ecology of plant-associated yeasts.

A list of speakers at the 13th Regular Meeting of the Seminar of Yeast Studies, Osaka, Japan, Oct 1963 was provided by **Dr. Minoru Yoneyama** of Hiroshima University, Japan. Dr. Yoneyama also described his work on yeasts associated with *Drosophila melanogaster* in Hiroshima.

The abstracts of two articles in press in the Journal of Bacteriology were submitted by **Dr. L. R. Hedrick**, Illinois Institute of Technology, USA, regarding the effects of cations on hydrophobicity of yeasts and the growth of *Acanthamoeba castellanii* with the yeast *Torulopsis famata*.

Dr. Colin H. Clarke, Institute of Animal Genetics, Edinburgh, Scotland described ongoing research projects with *Schizosaccharomyces pombe*, utilizing red-white sectored colonies to study revertants of adenine auxotrophs.

Dr. Carl C. Lindgren of Southern Illinois University, USA published eight manuscripts, on the morphology of cells and organelles, chromosome mapping, and cell wall structure in *Saccharomyces*.

Work in the laboratory of **Dr. Siegfried Windisch**, Institut für Garungsgewerbe, Berlin, Germany included the yeast flora of beers, and the genetic structure of ad_6 and ad_7 loci of *Schizosaccharomyces pombe*. Genetic analysis was applied to examine the factors affecting velocity of beer fermentation by *Saccharomyces*, formation of top fermenting cells by bottom yeasts, maltose fermentation, sucrose fermentation, and vitamin requirements. The relation between ploidy and fermentation intensity was studied.

Dr. Noboru Kawakami, Hiroshima University, Japan, announced that he planned to visit the USA to study electron microscopy.

Dr. Sumio Suehiro shared a list of five recent publications on isolation of yeasts from marine habitats including putrefied marine algae, diatoms, tideland mud, marine plankton, and seaweed.

Compiled by Dr. Kyria Boundy-Mills
Curator, Phaff Yeast Culture Collection, University of California Davis
