

# Yeast

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# Editorials

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## Ralph Kunkee - 1927-2011

I regret to announce the death of Prof. Ralph Kunkee, Professor Emeritus at the University of California at Davis and long-time reader of the Yeast Newsletter. Prof. Kunkee was well known for his contributions to the development of oenology in the United States and has participated in the education of many winemakers worldwide. I had the privilege of attending some of his lectures while I was a graduate student, during which time he served as my course advisor.

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## Johannes van der Walt - 1925-2011

A towering figure in the world yeast biodiversity, Dr. Johannes van der Walt, died recently after a short illness. Dr. van der Walt is remembered by many of us for his prolific output of new yeast species or genera from South African insects, plants, and soil. His training, however, was in biochemistry: his doctoral thesis in Kluyver's lab reported on the structure of pulcherrimin, the beautiful pigment of *Metschnikowia pulcherrima* and some *Kluyveromyces* species (PNAS 39:583). He once confided to me that his original dream was to discover new chemical compounds, but that he changed his mind upon realizing that there was, out there, a universe of new yeast species. One of his proudest moments was the observation of the multispored asci of *Kluyveromyces polysporus*, now more aptly *Vanderwaltozyma polyspora*. Johannes was a trailblazer in the discovery of ascomycetous life cycles and was a strong advocate of the use of interfertility as the primary criterion for species delineation. Students of yeast biodiversity should be aware of the tremendous debt owed to Dr. van der Walt.

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## ISSY 29 – Guadalajara, Mexico

Congratulations to Patricia Lappe, Anne Gschaedler-Mathis, and their colleagues for an outstanding International Specialized Symposium on Yeasts. Held jointly with the First International Symposium on Agave, August 29 to September 2 2011, ISSY 29 was entitled “The Relevance of Yeasts and Microbial Consortia in Traditional and Industrial Fermentations”. This topic served as a strong theme throughout the meeting, which covered numerous aspects of yeasts in food, beverages, and other industrial products, from systematics to ecology, physiology, and genomics. The scientific committee deserves special praise for having recruited an impressive list of speakers internationally, but featuring many distinguished yeast researchers from Latin America. The venue, Hotel Camino Real in the centre of Guadalajara, offered delightful Mexican gastronomy in a comfortable setting. Three major social events provided participants with an introduction to traditional Tapatío culture, including a tour of Casa Herradura, where guests were treated to rural delicacies and premium tequila.

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### Printed subscriptions

Due to ever increasing postal rates, it is once again necessary to increase the subscription fees. Henceforth, the cost of printed copies will be USD 10.00 (USA and Canada) and USD 15.00 (elsewhere). We now offer the possibility of making credit card payments using *Paypal*. Interested readers should access the Yeast Newsletter website and select [Subscriptions - Renewals](#). To ensure proper credit, please ensure that your payment information matches your mailing address as it appears on your invoice. The option to receive the Yeast Newsletter electronically, free of charge, will continue for all readers whose accounts are in good standing.

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### Electronic mailing list

The Yeast Newsletter is available electronically, free of charge. Back issues can be downloaded from the Yeast Newsletter website by selecting the “Back Issues” icon. The current issue will be sent to individuals upon request to be added to the electronic mailing list. Readers are encouraged to inform colleagues of the availability of this service by forwarding the link sent by email in June or December.

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

M. A. Lachance, Editor

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Recent publication.

- 1 Kotik AN, Trufanova VA, Golubev WI 2011 Sensitivity of yeasts to mycotoxins. *Problems in Medical Mycology* **13**:85.

The yeasts (members of more than 30 genera) are resistant to aflatoxin B1 and ochratoxin A, and rare strains of only basidiomycetous species are sensitive to zearalenone. The sensitivity to aurofusarin and T-2 toxin is a strain-specific

property but on the whole the proportion of resistant species is larger among basidiomycetous yeasts than ascomycetous ones. Rather many species are resistant to all mycotoxins examined.

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The following article was accepted recently.

- 1 Basso TO, de Kok S, Dario M, d, do Espirito-Santo JCA, Müller G, Schlögl PS, Silva CP, Tonso A, Daran JM, Gombert AK, van Maris AJA, Pronk JT, Stambuk BU 2011 Engineering topology and kinetics of sucrose metabolism in *Saccharomyces cerevisiae* for improved ethanol yield. *Metab Eng* – doi:10.1016/j.ymben.2011.09.005.

Sucrose is a major carbon source for industrial bioethanol production by *Saccharomyces cerevisiae*. In yeasts, two modes of sucrose metabolism occur: (i) extracellular hydrolysis by invertase, followed by uptake and metabolism of glucose and fructose, and (ii) uptake via sucrose-proton symport followed by intracellular hydrolysis and metabolism. Although alternative start codons in the *SUC2* gene enable synthesis of extracellular and intracellular invertase isoforms, sucrose hydrolysis in *S. cerevisiae* predominantly occurs extracellularly. In anaerobic cultures, intracellular hydrolysis theoretically enables a 9% higher ethanol yield than extracellular hydrolysis, due to energy costs of sucrose-proton symport. This prediction was tested by engineering the promoter and 50 coding sequences of *SUC2*, resulting in predominant (94%) cytosolic localization of invertase. In anaerobic sucrose-limited chemostats, this iSUC2-strain showed an

only 4% increased ethanol yield and high residual sucrose concentrations indicated suboptimal sucrose-transport kinetics. To improve sucrose-uptake affinity, it was subjected to 90 generations of laboratory evolution in anaerobic, sucrose-limited chemostat cultivation, resulting in a 20-fold decrease of residual sucrose concentrations and a 10-fold increase of the sucrose-transport capacity. A single-cell isolate showed an 11% higher ethanol yield on sucrose in chemostat cultures than an isogenic *SUC2* reference strain, while transcriptome analysis revealed elevated expression of *AGT1*, encoding a disaccharide-proton symporter, and other maltose-related genes. After deletion of both copies of the duplicated *AGT1*, growth characteristics reverted to that of the unevolved *SUC2* and *iSUC2* strains. This study demonstrates that engineering the topology of sucrose metabolism is an attractive strategy to improve ethanol yields in industrial processes.

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Two papers accepted in August 2011.

- 1 Kopecká M, Golubev W, Ramíková V, Klemová D, Ilkovic L 2011 Ultrastructural characteristics and variability of vegetative reproduction in *Fellomyces penicillatus*. *J Basic Microbiol* (in press).
- 2 Kopecká M, Yamaguchi M 2011 Ultrastructural disorder of actin mutant suggests uncoupling of actin-dependent pathway from microtubule-dependent pathway in budding yeast. *J Electron Microscopy* (published online).

Application for a new research grant for the period 2012-2014 to the Czech Science Foundation GA CR, Prague, Czech Republic.

“The Cytoskeleton of Pathogenic Yeasts as a New Antifungal Target” *Applicants:* Marie Kopecká and Augustin Svoboda, Department of Biology Faculty of Medicine Masaryk University, Brno, Czech Republic in cooperation with Masashi Yamaguchi, Medical Mycology Research Center, Chiba University, Japan.

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**IV National Center for Agricultural Utilization Research, ARS, USDA, 1815 N. University St., Peoria, IL 61604-3999 USA. Communicated by Cletus P Kurtzman.**

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Recent publications.

- 1 Kurtzman CP 2011 A new methanol assimilating yeast, *Ogataea parapolyomorpha*, the ascosporic state of *Candida parapolyomorpha*. *Antonie van Leeuwenhoek* **100**:455–462.

*Ogataea parapolyomorpha* sp. n. (NRRL YB-1982, CBS 12304, type strain), the ascosporic state of *Candida parapolyomorpha*, is described. The species appears homothallic, assimilates methanol as is typical of most *Ogataea* species and forms hat-shaped ascospores in asci that become deliquescent. *Ogataea parapolyomorpha* is closely related to *O. angusta* and *O. polymorpha*. The three species can be resolved from gene sequence analyses but

are unresolved from fermentation and growth reactions that are typically used for yeast identification. On the basis of multiple isolates, *O. angusta* is known only from California, USA, in association with *Drosophila* and *Aulacigaster* flies, whereas *O. parapolyomorpha* is predominantly associated with insect frass from trees in the eastern USA, but *O. polymorpha* has been isolated from various substrates in the USA, Brazil, Spain and Costa Rica.

- 2 Kurtzman CP 2011 *Citeromyces hawaiiensis* sp. nov., a new ascosporic yeast associated with *Myoporium sandwicense* in Hawaii. *Int J Syst Evol Microbiol*. Published online.

*Citeromyces hawaiiensis* sp. nov. (NRRL Y-11581, CBS 12303, type strain) is described from 12 strains isolated from flux of the sandalwood (*Myoporium sandwicense*) and adjacent soil in Hawaii, USA. Analysis of gene sequences from the D1/D2 domains of nuclear large subunit rRNA, ITS, mitochondrial small subunit rRNA and translation elongation factor-1 $\alpha$  each separated the proposed

new species from *C. matritensis* and *C. siamensis*, the other known species of the genus *Citeromyces*. The three species are morphologically similar but they can be separated by growth reactions on standard assimilation tests. An additional strain of *C. siamensis* (NRRL Y-11788), a species previously known only from Thailand, was obtained from spoiled condensed milk in Ohio, USA.

- 3 Graham JR, Ellis JD, Benda ND, Kurtzman CP, Boucias DG 2011 *Kodamaea ohmeri* (Ascomycota: Saccharomycotina) presence in commercial *Bombus impatiens* Cresson and feral *Bombus pensylvanicus* DeGeer (Hymenoptera: Apidae) colonies. *J Apicultural Res* **50**:218- 226.

In this study, eight commercial and three feral bumble bee (*Bombus impatiens* Cresson and *Bombus pensylvanicus* DeGeer respectively, Hymenoptera: Apidae) colonies were tested for the presence of *Kodamaea ohmeri* (Ascomycota: Saccharomycotina), a yeast known to attract small hive beetles (SHB) (*Aethina tumida* Murray, Coleoptera: Nitidulidae) to honey bee (*Apis mellifera* L., Hymenoptera: Apidae) colonies. Swabs of commercial bumble bee colonies and homogenates of bumble bee colony components (adults, brood, honey, pollen and wax) were plated on selective media. The resulting yeast isolates were compared to *K. ohmeri* previously isolated from SHB. Yeasts were detected in all of the commercial bumble bee

colony swab samples (n = 56) and a selected subsample was shown through molecular, chemical, and microbiological evidence to be *K. ohmeri*. For the second part of the study, feral bumble bee colonies were excavated and evaluated for the presence of any SHB life stage (none was found). Adult bees and swabs from the colonies were plated on selective media. *Kodamaea ohmeri* was isolated in all samples collected from the feral bumble bee colonies. The presence of *K. ohmeri* in commercial and feral bumble bee colonies is of concern, as SHB, which harbour *K. ohmeri*, are attracted to the volatiles produced by *K. ohmeri* growing on bee collected pollen.

- 4 Kurtzman CP 2010 Description of new yeast species – is one strain enough? *Bulletin of BISMis* **1**:17-24.

The issue of description of new yeast species on the basis of a single strain is discussed. Single gene sequences,

such as those from D1/D2 LSU rRNA, or sequences from ITS1/ITS2 are commonly used as the basis for recognizing

new yeast species. Evidence is presented that hybrids and species with polymorphic gene sequences may not be recognized from a single gene analysis, but with multigene sequence comparisons, single-strain species can be accurately determined. Further, description of single-strain

species will add to an understanding of yeast phylogeny and species diversity, which would be unknown if new species descriptions were limited to those taxa for which multiple strains were available.

- 5 Kurtzman CP, Price NP, Ray KJ, Kuo TM 2010 Production of sophorolipid biosurfactants by multiple species of the *Starmerella (Candida) bombicola* yeast clade. FEMS Microbiol. Lett. **311**:140–146.

Sophorolipids are carbohydrate-based, amphiphilic biosurfactants that are of increasing interest for use in environmentally benign cleaning agents. Sophorolipid production was tested for 26 strains representing 19 species of the *Starmerella* yeast clade, including *Starmerella bombicola* and *Candida apicola*, which were previously reported to produce sophorolipids. Five of the 19 species tested showed significant production of sophorolipids: *S. bombicola*, *C. apicola*, *Candida riidocensis*, *Candida stellata* and a new species, *Candida* sp. NRRL Y-27208. A

high-throughput matrix-assisted laser desorption/ionization-time of flight MS assay was developed that showed *S. bombicola* and *C. apicola* to produce a lactone form of sophorolipid, whereas *C. riidocensis*, *C. stellata* and *Candida* sp. NRRL Y-27208 produced predominantly free acid sophorolipids. Phylogenetic analysis of sequences for the D1/D2 domains of the nuclear large subunit rRNA gene placed all sophorolipid-producing species in the *S. bombicola* subclade of the *Starmerella* clade.

- 6 Price NPJ, Ray KJ, Vermillion KE, Dunlap CA, Kurtzman CP 2011 Structural characterization of novel sophorolipid biosurfactants from a newly identified species of *Candida* yeast. Carbohydrate Res. [doi:10.1016/j.carres.2011.07.016](https://doi.org/10.1016/j.carres.2011.07.016).

Sophorolipids are a group of *O*-acylsophorose-based biosurfactants produced by several yeasts of the *Starmerella* clade. The known sophorolipids are typically partially acetylated 2-*O*- $\beta$ -D-glucopyranosyl-D-glucopyranose (sophorose) *O*- $\beta$ -glycosidically linked to 17-L-hydroxy- $\Delta$ 9-octadecenoic acid, where the acyl carboxyl group often forms a 4'-lactone to the terminal glucosyl residue. In a recent MALDI-TOFMS-based screen for sophorolipid-producing yeasts we identified a new species, *Candida* sp. NRRL Y-27208, that produces significant amounts of novel sophorolipids. This paper describes the structural

characterization of these new compounds, using carbohydrate and lipid analysis, mass spectrometry, and NMR spectroscopy. Unlike those reported previously, the NRRL Y-27208 sophorolipids contain an  $\omega$ -hydroxy-linked acyl group (typically 18-hydroxy- $\Delta$ 9-octadecenoate), and occur predominantly in a non-lactone, anionic form. In addition, seventeen dimeric and trimeric sophoroses were identified by MALDI-TOFMS from this strain. The surfactant-like properties of these sophorolipids have value as potential replacements for petroleum-based detergents and emulsifiers.

- 7 Koganti S, Kuo TM, Kurtzman CP, Smith N, Ju LK 2011 Production of arabitol from glycerol: strain screening and study of factors affecting production yield. Appl Microbiol Biotechnol **90**:257–267.

Glycerol is a major by-product from biodiesel production, and developing new uses for glycerol is imperative to overall economics and sustainability of the biodiesel industry. With the aim of producing xylitol and/or arabitol as the value-added products from glycerol, 214 yeast strains, many osmotolerant, were first screened in this study. No strains were found to produce large amounts of xylitol as the dominant metabolite. Some produced polyol mixtures that might present difficulties to downstream separation and purification. Several *Debaryomyces hansenii* strains produced arabitol as the predominant metabolite with high yields, and *D. hansenii* strain SBP-1 (NRRL Y-7483) was chosen for further study on the effects of several

growth conditions. The optimal temperature was found to be 30°C. Very low dissolved oxygen concentrations or anaerobic conditions inhibited polyol yields. Arabitol yield improved with increasing initial glycerol concentrations, reaching approximately 50% (w/w) with 150 g/L initial glycerol. However, the osmotic stress created by high salt concentrations ( $\geq 50$  g/L) negatively affected arabitol production. Addition of glucose and xylose improved arabitol production while addition of sorbitol reduced production. Results from this work show that arabitol is a promising value-added product from glycerol using *D. hansenii* SBP-1 as the producing strain.

- 8 Peter G, Dlauchy D, Tornai-Lehoczki J, Suzuki M, Kurtzman CP 2011 *Spencermartinsiella* gen. nov. and *Spencermartinsiella europaea* sp. nov., a new member of the family Trichomonascaceae. Int J Syst Evol Microbiol **61**:993-1000.

Ten strains of a novel heterothallic yeast species were isolated from rotten wood collected at different locations in Hungary. Analysis of gene sequences for the D1/D2 domain of the large subunit rRNA, as well as analysis of concatenated gene sequences for the nearly complete nuclear large subunit rRNA, nuclear small subunit rRNA and translation elongation factor 1- $\alpha$ , placed the novel species in the family Trichomonascaceae, but showed that it was distinct from all currently recognized genera. The

name *Spencermartinsiella europaea* gen. nov., sp. nov. is proposed to accommodate the new genus and novel species. The novel species could be distinguished from recognized species of neighbouring genera on the basis of standard phenotypic characteristics. The type and isotype strains of *Spencermartinsiella europaea* are NCAIM Y.01817<sup>T</sup> (= NRRL Y-48265<sup>T</sup> = CBS 11730<sup>T</sup>) and NCAIM Y.01819<sup>T</sup> (= NRRL Y-48266<sup>T</sup> = CBS 11731<sup>T</sup>), respectively.

- 9 Peter G, Dlačhy D, Tornai-Lehoczki J, Gouliamova D, Kurtzman CP 2011 *Ogataea saltuana* sp. nov., a novel methanol-assimilating yeast species. *Antonie van Leeuwenhoek* **100**:375–383.

Four ascosporeulating strains of an undescribed methanol-assimilating yeast species were isolated from forest habitats in Hungary. Three of them were recovered from rotten wood and one from leaves of a sessile oak. A closely related, but somewhat divergent strain was recovered from insect frass in a Ponderosa pine collected in New Mexico, USA. Analysis of the D1/D2 sequences of the LSU rRNA gene placed the new species in the *Ogataea* clade. The ITS and the D1/D2 LSU sequences of the rRNA gene repeats were compared for the above-noted strains and

that of the type strain of *Ogataea zsoltii*, the closest neighbour among currently recognized *Ogataea* species. Their relatedness was investigated by parsimony network analysis as well. As a result of the sequence analysis, it was concluded that the five strains isolated from tree associated habitats represent a single new yeast species. *Ogataea saltuosa* sp. nov. is proposed to accommodate these strains. The type strain NCAIM Y.01833<sup>T</sup> (CBS 10795<sup>T</sup>, NRRL Y-48448<sup>T</sup>) was recovered from rotten wood of Scotch pine (*Pinus silvestris*) in Hungary.

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The following are the abstracts of papers which were published recently.

- 1 Pinel D, F D'Aoust, SB del Cardayré, PK Bajwa, H Lee & VJJ Martin 2011 Genome shuffling of *Saccharomyces cerevisiae* through recursive population mating leads to improved tolerance to spent sulfite liquor. *Applied and Environmental Microbiology* **77**:4736-4743.

Spent sulfite liquor (SSL) is a waste effluent from sulfite pulping that contains monomeric sugars which can be fermented<sup>d</sup> to ethanol. However, fermentative yeasts used for the fermentation of the sugars in SSL are adversely affected by the inhibitory substances in this complex feedstock. To overcome this limitation, evolutionary engineering of *Saccharomyces cerevisiae* was carried out using genome-shuffling technology based on large-scale population cross mating. Populations of UV-light-induced yeast mutants more tolerant than the wild type to hardwood spent sulfite liquor (HWSSL) were first isolated and then recursively mated and enriched for more-tolerant populations. After five rounds of genome shuffling, three strains were isolated

that were able to grow on undiluted HWSSL and to support efficient ethanol production from the sugars therein for prolonged fermentation of HWSSL. Analyses showed that greater HWSSL tolerance is associated with improved viability in the presence of salt, sorbitol, peroxide, and acetic acid. Our results showed that evolutionary engineering through genome shuffling will yield robust yeasts capable of fermenting the sugars present in HWSSL, which is a complex substrate containing multiple sources of inhibitors. These strains may not be obtainable through classical evolutionary engineering and can serve as a model for further understanding of the mechanism behind simultaneous tolerance to multiple inhibitors.

- 2 Richardson TL, NK Harner, PK Bajwa, JT Trevors & H Lee 2011 Approaches to deal with toxic inhibitors during fermentation of lignocellulosic substrates. *In: Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass*. Eds. Zhu, J.Y., X. Zhang & X.J. Pan. American Chemical Society Publication. Washington, DC. ACS Symposium Series, Vol. **1067**, pp. 171-202 (Chapter 7). (<http://dx.doi.org/10.1021/bk-2011-1067.ch007>)

The recalcitrance of lignocellulose materials requires harsh pretreatment(s) to release sugar monomers for ethanol fermentation by microorganisms. Harsh pretreatment conditions result in the formation of compounds in the

hydrolysate that are inhibitory to the fermenting microorganism(s). The main inhibitors include furan derivatives, organic acids, and phenolic compounds. Research has focused on physical, chemical and/or

biological methods to deal with inhibitors. Many of these methods remove or convert inhibitors through a detoxification step prior to fermentation. Recently, biological methods have focused on increasing yeast

inhibitor tolerance. This review examines research on the different methods used to detoxify hydrolysates or increase yeast tolerance to inhibitors.

- 3 Bajwa PK, C Phaenark, N Grant, X Zhang, M Paice, VJJ Martin, JT Trevors & H Lee (2011) Ethanol production from selected lignocellulosic hydrolysates by genome shuffled strains of *Scheffersomyces stipitis*. *Bioresource Technology* **102**:9965-9969. (<http://dx.doi.org/10.1016/j.biortech.2011.08.027>)

Two genome-shuffled *Scheffersomyces stipitis* strains, GS301 and GS302, exhibiting improved tolerance to hardwood spent sulphite liquor, were tested for growth and fermentation performance on three wood hydrolysates: (a) steam-pretreated enzymatically hydrolyzed poplar hydrolysate from Mascoma Canada, (b) steam pretreated poplar hydrolysate from University of British Columbia Forest Products Biotechnology Laboratory, and (c) mixed hardwoods pre-hydrolysate from FPIinnovations (FPI). In the FPI hydrolysate, the wild type (WT) died off within

25 h, while GS301 and GS302 survived beyond 100 h. In fermentation tests, GS301 and GS302 completely utilized glucose and xylose in each hydrolysate and produced 0.39–1.4% (w/v) ethanol. In contrast, the WT did not utilize or poorly utilized glucose and xylose and produced non-detectable to trace amounts of ethanol. The results demonstrated cross tolerance of the mutants to inhibitors in three different wood hydrolysates and reinforced the utility of mating-based genome shuffling approach in industrial yeast strain improvement.

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Many thanks to José Paulo Sampaio for invitation to visit his lab in May 2011 and exchange of strains. We were glad to accept in our lab Taiwanese colleagues Wang Pin-Han (Tonghai University) and Lee Ching-Fu (National Hsinchu University of Education) during short visit in June 2011.

The following are papers for 2011 or in press.

- 1 Naumova ES, Serpova EV, Korshunova IV, Naumov GI 2011 Molecular polymorphism of  $\alpha$ -galactosidase *MEL* genes of *Saccharomyces* yeasts. *Microbiology (Moscow)*. **80**:502–513. © Pleiades Publishing, Ltd.
- 2 Naumov GI, Naumova ES, Martynenko NN, Masneuf-Pomarède I 2011 Taxonomy, ecology and genetics of the yeast *Saccharomyces bayanus* – a new object for science and practice. *Microbiology (Moscow)*. **80**:735–742. © Pleiades Publishing, Ltd.
- 3 Naumov GI, Lee C-F 2011 Species specificity action of killer toxins of *Zygowilliopsis californica* on *Saccharomyces* yeasts: study of Taiwanese populations. *Mikologiya i Fitopatologiya*. **45**:332-336 (in Russian).

Various soil strains of *Zygowilliopsis californica* were shown to have, at least, two different types of killer toxins. The first type acts against super sensitive tester *Candida nitratophila* VKPM Y-740 (=CBS 2027), while does not affect strains of *Saccharomyces cerevisiae* and

*S. kudriavzevii*. Strains of the second type do not kill only *S. kudriavzevii* testers. In this connection, association of *S. kudriavzevii*, but not *S. cerevisiae* yeasts, with soil is discussed.

- 4 Naumov GI, Kondratieva VI, Naumova ES, Chen G-Y, Lee C-F 2011 Polymorphism and species-specificity of killer activity formation in the yeast *Zygowilliopsis californica*. *Biotechnology (Moscow)*. **3**:29-33 (in Russian).
- 5 Naumov GI 2011 Are melibiose-fermenting intestinal and alpechin strains of *Saccharomyces cerevisiae* a novel type of yeast probiotics? *Doklady Biological Sciences* **439**:262-263. © Pleiades Publishing, Ltd.

- 6 Naumov G.I. 2011. Genus assignment of small-spored aquatic and terrestrial species of the yeast *Metschnikowia* (letter to editor). *Microbiology* (Moscow, in press).
- 7 Naumov GI, Naumoff DG 2011 Molecular genetic differentiation of yeast  $\alpha$ -glucosidases: maltases and isomaltases. *Microbiology* (Moscow, in press).
- 8 Naumova ES, Michailova YuV, Naumov GI 2011 Natural interspecies hybridization of *Saccharomyces* yeasts. 29th International Specialized Symposium on Yeasts (ISSY29), August 29-September 2, 2011, Guadalajara, Mexico, P27.
- 9 Naumov GI *Metschnikowia* sensu stricto is the oldest described genetic genus consisting of biological species. 1<sup>st</sup> International Symposium "Non-Conventional Yeasts in the Postgenomic Era", September 11-14, 2011, Lvov, Ukraine, P15.
- 10 Naumova E.S, Kondratieva V.I, Lee C.-F, Naumov G.I. Genetic diversity of the yeast *Zygowilliopsis* Kudrjavzev. 1<sup>st</sup> International Symposium "Non-Conventional Yeasts in the Postgenomic Era", September 11-14, 2011, Lvov, Ukraine, P16.

Progress in molecular biology has resulted in the use of new methods in yeast taxonomy and stimulated active development of molecular phylogeny (Suh et al, 2006). Taxonomists are now trying to design a classification that will indicate the genetic relatedness between yeast species and genera. Analysis of rRNA and some other nuclear and mitochondrial genes, enabling evaluation of yeast relationship both at the species and higher taxonomical levels, is currently used to separate yeast taxons and establish their phylogenetic relationship. However, many yeast genera accepted in Kurtzman, Fell & Boekhout (2011) are still heterogeneous, and, within them, there are groups of closely related species corresponding to a genetic genus, a good example being the genetic genus *Zygowilliopsis* included into the recently described genus *Barnettozyma* Kurtzman, Robnett & Basehoar-Powers. Using multigene sequence analysis and classical genetic hybridization, we studied the relatedness among the species assigned to a formal heterogeneous genus *Barnettozyma*. Over 100 *Zygowilliopsis* strains isolated in different geographic localities (Taiwan, Europe, North America and Japan) have been characterized. According to phylogenetic analysis,

there are two separate lineages. The first one includes *Zygowilliopsis* strains, *Pichia populi* and *P. hawaiiensis* (bootstrap values 100%). The second contains *Candida norvegica*, *C. montana*, *Pichia salicaria*, *P. wickerhamii* and *Komagataea pratensis*. The two lineages showed little phylogenetic affinity to each other (54% bootstrap support), suggesting that the *Barnettozyma* genus sensu Kurtzman is composed of a complex of separate genera, one of which is *Zygowilliopsis* Kudrjavzev. According to molecular and genetic analyses, the genus *Zygowilliopsis* includes at least seven biological species, four of which are novel: one in Taiwan and three in North America. Using comparative analysis of nucleotide sequences of internal transcribed spacers ITS1 and ITS2, we have determined phylogenetic relationships of *Z. californica* strains isolated in Taiwan and in other world regions. We found that in Taiwan the *Z. californica* species is represented almost solely by variety *Z. californica* var. *dimenna*. The role of island isolation in yeast evolution and speciation is discussed.

The study was supported by Russian-Taiwanese grant RFBR (No.10-04-92008) and NSC (N<sup>o</sup>99-2923-B-134-001-MY3).

- 11 Sadykova AZh, Naumova ES, Martynenko NN, Naumov GI Molecular-genetic differentiation of *Kluyveromyces lactis* and *Kluyveromyces marxianus* yeasts. 1<sup>st</sup> International Symposium "Non-Conventional Yeasts in the Postgenomic Era", September 11-14, 2011, Lvov, Ukraine, P18.

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The following are abstracts of recently published and in press papers of the group.

- 1 Diehl B, Hoffmann TM, Mueller NC, Burkhardt JL, Kazmaier U, Schmitt MJ 2011 A novel yeast bioassay for high-throughput screening of matrix metalloproteinase inhibitors. *Appl Environ Microbiol*, October 14 [Epub ahead of print].

Diverse malfunctions in the expression and regulation of matrix metalloproteinases (MMPs) are often the cause of severe human diseases, bringing the

identification of specific MMP-inhibitors into major focus particularly in anti-cancer treatment. Here we describe a novel bioassay based on recombinant yeast cells (*Pichia*

*pastoris*) which express, deliver and incorporate biologically active human MMP-2 and MMP-9 to the yeast cell surface. Using Sed1p for cell wall targeting and covalent anchorage a highly efficient bioassay was established which allows high-throughput screening and subsequent validation of novel MMP inhibitors as potential

anti-cancer drugs. In addition, we developed a straightforward synthesis of a new aspartate-derived MMP-inhibitor active in the nM range, bearing an amino functionality which should allow the introduction of a wide range of side chains to modify the properties of these compounds.

- 2 Becker B, Schmitt MJ 2011 Adapting yeast as model to study ricin toxin A uptake and trafficking. *Toxins* 3:834-47.

The plant A/B toxin ricin represents a heterodimeric glycoprotein belonging to the family of ribosome inactivating proteins, RIPs. Its toxicity towards eukaryotic cells results from the depurination of 28S rRNA due to the N-glycosidic activity of ricin toxin A chain, RTA. Since the extension of RTA by an mammalian-specific endoplasmic reticulum (ER) retention signal (KDEL) significantly increase RTA *in vivo* toxicity against mammalian cells, we here analyzed the phenotypic effect of RTA carrying the yeast-specific ER retention motif HDEL. Interestingly, such a toxin (RTA<sup>HDEL</sup>) showed a similar cytotoxic effect on yeast as a corresponding RTA<sup>KDEL</sup> variant on HeLa cells. Furthermore, we established a powerful yeast bioassay for

RTA *in vivo* uptake and trafficking which is based on the measurement of dissolved oxygen in toxin-treated spheroplast cultures of *S. cerevisiae*. We show that yeast spheroplasts are highly sensitive against external applied RTA and further demonstrate that its toxicity is greatly enhanced by replacing the C-terminal KDEL motif by HDEL. Based on the RTA resistant phenotype seen in yeast knock-out mutants defective in early steps of endocytosis (*end3*) and/or in RTA depurination activity on 28S rRNA (*rpl12B*) we feel that the yeast-based bioassay described in this study is a powerful tool to dissect intracellular A/B toxin transport from the plasma membrane through the endosomal compartment to the ER.

- 3 Walch B, Breinig T, Schmitt MJ, Breinig F 2011 Delivery of functional DNA and messenger RNA to mammalian phagocytic cells by recombinant yeast. *Gene Ther.*, August 25 [Epub ahead of print]

Among the different vaccination approaches, DNA/RNA vaccination represents a promising means in particular for the induction of effective cellular immune responses conferred by CD8-positive T lymphocytes. To achieve such immune responses, there is a need for novel delivery systems that allow the introduction of nucleic acids to the cytosol of immune cells. We show, for the first time, the delivery of functional DNA and messenger RNA (mRNA) to mammalian antigen-presenting cells, including murine macrophages and human dendritic cells, using the

yeast *Saccharomyces cerevisiae* as the delivery vehicle. After transfer of the particular nucleic acid, subsequent antigen processing and presentation were demonstrated in a human system. Remarkably, release of DNA/mRNA does not require additional 'helper' proteins such as listeriolysin. In conclusion, the yeast-based system described here is superior to many bacterial and viral systems in terms of efficacy, safety and targeting suggesting 'mycofection' as a promising approach for the development of a novel type of live vaccines.

- 4 Bazan SB, Geginat G, Breinig T, Schmitt MJ, Breinig F (2011). Uptake of various yeast genera by antigen-presenting cells and influence of subcellular antigen localization on the activation of ovalbumin-specific CD8 T lymphocytes. *Vaccine* 29: 8165-73.

Yeasts of the genus *Saccharomyces* expressing recombinant antigens are currently evaluated as candidate T cell vaccines. Here, we compared the interaction kinetics between four biotechnologically relevant yeast genera (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Pichia pastoris*) and human dendritic cells as well as the involvement of Dectin-1 and mannose receptor in phagocytosis. Further, we analyzed the activation capacity of recombinant yeasts expressing ovalbumin (OVA) either intracellular, extracellular or surface-displayed by OVA-specific CD8 T lymphocytes.

We found that the kinetic patterns of yeast uptake by phagocytic cells varied between the tested yeast genera and that both genus and subcellular OVA antigen localization influenced the strength of T cell activation. In particular, in *S. cerevisiae*, a secreted antigen was less effectively delivered than its cytosolic variant, whereas most efficient antigen delivery with *P. pastoris* was obtained by cell surface bound antigen. Our data indicate that protein secretion might not be an effective delivery pathway in yeast.

- 5 Walch B, Breinig T, Geginat G, Schmitt MJ, Breinig F (2011). Yeast-based protein delivery to mammalian phagocytic cells is increased by coexpression of bacterial listeriolysin. *Microbes Infect.* 13: 908-13.

Yeast-mediated protein delivery to mammalian antigen-presenting cells is a powerful approach for inducing cell-mediated immune responses. We show that coexpression of the pore-forming protein listeriolysin O from *Listeria monocytogenes* leads to improved translocation of a proteinaceous antigen and subsequent

activation of specific T lymphocytes. As the resulting yeast carrier is self-attenuated and killed after antigen delivery without exhibiting any toxic effect on antigen-presenting cells, this novel carrier system suggests itself as promising approach for the development of yeast-based live vaccines.

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Papers presented at this year's ASBC.

1 Beattie A, Eck E, Edney M, MacIntosh AJ, Rossnagel B, Speers RA Effects of fungal contamination of barley malt on yeast in suspension during fermentation.

Premature Yeast Flocculation (PYF) in brewing fermentations is a concern to both the malting and brewing industries. The exact causes of PYF are likely varied; however, it is hypothesized that exposure of barley grains to indigenous microflora has an effect upon the behavior of yeast cells during subsequent fermentations. In this study, the effect of microbial contamination on barley fermentation was investigated using small-scale assays. Two varieties of barley (CDC bold and AC Metcalf) were field inoculated with one of three common fungal infections: spot blotch (*C. sativus*), head blight (*F. graminearum*) and net blotch (*P. teres*). Each sample was malted, mashed, pitched and fermented using a high precision mill, automated mash bash and temperature-controlled fermentation vessel ( $\pm 0.1$  C). Fermentations were performed in triplicate using a small-scale (15 mL assay). During each fermentation, samples were taken at set intervals (1, 6, 22, 26, 30, 46, 50, 54, 70, 74 and 78 hr). Yeast turbidity was spectrophotometrically assessed at 600 nm while apparent extract was determined through density measurements of the wort. It was found that the final turbidity (linked to yeast in suspension) differed

significantly ( $p > 0.05$ ) between most control and infected samples. In the fermentation of most fungal-infected malts, the data show turbidity peaking sooner and declining more quickly than the control malt. It was also found that the degree of difference from the control correlated very well with established susceptibilities of the barley varieties to the introduced fungal species. In general, CDC Bold, which is reported to possess 'very poor' resistance to all three fungi, exhibited poor yeast in suspension behaviour when infected. AC Metcalf which is ranked higher than CDC Bold in resistance to these fungi, exhibited little or no PYF behaviour. For example, CDC Bold, when exposed to *F. graminearum*, showed a peak in turbidity 12 hr earlier than the control and declined to less than half the turbidity by 78hr when exposed to that fungus. Conversely, AC Metcalf, when exposed to *C. sativus*, displayed no significant ( $p > 0.05$ ) change in turbidity versus its' control. The observed changes to yeast in suspension are consistent with the phenomenon of premature yeast flocculation. Given that these fungal infections appeared to trigger PYF in laboratory studies, further examination is warranted.

2 AJ MacIntosh, J Adler, E Eck, RA Speers Suitability of the miniature fermentability method to monitor industrial fermentations.

Malt barley breeders and maltsters often strive to improve the quality of their product by improving malt fermentability. Small-scale assays are often used to assess the fermentability of malt; however anecdotal reports suggests that these assays have poor correlation with industrial fermentations in addition to inconsistency between assays. There are several factors that likely contribute to inconsistent fermentability between assays such as pitching rate, mashing regime, fermentation temperature, barley modification and batch size. This study aimed to isolate and examine the effect of fermenter size on malt fermentability through the use of a miniature-scale (15 mL) assay fermented in parallel to industrial sized operations. Using oxygenated wort mashed and pitched by local craft breweries, miniature fermentations were conducted at identical temperatures to their industrial scale counterparts. Throughout the fermentations, wort density was measured using a portable densitometer while the

Suitability of the miniature fermentability method to

turbidity was assessed spectrophotometrically (at 600 nm). It was found that fermentation vessel size had a significant effect on the apparent degree of fermentation, however observed disparities were consistent between the assay and fermentor dimensions. For example, a difference in final density of  $1.1 \text{ }^\circ\text{P} \pm 0.2 \text{ }^\circ\text{P}$ , was observed between the final density of a 19.6 hL craft brewery and the 15 mL assay over three consecutive experiments. However, when the wort from an 8.5 hL brew-pub was tested using the small assay, no significant differences in final attenuation were found. The shear generated through consumption of sugar and subsequent production of carbon dioxide was theoretically determined for each fermentation. A reduced shear generated within the shorter (miniature scale) fermentors likely influenced the yeast floc distributions and subsequent final density. To properly make use of miniature scale assays, a size correlation was proposed to rationalize the effect of fermentor size on fermentations.

Miniature Fermentation Method development (now accepted as a standard method of the ASBC).

- 3 Miniature Fermentation Assay. ASBC Methods of Analysis, Approved (2011), American Society of Brewing Chemists, St. Paul, MN.
- 4 Speers RA, Baugh C, Cook D, Eck E, Gibson B, Joy R, MacLeod A, Pantelougou A, Walker, S, Voetz M, Powell C 2011 Technical Committee Report: Mini-Fermentation Method. J ASBC **69**: (In Press).

Other related papers dealing with premature yeast flocculation.

- 5 Kuar M, Sheehy M, Stewart D, Bowman J, Speers RA, Evans DE 2011 Investigation of microbe induced premature yeast flocculation during brewery wort fermentation using terminal restriction fragment length polymorphism and clone library analysis. Submitted to J Appl Environ Microbiol
- 6 Patel JK, Speers RA, Lake JC 2011 Colloidal examination of worts associated with premature yeast flocculation. J ASBC **69**:81-90.

Book in press.

- 7 Speers RA (Editor) 2011 Yeast Flocculation, Vitality and Viability. Proceedings of the 2<sup>nd</sup> International Brewers Symposium. Master Brewers Association of the Americas, St. Paul, MN. In Press.

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Recent publications.

- 1 Ells R, Kock JLF, Pohl CH 2011 *Candida albicans* or *Candida dubliniensis*? Mycoses **54**:1-16.
- 2 Ncango DM, Pohl CH, Van Wyk PWJ, Kock JLF 2011 NSAIDs inhibit growth of yeast pathogens. African J Microbiol Res **5**:1123-1125.
- 3 Maartens MJ, Swart CW, Pohl CH, Kock JLF 2011 Antimicrobials, chemotherapeutics or antibiotics? Scientific Research and Essay **6**:3927-3929.
- 4 Ells R, Kock JLF, Albertyn J, Kemp G, Pohl CH 2011 Effect of inhibitors of arachidonic acid metabolism on prostaglandin E<sub>2</sub> production by *Candida albicans* and *Candida dubliniensis* biofilms. Medical Microbiology and Immunology **200**:23-28.

Arachidonic acid (AA) is released from infected host cells during *Candida albicans* infection and may serve as carbon source for yeast growth and as precursor for the production of biologically active eicosanoids, such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by *C. albicans*. However, the mechanism involved in this production is still unclear. Therefore, it was of interest to investigate the effect of different arachidonic acid metabolism inhibitors on PGE<sub>2</sub> production by biofilms of *C. albicans* and the closely related *C. dubliniensis*. This was done by growing *Candida*

biofilms in the presence of AA as well as cytochrome P450 (CYP), multicopper oxidase, cyclooxygenase or lipoxygenase inhibitors. The concentration of PGE<sub>2</sub> was determined by a monoclonal PGE<sub>2</sub> enzyme-linked immunosorbent assay and verified with LCMS/MS. The results obtained indicate the ability of *C. albicans* and *C. dubliniensis* biofilms to produce PGE<sub>2</sub> from exogenous AA. The use of different inhibitors suggested that CYPs and multicopper oxidases are involved in PGE<sub>2</sub> production by these *Candida* biofilms.

- 5 Kock JLF, Swart CW, Pohl CH 2011 The anti-mitochondrial antifungal assay for the discovery and development of new drugs. Expert Opinion on Drug Discovery **6**:671-681.

*Introduction:* New targets and drugs are constantly searched for to effectively combat fungal infections and diseases such as cancer. Mitochondria, as the main powerhouses of eukaryotic cells, must be regarded as important targets for the development of new therapies. This

has lead to the development of a fungal assay that shows potential in the selection of new antifungal and anticancer drugs as well as the identification of compounds that are toxic to human mitochondria.

*Areas covered:* In this review the authors discuss the

development of a potential method of drug discovery that targets mitochondrial function. The authors cover the application of new nanotechnology as well as yeast systematic research where the link between fungal fruiting structures, cell growth, increased mitochondrial activity and susceptibility to a variety of anti-mitochondrial drugs is assessed.

*Expert opinion:* This assay shows potential to select anti-mitochondrial drugs as a first screen. This should be

followed up by more specific *in vitro* and *in vivo* tests to pinpoint the type of anti-mitochondrial activity exerted by these drugs, if any. This is because the possibility exists that compounds regarded as anti-mitochondrial may not inhibit mitochondrial function but other fruiting structure developmental stages and therefore yield false positives. To enhance our knowledge on how these drugs act at the structural level, the authors recommend Nano Scanning Auger Microscopy as the tool of choice.

- 6 Motaung TE, Albertyn J, Kock JLF, Pohl CH 2011 *Cryptococcus cyanovorans* sp. nov., a basidiomycetous yeast isolated from cyanide contaminated soil. Int J Syst Evol Microbiol doi:10.1099/ijms.0.034181-0.

Eighteen yeast strains were isolated and identified from cyanide contaminated soil in South Africa. According to sequence-based analyses using the D1/D2 region of the large ribosomal subunit and ITS region three of these strains were identical and represent a novel species. Phylogenetic analysis based on the combined data set of the D1/D2 and ITS regions revealed a grouping with *Cryptococcus curvatus*, representing a defined clade (Curvatus) in the order Trichosporonales. The three strains are demarcated from *Cryptococcus curvatus* by standard physiological tests

such as assimilation of lactose, xylitol, 5-keto-D-gluconate, succinate and citrate as well as growth on media containing 10% NaCl and 5 % glucose. In addition, it was established that these strains could utilize up to 10 mM NaCN as sole carbon source on solid media and as sole nitrogen source in liquid media. On the basis of these findings, it is suggested that the three strains represent a new species for which the name *Cryptococcus cyanovorans* sp. nov. is given (Type strain CBS11948<sup>T</sup> = NRRL Y-48730<sup>T</sup>).

- 7 Pohl CH, Smit MS & Albertyn J 2011 *Rhodotorula bloemfonteinensis* sp. nov., *Rhodotorula eucalyptica* sp. nov., *Rhodotorula orientis* sp. nov. and *Rhodotorula pini* sp. nov., yeasts isolated from monoterpene rich environments. Int J Syst Evol Microbiol 61:2320-2327.

Recent rDNA sequencing of 25 isolates from a previous study, during which limonene-utilizing yeasts were isolated from monoterpene-rich environments by using 1,4-disubstituted cyclohexanes as sole carbon sources, led to the identification of four hitherto unknown *Rhodotorula* species. Analyses of the 26S rDNA D1/D2 region as well as the internal transcribed spacer (ITS) domain indicated that two isolates (CBS 8499T and CBS 10736) were identical and were closely related to *Rhodotorula cycloclastica*, a previously described limonene-utilizing yeast. These novel isolates differed from known yeast species and could be distinguished from *R. cycloclastica* by standard physiological tests. The other three isolates represent three

novel *Rhodotorula* species, closely related to *Sporobolomyces magnisporus*. These three species could also be distinguished from other *Rhodotorula* species by standard physiological tests. Based on these results, we suggest that the new isolates represent novel species, for which the names *Rhodotorula eucalyptica* sp. nov. (type strain CBS 8499T 5NRRL Y-48408T), *Rhodotorula pini* sp. nov. (type strain CBS 10735T 5NRRL Y-48410T), *Rhodotorula bloemfonteinensis* sp. nov. (type strain CBS 8598T 5NRRL Y-48407T) and *Rhodotorula orientis* sp. nov. (type strain CBS 8594T 5NRRL Y-48719T) are proposed. *R. eucalyptica* and *R. pini* can also utilize limonene. Video Lectures by Translational Biomedicine.

e-Conference 1:

<http://vimeo.com/24167863>

<http://obiocon.blogspot.com/2011/05/yeast-contraceptives-also-novel-drugs.html>

e-Conference 2:

<http://vimeo.com/21056636>

<http://obiocon.blogspot.com/2011/03/new-nanotechnology-for-translational.html>

Recent publication.

- 1 Košíková B, Sláviková E 2011 Use of lignin and PLA as renewable additives of polypropylene films, *Wood Research* 56:393-402.

The new type of films was prepared by blending of polypropylene with lignin and polylactic acid (PLA). Both used additives are particularly attractive as sustainable alternatives to petrochemical-derived products, since they can be obtained from renewable resources. Introduction of both additives into polypropylene blends allows to prepare the films with thickness 50-60  $\mu\text{m}$ . In order to increase biodegradability of polypropylene plastics, the films of blends of polypropylene, containing 2 wt % lignin and 3-10

wt % polylactic acid were prepared in the absence of the commercial stabilizers and investigated from the view point of their stability during thermo-oxidative aging and accelerated weathering. The obtained results showed that lignin acts as a stabilizer of polypropylene matrix against thermo-oxidation. In contrast to neat PP, the mechanical properties of the films containing lignin and PLA were decreased during biodegradation as well as during long-term artificially accelerated weathering.

Book chapter.

- 2 Márová I, Čertík M, Breierová E 2011 Production of Enriched Biomass by Carotenogenic Yeasts - Application of Whole-Cell Yeast Biomass to Production of Pigments and Other Lipid Compounds, In: *Remote Sensing of Biomass: Principles and Applications / Book 4*, Ed. Matovic D., 40 s., INTECH, 2011. ISBN 978-953-307-492-4.

Biomass has been an intimate companion of humans from the dawn of civilization to the present. Its use as food, energy source, body cover and as construction material established the key areas of biomass usage that extend to this day. Given the complexities of biomass as a source of multiple end products, this volume sheds new light to the

whole spectrum of biomass related topics by highlighting the new and reviewing the existing methods of its detection, production and usage. We hope that the readers will find valuable information and exciting new material in its chapters.

- 3 Mazáň M, Ragni E, Popolo I, Farkaš V 2011 Catalytic properties of the Gas family  $\beta$ -(1,3)-glucanotransferases active in fungal cell-wall biogenesis as determined by a novel fluorescent assay. *Biochem J* 438:275-282.

Glucanotransferases ( $\beta$ -1,3-glucanotransglycosylases, EC 2.4.1.-) of the glycosyl hydrolase family GH72 are glycosyl phosphatidylinositol-anchored proteins that play an important role in the biogenesis of fungal cell walls. They randomly cleave glycosidic linkages in  $\beta$ -(1,3)-glucan chains and ligate the polysaccharide portions containing newly formed reducing ends to C3(OH) at non-reducing ends of other  $\beta$ -(1,3)-glucan molecules. We have developed a sensitive fluorescence-based method for the assay of transglycosylating activity of GH72 enzymes. In the new assay, laminarin [ $\beta$ -(1,3)-glucan] is used as the glucanotransferase donor and laminarioligosaccharides fluorescently-labeled with sulforhodamine (SR-laminarioligosaccharides) serve as the acceptors. The new fluorescent assay was employed for

partial biochemical characterization of the heterologously expressed Gas family proteins from the yeast *Saccharomyces cerevisiae*. All the Gas enzymes specifically used laminarin as the glucanotransferase donor and SR-laminarioligosaccharides of  $\text{DP} \geq 5$  as the acceptors. Gas proteins expressed in distinct stages of the yeast life cycle showed differences in their pH optima. Gas1p and Gas5p, which are expressed during vegetative growth, had the highest activity at pH 4.5 and 3.5 respectively, whereas the sporulation-specific Gas2p and Gas4p were most active between pH 5 and 6. The novel fluorescent assay provides a suitable tool for the screening of potential glucanotransferases or their inhibitors.

- 4 Vadkertiová R, Sláviková E 2011 Influence of pesticides on yeasts colonizing leaves. *Zeitschrift für Naturforschung C* (in press).

The effect of nine different pesticides on the growth of yeasts isolated from the leaves of fruit and forest trees was investigated. Four insecticides (with the active ingredients: thiacloprid, deltamethrin, lambda-cyhalothrin,

and thiamethoxam) and five fungicides (with the effective substances: bitertanol, kresoxim-methyl, mancozeb, trifloxystrobin, and cupric oxychloride) were tested. The concentrations of chemicals were those recommended by

the manufacturers for the spraying of trees. The yeast strains isolated from the leaves of fruit trees were not sensitive to any of the insecticides. The majority of yeast strains isolated from the leaves of forest trees were either not sensitive or only to a small extent. While *Rhodotorula mucilaginosa* and *Pichia anomala* were not affected by any insecticides, the strains of *Cryptococcus laurentii* and *Rhodotorula glutinis* showed the highest sensitivity. The effects of fungicides on the growth of isolated yeasts were more substantial. The fungicide Dithane® DG (mancozeb)

completely inhibited the growth of all yeasts. All strains isolated from fruit tree leaves were more resistant to the tested fungicides than those isolated from the leaves of forest trees. The most resistant strains from the leaves of fruit trees belonged to the species *Metschnikowia pulcherrima*, *Pichia anomala*, and *Saccharomyces cerevisiae*, whereas *Cryptococcus albidus* and *C. laurentii*, originating from the leaves of forest trees, showed the highest sensitivity to fungicides.

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Recent book chapter.

- 1 Carvajal Barriga EJ, Libkind D, Briones AI, Úbeda Iranzo J, Portero P, Roberts I, James S, Morais PB, Rosa CA 2011 Yeasts biodiversity and its significance: case studies in natural and human-related environments, ex situ preservation, applications and challenges. In: Grillo O, Venora G (Ed.) Changing Diversity in Changing Environment, InTech, ISBN: 978-953-307-796-3. A PDF of the chapter can be downloaded from this site:

<http://www.intechopen.com/articles/show/title/yeasts-biodiversity-and-its-significance-case-studies-in-natural-and-human-related-environments-ex-s>

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Recent papers.

- 1 Yurkov AM, Kemler M & Begerow D 2011 Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. PLoS ONE 6(8): e23671.

*Background:* Yeast-like fungi inhabit soils throughout all climatic zones in a great abundance. While recent estimations predicted a plethora of prokaryotic taxa in one gram of soil, similar data are lacking for fungi, especially yeasts.

*Methodology/Principal findings:* We assessed the diversity of soil yeasts in different forests of central Germany using cultivation-based techniques with subsequent identification based on rDNA sequence data. Based on experiments using various pre-cultivation sample treatment and different cultivation media we obtained the highest number of yeasts by analysing mixed soil samples with a single nutrient-rich medium. Additionally, several

species richness estimators were applied to incidence-based data of 165 samples. All of them predicted a similar range of yeast diversity, namely 14 to 16 species. Randomized species richness curves reached saturation in all applied estimators, thus indicating that the majority of species is detected after approximately 30 to 50 samples analysed.

*Conclusions/Significance:* In this study we demonstrate that robust species identification as well as mathematical approaches are essential to reliably estimate the sampling effort needed to describe soil yeast communities. This approach has great potential for optimisation of cultivation techniques and allows high throughput analysis in the future.

- 2 Lumbsch T, Miller AN, Begerow D & Penev L 2011 MycoKeys, or why we need a new journal in mycology? MycoKeys 1:1-6.

The launch of MycoKeys coincided with several revolutionary changes to the International Code of Botanical Nomenclature (ICBN) (Knapp et al. 2011 and Hawksworth 2011, in this volume, Miller et al. 2011) that occurred during the Nomenclature Section of the XVIII International Botanical Congress (IBC2011) in Melbourne,

Australia. The path to the present launch, however was paved years ago by the lively discussions on electronic publication and dissemination of biodiversity information in the Internet era (e.g., Knapp 2010, Penev et al. 2010a), as well as by the successful start of its sister journals, PhytoKeys and ZooKeys.

- 3 Cai L, Giraud T, Zhang N, Begerow D, Cai G & Shivas RG 2011 The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Diversity* **50**:121-133.

In this paper, we review historical and contemporary species concepts and species recognition criteria for plant pathogenic fungi. Previous incongruent and unstable classification based on subjective and changing criteria have led to some confusion, especially amongst plant pathologists. The goal of systematics is to provide an informative and robust framework that stands the test of time. The taxonomic histories of *Cercospora*, *Colletotrichum*, *Fusarium*, as well as the rust and smut fungi, are used as examples, to show how concepts and criteria used to delimit and recognize species have changed. Through these examples we compare the Genealogical

Concordance Phylogenetic Species Recognition, an extension of the Phylogenetic Species Criterion, with other species recognition criteria and show that it provides a better discrimination for delimiting species. A rapidly increasing number of cryptic species are being discovered amongst plant pathogenic fungi using the Genealogical Concordance Phylogenetic Species Recognition, and it is important to determine their host range, the severity of diseases they cause and their biosecurity significance. With rapidly expanding global trade it has become imperative that we develop effective and reliable protocols to detect these previously unrecognized pathogens.

- 4 Yurkov AM, Krüger D, Begerow D, Arnold N & Tarkka MT 2011 Basidiomycetous yeasts from Boletales fruiting bodies and their interactions with the mycoparasite *Sepedonium chrysospermum* and the host fungus *Paxillus*. *Microbial Ecology* - DOI: 10.1007/s00248-011-9923-7.

Interactions between mushrooms, yeasts, and parasitic fungi are probably common in nature, but are rarely described. Bolete fruiting bodies are associated with a broad spectrum of microorganisms including yeasts, and they are commonly infected with filamentous mycoparasites of the genus *Sepedonium* (teleomorph Hypomyces). We report the isolation of 17 yeast strains from *Paxillus* and *Xerocomus*, 16 of which were obtained from the surface tissue, the primary site of *Sepedonium* infection. Phylogenetic analyses with the D1/D2 region of the 28S ribosomal gene and the internal transcribed spacers placed the yeasts as *Rhodotorula*, *Rhodospordium*, and *Mastigobasidium* from the Pucciniomycotina, *Cryptococcus*, *Cystofilobasidium*, *Holtermanniella*, and *Trichosporon* from the Agaricomycotina, and *Kluyveromyces* from the Saccharomycotina including the first isolation of *Rhodotorula graminis* from Europe. To

investigate the influence of the yeast strains on the mycoparasite and the host fungus, *in vitro* assays were conducted with *Sepedonium chrysospermum* and *Paxillus involutus*. Both *S. chrysospermum* growth inhibitory and stimulating yeast strains were detected among the isolates. The number of *S. chrysospermum* inhibitory yeast strains increased and the number of *S. chrysospermum* stimulatory yeast strains decreased in the presence of *P. involutus* in co-cultures. Low nutrient levels in the culture medium also led to an increased number of *S. chrysospermum* inhibitory yeast strains and ten yeasts inhibited the mycoparasite in spatial separation by a crosswall. Six yeast strains inhibited *P. involutus* in dual culture, and the inhibitory *P. involutus* yeast interactions increased to nine in the presence of *S. chrysospermum*. Our results suggest that the bolete-associated yeasts influence the growth of the mycoparasitic fungus, which may affect the health of the fruiting bodies.

- 5 Yurkov A, Kemler M & Begerow D 2011 Assessment of yeast diversity in soils under different management regimes. *Fungal Ecology* - DOI: 10.1016/j.funeco.2011.07.004.

Human activities, land management and climate change all have great impact on soil biology, but our knowledge on biodiversity of soil organisms is still very limited. Therefore, we wanted to assess responses of soil yeasts to land management and analysed 57 soils showing different land use from three distinct localities. We isolated and identified molecularly a total of 40 yeast fungi including several new species. Overall, species composition of different localities was very heterogeneous and nearly half of the species were found in a single site only. The

analysis of species abundance and community composition revealed a strong long-term effect of forest replacement by grassland vegetation. Unlike forests, grasslands harbour predominantly ascomycetous yeasts and their proportion increases with management intensity. In forests, evenness of yeast communities follows the gradient of land management intensity and natural beech forests harboured the most unevenly structured community, thereby mirroring the evenness of plant communities.

- 6 Kellner R, Vollmeister E, Feldbrügge M & Begerow D 2011 Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. *PLoS Genetics*. DOI: 10.1371/journal.pgen.1002436.

The grass smuts comprise a speciose group of biotrophic plant parasites, so-called Ustilaginaceae, which

are specifically adapted to hosts of sweet grasses, the Poaceae family. Mating takes a central role in their life

cycle as it initiates parasitism by a morphological and physiological transition from saprobic yeast cells to pathogenic filaments. As in other fungi sexual identity is determined by specific genomic regions encoding allelic variants of a pheromone-receptor (PR) system and heterodimerising transcription factors. Both operate in a biphasic mating process that starts with PR-triggered recognition, directed growth of conjugation hyphae and plasmogamy of compatible mating partners. So far, studies on the PR system of grass smuts revealed diverse interspecific compatibility and mating type determination. However, many questions concerning the specificity and evolutionary origin of the PR system remain unanswered. Combining comparative genetics and biological approaches we report on the specificity of the PR system and its genetic

diversity in 10 species spanning about 100 million years of mating type evolution. We show that three highly syntenic PR alleles are prevalent among members of the Ustilaginaceae favouring a triallelic determination as the plesiomorphic characteristic of this group. Furthermore, the analysis of PR loci revealed increased genetic diversity of single PR locus genes compared to genes of flanking regions. Performing interspecies sex tests we detected a high potential for hybridisation that is directly linked to pheromone signalling as known from intraspecies sex. Although the PR system seems to be optimised for intraspecific compatibility, the observed functional plasticity of the PR system increases the potential for interspecific sex, which might allow the hybrid-based genesis of newly combined host specificities.

- 7 Koepke J, Kaffarnik F, Haag C, Zarnack K, Luscombe NM, König J, Ule J, Kellner R, Begerow D & Feldbrugge M 2011 The RNA-binding protein Rrm4 is essential for efficient secretion of endochitinase Cts1. *Molecular & Cell Proteomics* - DOI: 10.1074/mcp.M111.011213

Long-distance transport of mRNAs is crucial to determine spatio-temporal gene expression in eukaryotes. The RNA-binding protein Rrm4 constitutes a key component of microtubule-dependent mRNA transport in filaments of *Ustilago maydis*. Although a number of potential target mRNAs could be identified, cellular processes that depend on Rrm4-mediated transport remain largely unknown. Here, we used differential proteomics to show that ribosomal, mitochondrial and cell wall-remodelling proteins, including the bacterial-type endochitinase Cts1, are differentially regulated in rrm4-delta filaments. In vivo UV crosslinking and immunoprecipitation (CLIP) and fluorescence in situ hybridization (FISH)

revealed that cts1 mRNA represents a direct target of Rrm4. Filaments of cts1-delta mutants aggregate in liquid culture suggesting an altered cell surface. In wild type cells Cts1 localizes predominantly at the growth cone, whereas it accumulates at both poles in rrm4-delta filaments. The endochitinase is secreted and associates most likely with the cell wall of filaments. Secretion is drastically impaired in filaments lacking Rrm4 or conventional kinesin Kin1 as well as in filaments with disrupted microtubules. Thus, Rrm4-mediated mRNA transport appears to be essential for efficient export of active Cts1, uncovering a novel molecular link between mRNA transport and the mechanism of secretion.

PhD thesis.

René Prior is starting his PhD thesis supported by the Deutsche Bundesstiftung Umwelt. The title of the project is "Epi- and endophytic fungi as biocontrol agents – biodiversity and antagonistic effects". The project is aimed to explore diversity of yeasts and yeast-like fungi colonizing leaf surfaces comparing it with the species growing within plant tissues. Special attention is given to the study of antagonistic effects of phyllosphere-inhabiting fungi on plant parasites, e.g. bean rust (*Uromyces*).

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Recent publications.

- 1 Flores CL and Gancedo C 2011 Unraveling moonlighting functions with yeasts. *IUBMB Life*. **63**:457-462.

This review considers the use of yeasts to study protein moonlighting functions. The cases discussed highlight the possibilities offered by the well-developed yeast genetics for the study of moonlighting mechanisms. The possibility to generate sets of mutants encoding different protein variants has allowed in some cases to map the regions that participate in the moonlighting function. We discuss cases of enzymes that moonlight in such

different activities as control of transcription, assembly of multimeric proteins, stabilization of mitochondrial DNA or biosynthesis of CoA. The moonlighting role of an enzyme and its metabolic function seems to have evolved independently as indicated by the finding that a protein may moonlight in a yeast species but not in others. Yeasts may open ways to study possible evolutionary relationships among moonlighting proteins.

- 2 Flores CL, Gancedo C, Petit T 2011 Disruption of *Yarrowia lipolytica* *TPS1* gene encoding trehalose-6-P synthase does not affect growth in glucose but impairs growth at high temperature. PLoS One. 6(9):e23695.

We have cloned the *Yarrowia lipolytica* *TPS1* gene encoding trehalose-6-P synthase by complementation of the lack of growth in glucose of a *Saccharomyces cerevisiae* *tps1* mutant. Disruption of *YITPS1* could only be achieved with a cassette placed in the 3' half of its coding region due to the overlap of its sequence with the promoter of the essential gene *YITFC1*. The *Yltps1* mutant grew in glucose although the *Y. lipolytica* hexokinase is extremely sensitive to inhibition by trehalose-6-P. The presence of a glucokinase, insensitive to trehalose-6-P, that constitutes about 80% of the glucose phosphorylating capacity during growth in glucose may account for the growth phenotype. Trehalose content was below 1 nmol/mg dry weight in *Y.*

*lipolytica*, but it increased in strains expressing *YITPS1* under the control of the *YITEF1* promoter or with a disruption of YALI0D15598 encoding a putative trehalase. mRNA levels of *YITPS1* were low and did not respond to thermal stresses, but that of *YITPS2* (YALI0D14476) and *YITPS3* (YALIOE31086) increased 4 and 6 times, respectively, by heat treatment. Disruption of *YITPS1* drastically slowed growth at 35°C. Homozygous *Yltps1* diploids showed a decreased sporulation frequency that was ascribed to the low level of YALI0D20966 mRNA an homolog of the *S. cerevisiae* *MCK1* which encodes a protein kinase that activates early meiotic gene expression.

- 3 Livas D, Almering MJ, Daran JM, Pronk JT, Gancedo JM 2011 Transcriptional responses to glucose in *Saccharomyces cerevisiae* strains lacking a functional protein kinase A. BMC Genomics. 12:405. doi: 10.1186/1471-2164-12-405.

**BACKGROUND:** The pattern of gene transcripts in the yeast *Saccharomyces cerevisiae* is strongly affected by the presence of glucose. An increased activity of protein kinase A (PKA), triggered by a rise in the intracellular concentration of cAMP, can account for many of the effects of glucose on transcription. In *S. cerevisiae* three genes, *TPK1*, *TPK2*, and *TPK3*, encode catalytic subunits of PKA. The lack of viability of *tpk1 tpk2 tpk3* triple mutants may be suppressed by mutations such as *yak1* or *msn2/msn4*. To investigate the requirement for PKA in glucose control of gene expression, we have compared the effects of glucose on global transcription in a wild-type strain and in two strains devoid of PKA activity, *tpk1 tpk2 tpk3 yak1* and *tpk1 tpk2 tpk3 msn2 msn4*.

**RESULTS:** We have identified different classes of genes that can be induced -or repressed- by glucose in the absence of PKA. Representative examples are genes required for glucose utilization and genes involved in the metabolism of other carbon sources, respectively. Among the genes responding to glucose in strains devoid of PKA

some are also controlled by a redundant signalling pathway involving PKA activation, while others are not affected when PKA is activated through an increase in cAMP concentration. On the other hand, among genes that do not respond to glucose in the absence of PKA, some give a full response to increased cAMP levels, even in the absence of glucose, while others appear to require the cooperation of different signalling pathways. We show also that, for a number of genes controlled by glucose through a PKA-dependent pathway, the changes in mRNA levels are transient. We found that, in cells grown in gluconeogenic conditions, expression of a small number of genes, mainly connected with the response to stress, is reduced in the strains lacking PKA.

**CONCLUSIONS:** In *S. cerevisiae*, the transcriptional responses to glucose are triggered by a variety of pathways, alone or in combination, in which PKA is often involved. Redundant signalling pathways confer a greater robustness to the response to glucose, while cooperative pathways provide a greater flexibility.

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Recent publications.

- 1 Ilmén M, den Haan R, Brevnova E, McBride J, Wiswall E, Froehlich A, Koivula A, Voutilainen SP, Siika-Aho M, la Grange DC, Thorngren N, Ahlgren S, Mellon M, Deleault K, Rajgarhia V, van Zyl WH and Penttilä M 2011 High level secretion of cellobiohydrolases by *Saccharomyces cerevisiae*. Biotechnol Biofuels. 12:30.

**BACKGROUND:** The main technological impediment to widespread utilization of lignocellulose for the production of fuels and chemicals is the lack of low-cost technologies to overcome its recalcitrance. Organisms that hydrolyze lignocellulose and produce a valuable product

such as ethanol at a high rate and titer could significantly reduce the costs of biomass conversion technologies, and will allow separate conversion steps to be combined in a consolidated bioprocess (CBP). Development of *Saccharomyces cerevisiae* for CBP requires the high level

secretion of cellulases, particularly cellobiohydrolases.

RESULTS: We expressed various cellobiohydrolases to identify enzymes that were efficiently secreted by *S. cerevisiae*. For enhanced cellulose hydrolysis, we engineered bimodular derivatives of a well secreted enzyme that naturally lacks the carbohydrate-binding module, and constructed strains expressing combinations of *cbh1* and *cbh2* genes. Though there was significant variability in the enzyme levels produced, up to approximately 0.3 g/L CBH1 and approximately 1 g/L CBH2 could be produced in high cell density fermentations. Furthermore, we could show

activation of the unfolded protein response as a result of cellobiohydrolase production. Finally, we report fermentation of microcrystalline cellulose (Avicel™) to ethanol by CBH-producing *S. cerevisiae* strains with the addition of beta-glucosidase.

CONCLUSIONS: Gene or protein specific features and compatibility with the host are important for efficient cellobiohydrolase secretion in yeast. The present work demonstrated that production of both CBH1 and CBH2 could be improved to levels where the barrier to CBH sufficiency in the hydrolysis of cellulose was overcome.

- 2 Koivistoinen OM, Arvas M, Headman JR, Andberg M, Penttilä M, Jeffries TW and Richard P In press Characterisation of the gene cluster for L-rhamnose catabolism in the yeast *Scheffersomyces (Pichia) stipitis*. Gene.

In *Scheffersomyces (Pichia) stipitis* and related fungal species the genes for L-rhamnose catabolism *RHA1*, *LRA2*, *LRA3* and *LRA4* but not *LADH* are clustered. We find that located next to the cluster is a transcription factor, *TRC1*, which is conserved among related species. Our transcriptome analysis shows that all the catabolic genes and all genes of the cluster are up-regulated on L-rhamnose. Among genes that were also up-regulated on L-rhamnose were two transcription factors including the *TRC1*. In addition, in 16 out of the 32 analysed fungal species only *RHA1*, *LRA2* and *LRA3* are physically clustered. The clustering of *RHA1*, *LRA3* and *TRC1* is also conserved in

species not closely related to *S. stipitis*. Since the *LRA4* is often not part of the cluster and it has several paralogs in L-rhamnose utilising yeasts we analysed the function of one of the paralogs, *LRA41* by heterologous expression and biochemical characterization. *Lra41p* has similar catalytic properties as the *Lra4p* but the transcript was not up-regulated on L-rhamnose. The *RHA1*, *LRA2*, *LRA4* and *LADH* genes were previously characterized in *S. stipitis*. We expressed the L-rhamnonate dehydratase, *Lra3p*, in *S. cerevisiae*, estimated the kinetic constants of the protein and showed that it indeed has activity with L-rhamnonate.

- 3 Gibson BR 2011 125<sup>th</sup> Anniversary Review. Improvement of higher gravity brewery fermentation via wort enrichment and supplementation. J Inst Brew 117:264-284.

Intensification of the industrial brewing process, particularly the use of higher gravity worts, has been driven by increasing competition within the industry as well as the need to maximise the use of raw materials and minimise energy expenditure. These developments have, however, placed greater demands on brewing yeast strains, whose evolutionary history has not prepared them for the extreme conditions associated with higher gravity brewing. Various yeast nutrient supplements have been used or proposed to maintain yeast performance under stressful conditions. These have included specific metal ions, lipids and lipid

components such as fatty acids and sterols and free amino nitrogen usually supplied in the form of a complex yeast food. Correction of wort nutritional deficiencies may reduce stress sensitivity of yeast and improve fermentation performance. Potential negative consequences of altering wort composition must however be considered as important beer quality attributes such as taste, stability and foam can be affected. Here, the various options for nutrient supplementation and their influence on yeast physiology and performance as well as beer characteristics are considered.

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The following papers were recently published.

- 1 Gonçalves P, Valério E, Correia C, Almeida JMGCF, Sampaio JP 2011 Evidence for divergent evolution of growth temperature preference in sympatric *Saccharomyces* species. PLoS ONE 6(6): e20739.
- 2 Libkind D, Hittinger CT, Valério E, Gonçalves C, Dover J, Johnston M, Gonçalves P, Sampaio JP 2011 Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Nat Acad Sci USA 108:14539-14544.

Domestication of plants and animals promoted humanity's transition from nomadic to sedentary lifestyles, demographic expansion, and the emergence of civilizations. In contrast to the well-documented successes of crop and livestock breeding, processes of microbe domestication remain obscure despite the importance of microbes to the production of food, beverages, and biofuels. Lager-beer, first brewed in the fifteenth century, employs an allotetraploid hybrid yeast, *Saccharomyces pastorianus*, a domesticated species created by the fusion of a *S. cerevisiae* ale-yeast with an unknown cryotolerant *Saccharomyces* species. We report the discovery of that species and designate it *S. eubayanus* sp. nov. because of its resemblance to *S. bayanus* (a complex hybrid of *S. eubayanus*, *S. uvarum*, and *S. cerevisiae* found only in

the brewing environment). Individuals from populations of *S. eubayanus* and its sister species, *S. uvarum*, exist in apparent sympatry in *Nothofagus* (Southern beech) forests in Patagonia, but are isolated genetically through intrinsic post-zygotic barriers, and ecologically through host-preference. The draft genome sequence of *S. eubayanus* is 99.5% identical to the non-*S. cerevisiae* portion of the *S. pastorianus* genome sequence and suggests specific changes in sugar and sulfite metabolism that were crucial for domestication in the lager-brewing environment. This study shows that combining microbial ecology with comparative genomics facilitates the discovery and preservation of wild genetic stocks of domesticated microbes to trace their history, identify genetic changes, and suggest paths to further industrial improvement.

- 2 Coelho MA, Gonçalves P, Sampaio JP 2011 Evidence for maintenance of sex determinants but not of sexual stages in red yeasts, a group of early diverged basidiomycetes. *BMC Evolutionary Biology* 11:249.

The red yeasts are an early diverged group of basidiomycetes comprising sexual and asexual species. Sexuality is based on two compatible mating types and sexual identity is determined by *MAT* loci that encode homeodomain transcription factors, peptide pheromones and their receptors. The objective of the present study was to investigate the presence and integrity of *MAT* genes throughout the phylogenetic diversity of red yeasts belonging to the order Sporidiobolales. We surveyed 18 sexual heterothallic and self-fertile species and 16 asexual species. Functional pheromone receptor homologues (*STE3.A1* and *STE3.A2*) were found in multiple isolates of most of the sexual and asexual species. For each of the two mating types, sequence comparisons with whole-genome data indicated that synteny tended to be conserved along the pheromone receptor region. For the homeodomain

transcription factor, likelihood methods suggested that diversifying selection acting on the self/non-self recognition region promotes diversity in sexual species, while rapid evolution seems to be due to relaxed selection in asexual strains. The majority of both sexual and asexual species of red yeasts have functional pheromone receptors and homeodomain homologues. This and the frequent existence of asexual strains within sexual species, makes the separation between sexual and asexual species imprecise. Events of loss of sexuality seem to be recent and frequent, but not uniformly distributed within the Sporidiobolales. Loss of sex could promote speciation by fostering the emergence of asexual lineages from an ancestral sexual stock, but does not seem to contribute to the generation of exclusively asexual lineages that persist for a long time.

- 4 Sun S, Metin B, Findley K, Fonseca A, and Heitman J 2011 Validation of *Kwoniella heveanensis*, teleomorph of the basidiomycetous yeast *Cryptococcus heveanensis*. *Mycotaxon* 116:227-229. DOI: 10.5248/116.227

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**XV National Collection of Agricultural and Industrial Micro-organisms, Corvinus University of Budapest, Faculty of Food Sciences, Somlói út 14–16, H-1118 Budapest, Hungary. Communicated by G. Péter <[gabor.peter@uni-corvinus.hu](mailto:gabor.peter@uni-corvinus.hu)>.**

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The following articles have been published since our last report.

- 1 Péter G, Tornai-Lehoczki J and Dlačny D 2009 *Ogataea populialbae* sp. nov., a yeast species from white poplar. *FEMS Yeast Res* 9:936-941.

During a survey of methylotrophic yeasts in natural habitats in Hungary, the yeast community associated with the exudates of white poplar (*Populus alba*) was found to be unique among the tree exudates hitherto investigated. Nineteen methanol-assimilating yeast strains representing an undescribed ascomycetous species were isolated from tree

exudates of *P. alba* collected at different locations in Hungary. Analysis of the D1/D2 large subunit rRNA gene sequences placed the strains in the *Ogataea* clade and the new species is described as *Ogataea populialbae*. The type culture is NCAIM Y.01853<sup>T</sup> (CBS 11363, NRRL Y-48632).

- 2 Péter G, Tornai-Lehoczki J and Dlačny D 2010 *Ogataea pignaliae* sp. nov., the teleomorph of *Candida pignaliae*. Int J Syst Evol Microbiol **60**:2496–2500.

Six ascospore-producing *Candida pignaliae* strains were isolated from epigeal plant parts in Hungary. They share identical D1/D2 LSU rRNA gene sequences with the type strain of *C. pignaliae*, and the physiological characteristics investigated are also very similar to that of the type strain. The only substantial difference compared to the type strain of *C. pignaliae* is their ability to assimilate  $\beta$ -glucosides (cellobiose, salicin and arbutin). The majority of the

isolation sources of the strains reported in this study have the common feature of containing tannic acid, while the type strain of *C. pignaliae* was recovered from tanning fluid. We were able to induce ascospore production also in the type strain of *C. pignaliae*. Therefore, *Ogataea pignaliae* Péter, Tornai-Lehoczki & Dlačny sp. nov. is proposed as the teleomorph of *C. pignaliae* (F. H. Jacob) S. A. Meyer & Yarrow. The type strain is CBS 6071<sup>T</sup>.

- 3 Péter G, Dlačny D, Tornai-Lehoczki J, Suzuki M and Kurtzman CP 2011 *Spencermartinsiella europaea* gen. nov., sp. nov., a new member of the family Trichomonascaceae. Int J Syst Evol Microbiol **61**:993-1000.

Ten strains of a novel heterothallic yeast species were isolated from rotten wood collected at different locations in Hungary. Analysis of gene sequences for the D1/D2 domain of the large subunit rRNA, as well as analysis of concatenated gene sequences for the nearly complete nuclear large subunit rRNA, nuclear small-subunit rRNA and translation elongation factor 1- $\alpha$ , placed the novel species in the family Trichomonascaceae, but showed that it was distinct from all currently recognized genera. The

name *Spencermartinsiella europaea* gen. nov., sp. nov. is proposed to accommodate the new genus and novel species. The novel species could be distinguished from recognized species of neighbouring genera on the basis of standard phenotypic characteristics. The type and isotype strains of *Spencermartinsiella europaea* are NCAIM Y.01817<sup>T</sup> (NNRRL Y-48265<sup>T</sup> - CBS 11730<sup>T</sup>) and NCAIM Y.01819<sup>T</sup> (NNRRL Y-48266<sup>T</sup> - CBS 11731<sup>T</sup>), respectively.

- 4 Dlačny D, Tornai-Lehoczki J, Sedláček I, Audy M and Péter G 2011 *Debaryomyces psychrosporus* sp. nov., a yeast species from a Venezuelan cave. Antonie van Leeuwenhoek **99**:619-628.

Three yeast strains, which are phenotypically indistinguishable from *Debaryomyces hansenii*, were recovered from secondary mineral deposits (stalactites and stromatolites) obtained in the Crystal Eyes Cave, Roraima Tepui Mountain, Venezuela. Analyses of the D1/D2 domains of the LSU rRNA gene as well as the concatenated sequences of the nearly entire SSU rRNA gene, the ITS regions and the D1/D2 domains of the LSU rRNA gene

confirmed the placement of these strains in the genus *Debaryomyces*, but relationship with all valid species of *D. hansenii* complex was distant. Based on the observed considerable sequence divergence the three strains are proposed as a new species, *D. psychrosporus* sp. nov., with the type strain NCAIM Y.01972<sup>T</sup> (CBS 11845<sup>T</sup>, NRRRL Y-48723<sup>T</sup>).

- 5 Péter G, Dlačny D, Szűcs E and Tornai-Lehoczki J 2011 Enrichment in methanol-containing broth – a simple method for the isolation of *Saccharomyces* from grapes. Acta Alimentaria **40**:376–384.

In this study a simple and effective method was developed for the isolation of *Saccharomyces* strains from grapes. Aseptically collected grape samples were processed by enrichment in a nutritive basal medium supplemented with 10% (v/v) methanol followed by isolation of yeast strains. Sixteen of the 18 grape samples yielded *Saccharomyces* strain(s). More than 70% of the isolates belonged to the genus *Saccharomyces*. Based on phenotype and electrophoretic karyotyping, all strains of *Saccharomyces* were identified as *S. cerevisiae*. For several grape samples, varying physiological characters, the number

of spores per asci, and the observed chromosome length polymorphisms provided evidence for diversity of *S. cerevisiae* strains obtained by this enrichment in methanol-containing broth. Results indicated that enrichment in methanol-containing broth is an effective alternative method to facilitate isolation of *Saccharomyces* strains from grapes. The enrichment method described in this work provides a simple and effective tool for isolation of *Saccharomyces* strains from grapes. The method may be applied in studying wine fermentation ecology, as well as for the isolation of potential starter strains from grapes.

- 6 Péter G, Dlačny D, Tornai-Lehoczki J, Gouliamova D and Kurtzman CP 2011 *Ogataea saltuana* sp. nov., a novel methanol-assimilating yeast species. Antonie van Leeuwenhoek **100**:375-383.

Four ascospore-producing strains of an undescribed methanol-assimilating yeast species were isolated from

forest habitats in Hungary. Three were recovered from rotten wood and one from leaves of a sessile oak (*Quercus*

*petraea*). An additional isolate of the undescribed species sharing similar phenotypic characters with the above-noted strains was recovered from the gut of an unidentified beetle collected from under the bark of a coniferous tree in Bulgaria. A closely related, but somewhat divergent strain was recovered from insect frass in a Ponderosa pine (*Pinus ponderosa*) collected in New Mexico, USA. Analysis of the D1/D2 sequences of the LSU rRNA gene placed the new species in the *Ogataea* clade. The ITS and the D1/D2 LSU sequences of the rRNA gene repeats were compared for the above-noted strains and that of the type strain of *Ogataea zsoltii*, the closest neighbour among currently recognized *Ogataea* species. Their relatedness was investigated by

parsimony network analysis as well. As a result of the sequence analysis, it was concluded that the six strains isolated from tree associated habitats represent a single new yeast species. *Ogataea saltuana* sp. nov. is proposed to accommodate these strains. The type strain NCAIM Y.01833<sup>T</sup> (CBS 10795<sup>T</sup>, NRRL Y-48448<sup>T</sup>) was recovered from rotten wood of Scotch pine (*Pinus silvestris*) in Hungary. The GenBank accession number for the D1/D2 domain nuclear large subunit rRNA gene sequence of strain NCAIM Y.01833<sup>T</sup> (CBS 10795<sup>T</sup>, NRRL Y-48448<sup>T</sup>) is EU327033. The MycoBank number of the new species is MB 519966.

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Recent publications.

- 1 Strómayer-Rácz T, Gazdag Z, Belágyi J, Zhao RJ, Vágvölgyi Cs, Pesti M 2010 Oxidative stress induced by HIV-1 F34Ivpr in *Schizosaccharomyces pombe* is one of its multiple functions. *Exp Mol Pathol* **88**:38-44.
- 2 Horváth E, Papp G, Gazdag Z, Belágyi J, Vágvölgyi Cs, Pesti M 2010 In vivo direct patulin-induced fluidization of the plasma membrane of fission yeast *Schizosaccharomyces pombe*. *Food Chem Toxicol* **48**:1898-1904.
- 3 Poljsak B, Pócsi I, Raspor P, Pesti M 2010 Interference of chromium with biological systems in yeasts and fungi: a review. *J Basic Microbiol* **50**:21-36.
- 4 Nyilasi I, Kocsubé S, Pesti M, Lukács Gy, Papp T, Vágvölgyi Cs 2010 In vitro interactions between primycin and different statins in their effects against some clinically important fungi. *Med Microbiol* **59**:200-205.
- 5 Virág E, Pesti M, Kunsági-Máté S 2010 Competitive hydrogen bonds associated to the effect of primycin antibiotic on the oleic acid as building block of plasma membrane. *J Antibiot* **63**:113-117.
- 6 Horváth E, Papp G, Gazdag Z, Belágyi J, Blaskó Á, Deli Cs, Pesti M 2010 Characterization of stress processes of *Phaffia rhodozyma* stress-resistant mutant. *Acta Biol Hung* **62**:204-210.
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## Essay

### Biodiversity of yeast species in olive fermentations

J Geraldine Sandana Mala and C Rose

Microorganisms play an indispensable role in the ecological niche offering physiological and environmental attributes thereby establishing a protective microflora. Yeast species are essential counterparts by virtue of their biodiversity in the ecosystem with inherent industrial potentials. In the recent years, there has been increased attention in the identification and characterization of yeast populations associated with olive fermentations. We describe here the biodiversity of yeast sp. in oleic ecosystems, with special relevance to table olive industrial fermentations (Bautista-Gallego et al. 2011), extra virgin olive oil (Zullo et al. 2010) and olive fruits (Romo-Sanchez et al. 2010).

The worldwide production of table olive oil amounts to 2,153,500 tonnes in 2007/2008 season (IOOC, 2008) in the main regions of Mediterranean Basin, Australia, USA and South America. Castilla La Mancha is the second largest olive growing region in Spain with an average of 350,000 ha. Diverse microflora are involved throughout olive fermentation which determine the quality and flavor of the final product; and, yeasts are the most ubiquitous species (Garrido-Fernandez et al. 1997). The positive influences of yeasts on table olive fermentation have recently been reconsidered (Arroyo-Lopez et al. 2008; Hernandez et al. 2007). Apart from yeast sp., the

other microbial flora are those of lactic acid bacteria, *Enterobacteriaceae* and filamentous fungi. However, yeasts are more relevant in directly brined green and black natural olive fermentations, where fruits are not treated with NaOH and lactic acid bacteria are partially inhibited due to the presence of phenolic compounds (Garrido-Fernandez et al. 1997). Yeasts also tend to cause spoilage of olive fruits during table olive storage and packaging (Arroyo-Lopez et al. 2008). The enzymatic properties of yeasts in olive processing also impart useful characteristics for exploitation in table olive elaboration. Lipolytic activity,  $\beta$ -glucosidase, esterase and catalase activities thereby contribute towards the quality and flavor of olive with organoleptic attributes. The opalescent appearance of newly produced olive oil is due to the presence of solid particles and micro-drops of vegetation water containing a rich microflora (Ciafardini & Zullo, 2002). Yeasts present in newly produced oil are active throughout the products entire preservation period and affects the oil quality according to species. The taste and antioxidant capability of the oil can be improved by the  $\beta$ -glucosidase and esterase producing sp. of yeasts capable of hydrolysing oleuropein into

simpler and no longer bitter compounds characterized by high antioxidant activities (Ciafardini & Zullo, 2002). Lipase-producing yeasts deteriorates the oil quality through triacylglycerol hydrolysis. Thus, the flavor, nature and quality of the olive product are determined by the properties as well as species of yeasts during olive growth and processing.

*Candida* and *Pichia* are common genera during directly brined green olive fermentations reaching population levels of around 10<sup>6</sup> CFU/ml (Arroyo-Lopez et al. 2008). Bautista-Gallego et al. (2011) have observed a reduced range of yeast biodiversity of around 5, 3 and 6 species for Aloreña, Gordal and Manzanilla cultivars respectively during spontaneous industrial fermentations, similar to observations of Aponte et al. (2010). *S.cerevisiae*, *C. diddensiae* and *P. membranifaciens* were the prominent sp. related to Aloreña cultivar (Bautista-Gallego et al. 2011). In the case of directly brined Gordal and Manzanilla olive fermentations, the most important species involved were *C. tropicalis*, *P. galeiformis* and *W. anomalus*. A lower yeast population of only ~6.0 x 10<sup>3</sup> CFU/ml was found in Gordal and Manzanilla green olives processed according to Spanish style. A greater yeast biodiversity of a total of 10 species was found during fermentation of both Gordal and Manzanilla cultivars and 4 species *D. etchellsii*, *P. galeiformis*, *C. tropicalis* and *K. lactis* were isolated. Among all yeasts, 2 isolates of *W. anomalus* (SG4 and DG3) exhibited interesting technological properties for their application during table olive processing. This species have ideal adaptation to the environmental conditions that govern table olive fermentations such as low pH and high NaCl concentrations and displayed strong  $\beta$ -glucosidase and esterase activity, as well as moderate catalase and lipolytic activity. This species lacked xylanase and a weak protease activity, which are undesirable and produce softening of fruits (Hernandez et al. 2007). Dimorphic yeast forms in 26% of the commercial extra virgin olive oil originating from different geographical areas, and the dimorphic yeasts are represented by 3-99% of the total yeasts (Zullo et al. 2010). A total of 108 yeast strains have been isolated from olive fruits, olive paste and olive pomace. Identification of 14 different species of yeast belonging to *Zygosaccharomyces*, *Pichia*, *Lachancea*, *Kluyveromyces*, *Saccharomyces*, *Candida* and *Torulaspota* genera demonstrated species diversity. *P. holistii*, *P. mississippiensis* and *Lachancea* sp. were typically found in olive paste while *S. cerevisiae*, *S. rosinii*, *Candida* sp. *C. diddensiae*, *Zygosaccharomyces florentinus* and *Torulaspota delbrueckii* were found only in pomace.

Biodiversity was greater in olive by-products than olive fruits.

Presence of lipolytic activity in yeast sp. modify the nutritional composition of the final product (Arroyo-Lopez et al. 2008). However, Zullo and Ciafardini (2008) did not find lipase activity in yeasts isolated from *Leccino* olive oil and from olive brine. Thereby, enzyme activities are strain-dependent and influence the quality of the end-product. Romo-Sanchez et al. (2010) have evaluated cellulase, polygalacturonase, peroxidase and  $\beta$ -glucosidase activities of yeast sp. isolated from olive products which are determinants of quality and yield of the products. Thus, characterization of yeast species from olive by-products contributes to the development of microbial banks for potential industrial applications. Thereby, the biodiversity of yeast species in olive fermentations demonstrate significant implications in the food and biotechnology sectors offering potential avenues for commercial exploitations.

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Recent publication.

- 1 Sandana Mala JG, Gayathiri M, Rose C, Mandal AB 2011 Osmotic shock augments ethanol stress in *Saccharomyces cerevisiae* MTCC 2918 (Current Microbiology, 2011, in press).

Yeast cells sense and respond to hypertonicity. *Saccharomyces cerevisiae* MTCC 2918 was tested for its metabolic status in 1M NaCl by cell viability analysis, intracellular glycerol content and total antioxidant capacity. Yeast cell viability was maximum in 1M NaCl and 24 h addition of 1M NaCl was effective in induction of hyperosmolarity. Increased glycerol contents in cells treated with salt indicated adaptation to osmotic stress with a maximum of  $240.87 \pm 0.38$  mg/g dry weight (DW) at 72 h. The total antioxidant status with 1M NaCl was  $9.29 \pm 0.39$  mM/g DW at 96 h reflecting free radical quenching to

overcome stress with increasing growth period. Considering that pre-adaptation to one type of stress evoked a protective response to other stress factors, we have attempted the cross adaptation of osmotic shock to high ethanol concentrations. In effect, we observed that osmotic shock lowered the cell survival by augmentation of cell toxicity by ethanol due to stress induction during exponential phase. Glycerol accumulation to an order of  $470.27 \pm 0.53$  mg/g DW at 48 h in 1M NaCl and 12% ethanol indicated that both stresses culminated in membrane disruption further leading to cell burst and contributed to the stress overload.

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The following papers were read at recent conferences.

- 1 Lachance MA 2011 The genus *Kluyveromyces*: the new taxonomy of some old tequila yeasts. 29th International Symposium on Yeasts, Guadalajara, Mexico.

**A brief history.** The genus *Kluyveromyces* was established in 1956 by van der Walt upon the discovery of a remarkable yeast capable of forming multi-spored asci. In 1965, van der Walt expanded the genus to include a number of species formerly described, validly or not, as *Saccharomyces*, *Dekkeromyces*, *Fabospora*, *Guilliermondella*, *Zygofabospora*, or *Zygosaccharomyces*. The number of recognized species in the genus grew to 18, but was later reduced (van der Walt and Johannsen 1984) to 11, primarily on the basis of somatic hybridization data. This treatment collapsed nine previously described taxa into an expanded concept of *K. marxianus*, with seven varieties. Integrating primarily DNA reassociation and isoenzyme data, Lachance (1998) recognized 15 species, restored *K. dobzhanskii*, *K. lactis*, and *K. marxianus* as distinct species and retained two artificial varieties of *K. lactis*, separated based on lactose utilization. The genus as understood at that time was heterogeneous and clearly polyphyletic, with three apparent groups: the largest consisted of species with a greater affinity for *Saccharomyces* and included *K. polysporus*, the type species; the smallest comprised two species that exhibited much resemblance to *Saccharomyces kluyveri*; the remainder constituted a cohesive assemblage that included the better known species *K. lactis* and *K. marxianus*, with the special distinction that some members possessed the ability to utilize lactose. Various DNA sequencing studies have since confirmed polyphyly, culminating in Kurtzman's (2003) reorganization of all *Saccharomyces sensu lato* species into 11 genera based on a multigene phylogenetic analysis. The majority of species assigned to *Kluyveromyces* in 1998 were reassigned to five other genera, leaving behind a well-circumscribed, monophyletic assemblage that unfortunately did not include the original

type species, now *Kazachtania polyspora*. This made necessary a proposal (Kurtzman et al 2001) for the conservation of the name *Kluyveromyces* with a new type, the first-described (1888) species, *K. marxianus*. It is worth noting that every single step of the history described above, ranging from the original use of the name *Kluyveromyces*, the repeated fusions and splits of various *Kluyveromyces* species, the conservation proposal, its acceptance by the International Committee, and even the choice of *K. marxianus*, and not *K. lactis*, as the conserved type, has generated impassioned debate, be it at conferences, in the printed literature, or in private exchanges. Reluctantly faced with the task of preparing the 2011 (The Yeasts, 5<sup>th</sup> Edition) monograph on this coveted and contentious genus, and taking into account multifarious data published by numerous researchers with a focus on DNA sequences, I have accepted six species, namely *K. aestuarii*, *K. dobszhanskii*, *K. lactis*, *K. marxianus*, *K. nonfermentans*, and *K. wickerhamii*. It is abundantly clear that the internal structure of *K. lactis* is complex. However, attempts to subdivide the species along coherent genetic lines (Naumov-Naumova, Belloch et al; reviewed in Lachance 2011) have yielded contradictory arguments, leading me to retain the artificial varieties proposed previously, for pure convenience.

***Kluyveromces marxianus* and allied species.** A network analysis of rDNA ITS sequences supports, in proper context, the separation of *K. dobzhanskii*, *K. lactis*, and *K. marxianus* as distinct species and demonstrates that the polymorphism present in *K. lactis* does not call for obvious rearrangements.

**Tequila.** *K. marxianus* is of special interest in the context of the venue of ISSY29, the state of Jalisco, known the world over for the unique beverage that is tequila. In a

study of the yeasts associated with natural agave fermentations conducted in 1992 and 1994, I isolated a large number of yeasts in several genera, including, but not limited to *Saccharomyces*, *Dekkera/Brettanomyces*, *Hanseniaspora*, *Clavispora*, *Metschnikowia*, *Candida*, and of course, *Kluyveromyces*. One objective was to assess to what extent the yeast community of agave in the field contributed to the unique characteristics of premium quality tequila (Herradura SA de CV). At the time, the over thirty *Kluyveromyces* isolates were identified as *K. marxianus* on the basis of growth responses, despite considerable variation in the utilization of  $\beta$ -glucosides, polyols, and organic acids, as well as halo- and osmotolerance. These identifications were recently confirmed by rDNA ITS sequencing, which further revealed a small amount of polymorphism (Fig 1). The isolates came from blue agave in the field, processed agave in the distillery, and from the fermentation itself. In addition a few cultures were recovered from various materials associated with processed agave in other distilleries, including one that produces mezcal, a more generic product made from other types of agave. These were compared to isolates obtained from fermenting pulque, a non-distilled alcoholic beverage made from agave, from a drosophilid collected in a mesquite sap flux in Arizona, and from a cactus fruit in the Caribbean. Other than an interesting surprise for the pulque isolate, the rest of the strains had two nearly identical ITS haplotypes that appeared randomly distributed with respect to habitat or geography (Fig 1). As to the original question of the role of *K. marxianus* in tequila production, it had been informally

hypothesized that the ability of some strains to utilize inulin and the relatively vigorous fermentative ability of the species might be of significance. However, the yeast was more or less absent in mature fermentations, which were overwhelmingly dominated by *S. cerevisiae*. In 1994, laboratory-scale fermentations of agave must were conducted with strains of *S. cerevisiae*, *K. marxianus*, *C. humilis*, *B. bruxellensis*, *H. guilliermondii*, and *Z. bailii* collected in 1992. The wines were distilled into 'ordinario', analyzed by gas chromatography, and tasted. Only the products fermented with *S. cerevisiae* resembled the true product, although the *B. bruxellensis*-fermented tequila was not unpleasant and possessed a superior volatile profile. The chromatographic trace of the tequila made with *K. marxianus* resembled that obtained with *S. cerevisiae*, but featured elevated amounts of butanol resulting in an unacceptable taste. Other yeasts gave a variety of poor results.

**Conclusions.** The taxonomy of *Kluyveromyces* has significantly improved from the advent of DNA sequence analysis. *K. marxianus* occurs frequently in agave and derivatives, but plays a minor role in the complexity of naturally fermented tequila.

**Acknowledgements.** I acknowledge funding by the Natural Science and Engineering Council of Canada and Tequila Herradura SA de CV. The field assistance of C. Cone, L. Solis, and A. Rabago are gratefully acknowledged, as is laboratory assistance from L. Delgadillo and T. Wijayanayaka.

## 2 Lachance MA 2011 Microbial species descriptions: the importance of multiple strains. IUMS Congress, Sapporo, Japan.

Given the right cautionary preamble, most microbial systematists would probably agree that species can be defined as groups of individuals that share certain common characteristics and are distinct from other such groups. To establish groups as distinct requires an appreciation for the variance within the groups. It follows that species cannot be properly delineated if only a single representative of each species is available for study. The view presented here is that descriptions based on multiple isolates make for better science! If, for example, a sequence-based phylogenetic species concept is implied, as it often is, reciprocal monophyly must be established, which requires that the extent of sequence polymorphism be evaluated, at the very least in the new species. However, significant proportions of yeast species have been and continue to be described on the basis of a single isolate or a few isolates of dubious independence. This practice is even the object of vigorous

advocacy (Kurtzman 2010). In recent years, the task of discovering new yeast species has been greatly enhanced by the availability of a continuously updated database of sequences for the D1/D2 domains of the large subunit rRNA gene. Kurtzman and Robnett (1998) have shown empirically that polymorphic species seldom exhibit more than three substitutions in that region and that well-defined species seldom differ by less than 1% substitutions. This observation has triggered a veritable avalanche of species descriptions based solely on this criterion, and often applied to single strains. Recent evidence suggests that species that are sampled thoroughly may exhibit substantial amounts of polymorphism at the level of barcoding sequences. I shall review examples of such cases and discuss their potential impact on the problem of correct delineation and typification.

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## Obituaries

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### Some brief memories on Professor Sandro Martini

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Professor Martini graduated from the University of Perugia in 1967. In the early 70s, he joined the lab of Professor Herman J. Phaff at the University of California, Davis, where he met his wife, Ann Vaughan. Martini became Full Professor of Microbiology at the University of Perugia in 1975, later to become Director of the Department of Applied Biology and Coordinator of the Industrial Yeasts Collection, DBVPG. Under his leadership, DBVPG became part of ECCO (European Culture Collection Organization) and WFCC (World Federation of Culture Collections). In 1988, he became Chair of the International Commission on Yeasts and organized the 7<sup>th</sup> International Symposium on Yeasts, held at the University of Perugia. Martini co-authored over a hundred peer-reviewed publications and retired in 2005. During retirement, he continued his editorial activities in front of his home computer. His last scientific work, a DVD entitled “Yeast Biology”, is currently available from Insight Media.



I met Prof. Martini in 1998. I had known him for my eight years spent in another Department of the University of Perugia, but I joined his group in 1998. At that time, I had never worked with yeasts, but only with certain types of food-associated bacteria. The yeasts were for me an “obscure” group of eukaryotic microorganisms. Prof. Martini passed me his profound love for all aspects of the yeast world, not only yeast taxonomy, but also technology and biotechnology. He was a true master and mentor. He taught me scientific rigor, a critical sense, and, not least, a deep sense of modesty. A notable feature that fascinated me tremendously was Prof. Martini’s gift for finding new and original solutions to daily scientific problems. How many times did he cause me to exclaim: “Why did not I think about that before?” I shall always remember his first and friendly encouragement when I joined his group: “Pietro, please, find as soon as possible a few topics of interest for you within the yeast world and study them methodically.” I am honoured to have always followed this treasurable suggestion, as well as all others that he gave me in subsequent years. Above all, I especially value remembering Prof. Martini in his times outside of work. On such occasions, I was able to appreciate his honesty, his sense of humour, and his ideas about life, family, and politics. I also fondly remember his altruism towards younger colleagues: for example, he generously made the decision of retiring three years early to allow the creation of a researcher position for a young biologist. I can only conclude these brief memories of my unforgettable experience with him by saying: thank you Prof. Martini; your scientific, professional, and personal life will be always an example for me.

Pietro Buzzini  
Dip. di Biologia Vegetale e Biotecnologie Agroambientali  
Università di Perugia, Italy.

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### Professor Emeritus Ralph Kunkee

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Ralph Kunkee, Professor Emeritus of Enology at the University of California at Davis, passed away on November 12, 2011 from complications due to cancer. We in the Department of Viticulture and Enology will all miss him—his love of life and learning were infectious.

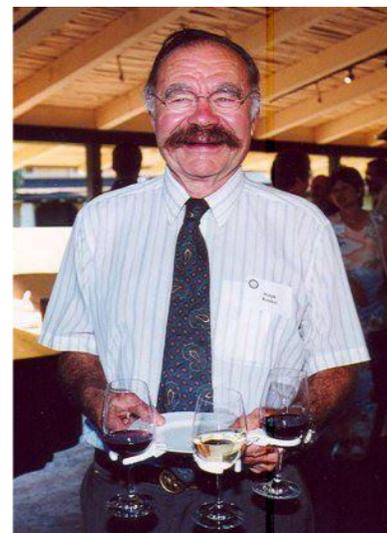
Ralph joined the department in the early 1960’s and taught in the department until his retirement in 1991. Ralph’s major research and teaching interests had to do with wine yeast, the malolactic fermentation and the sources and controls of microbiological spoilages of wines. The wine yeast studies that he completed involved the characterizations, descriptions and utilities of various yeast strains. The data from these studies are now standard wine making tools in the global wine industry. Prof. Kunkee’s work on malolactic fermentation helped bring

understanding to this bacterial activity, and how to control it. These research efforts resulted in the publication of nearly 150 scientific articles and Ralph was also co-author of two enological texts. Several of the research articles, and one of the texts, received prize-winning acclaim.

In addition, Ralph played a helpful role in the transition of the American taste in wines--and the corresponding change in California wine production--from high alcohol dessert/appetizer wines of the time to the lower alcohol table wines of today, by indoctrinating and urging the use of sterile filtration and sterile bottling as the standard means for wine stabilization. He visited essentially all of major wine growing regions of the world, and spent twelve-month sabbatical leaves in two of them (Germany and France).

Concerning his teaching, Ralph calculated that he taught over 1000 students in his specialty laboratory course: Microbiology of Winemaking—and most of those students are now widely distributed throughout the wineries of California and of the rest of the world. Even after he retired Ralph, Ralph was still involved in lecture presentations, in consultations and in wine judgments. Ralph also instructed a Distance Learning class, “Introduction to Winemaking,” through UCD Extension, with about 100 students annually.

Aside from his professional accomplishments, Ralph was a wonderful, warm person. His hospitality was legendary, and it was impossible to not have a good time in his presence, especially when he would flash his trademark smile under that bushy mustache, inevitably with a glass of wine in one of his hands.



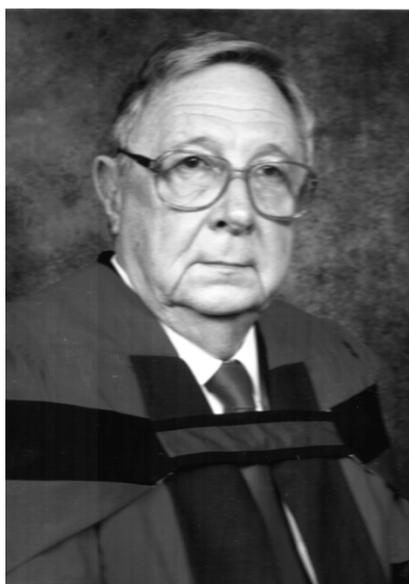
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Davis, California, USA

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## Professor J.P. van Der Walt (1925-2011)

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Professor Johannes van der Walt, internationally recognized yeast taxonomist, passed away in Pretoria, South Africa on the 13<sup>th</sup> of November 2011. Although challenged by numerous health problems in recent years, he remained active in research until a few weeks before his death, which was unexpectedly sudden.



Johannes van der Walt was born in Pretoria, South Africa on the 10<sup>th</sup> of February 1925. He was a born biologist, and as a boy was encouraged to collect and classify arid-dwelling plants in the Karoo of South Africa by his father. Visits to this part of the world were part of an annual vacation and he would accompany his father, hiking through the veld to identify the unusual lithops and other succulent plants native to this area. There is little doubt that these vacation excursions contributed substantially to his love of nature and to his profession as a scientist.

Johannes graduated from the University of Pretoria with a B.Sc. degree *cum laude*, majoring in chemistry and physics, and later obtained an M.Sc. degree in Chemistry at the same University. In 1948 he joined the Council for Scientific and Industrial Research (CSIR) as an assistant scientist where he was mentored by Professor Adrianus Pijper. That same year he was awarded a bursary from the Netherlands-South African Society and enrolled for a Doktorandus at Leiden University, The Netherlands under the guidance of Professor A.J. Kluyver with chemistry as his major subject and microbiology as a secondary subject, which he completed in 1949. Three years later he graduated

with a D.Sc. from the Delft University of Technology, having completed a thesis entitled: “On the yeast *Candida pulcherrima* and its pigment”.

Subsequently he returned to South Africa where he was employed by the CSIR until his retirement in 1988. During the early stages of his career he established the first modern microbiological investigation into sorghum beer. In 1957 he moved to Stellenbosch, where his research focused on the industrial problems being experienced by the wine industry with yeasts that acidified wine. In 1961 he was appointed as head of the microbiology research group where he pursued his passion for the systematics, genetics and ecology of yeasts. Between the years 1963-1967 he led the first mycotoxin research programme at the CSIR.

Johannes was regularly invited to present his research internationally. He published 131 scientific papers and four significant books on yeast taxonomy during the course of his career. He was a member of the Royal Society of South Africa and in 1969 he was invited by IUMS to serve as a member of the International Commission of Yeasts. In 1980 he served on the Special Committee for Fungi and Lichens of the International Association for Plant Taxonomy.

On his retirement from the CSIR he transferred his substantial yeast culture collection to the University of the Free State (UFS) in Bloemfontein, which formed the beginnings of the current MIRCEN yeast culture collection of the Department of Microbial, Biochemical & Food Biotechnology at the UFS. He was appointed by UFS as Professor Extraordinary from 1 Jan. 1993 to 31 March 1998. In 1994 he was awarded an honorary doctorate from the UFS for his important contributions to yeast taxonomy. He was appointed as honorary professor at the University of Pretoria between 1980 and 1983 and again between 1999 and 2001.

Johannes van der Walt made many important contributions to science nationally and internationally. His best-known contributions scientifically were in the field of yeast taxonomy where he described many new taxa. He had an amazing ability to discover novel yeasts and was a passionate advocate of enrichment techniques that enabled him to discover yeasts overlooked by others in similar environments. He contributed important chapters to books such as the 2<sup>nd</sup> and 3<sup>rd</sup> editions of “The Yeasts, a taxonomic study” and the 2<sup>nd</sup> edition of “The Yeasts”. A number of yeast taxa are named after him, namely the genus *Vanderwaltozyma* and the species *Bullera waltii*, *Candida vanderwaltii*, *Dexomyces waltii*, *Myxozyma vanderwaltii* and *Lachancea waltii* (formerly *Klyuveromyces waltii* and *Zygodafospora waltii*). Previous genera named after him included *Vanderwaltia*, *Waltomyces* and *Waltiozyma*. A new species recently isolated at UFS is to be named *Trichosporon vanderwaltii*.

Johannes became involved in the fledgling Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria when it was established in 1998. In this environment he was able to share his passion for science, particularly with post-graduate students and academic staff. He thoroughly enjoyed challenging students, sometimes mischievously, to think and to question. Johannes had an amazing knowledge of languages and was especially passionate about Latin and Greek. This interest led him to delight in deciding on names for new fungi and bacteria and here he always preferred Greek. In his later years, Johannes made weekly visits to the FABI laboratories to isolate yeasts, particularly from lichens, and these visits were the highlight of his week.

Johannes lived life to the full. He loved people and was always wonderful company. He was a great man of science and mentor and friend to many.

Teresa Coutinho & Mike Wingfield  
Forestry and Agricultural Biotechnology Institute (FABI)  
University of Pretoria

Amended by James du Preez  
Dept. of Microbial, Biochemical & Food Biotechnology  
University of the Free State, Bloemfontein

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**International Commission On Yeasts (ICY)**  
**Meeting of Commissioners, September 1, 2011**  
**International Specialized Symposium on Yeasts (ISSY 29)**  
**Hotel Hotel Camino Real, Guadalajara, Mexico**  
**Minutes of Meeting**

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**Present:** Andriy Sibirny (Ukraine, Chair), Leda C. Mendonça-Hagler (Brazil, Vice-Chair), Graham Fleet (Australia), Johan Thevelein (Belgium), Marc-André Lachance (Canada), Vladimir Mrsa (Croatia), Hana Sychrova (Czech Republic), Anna Maraz (Hungary), Pietro Buzzini (Italy), Patrizia Romano (Italy), Hiroshi Takagi (Japan), Patricia Lappe Oliveras (Mexico), Grzegorz Bartosz (Poland), Gennadi Naumov (Russia), Elena Naumova (Russia), Peter Raspor (Slovenia), James Du Preez (South Africa), Bernard Prior (South Africa), Amparo Querol (Spain), Charoen Charoentai (Thailand), Charles Abbas (USA), Kyria Boundy-Mills (USA), Sally Meyer (USA).

**Invited:** Angelica Ganga (Chile), Anne Gschaedler (Mexico) and Rosane Freitas Schwan (Brazil).

**Apologies:** T. Alamae, P. Biely, T. Boekout, M. Bolotin-Fukuhara, S. Dequin, J. Douglas, H. Erten, M. Gonchar, B. Hahn-Hagerdal, I. Hapala, Y. Kassir, M. Kopecka, M. Korhola, C. Kurtzman, J.M. Peinado, M. Penttilä, V. Pidhorsky, J. Piskur, I. Pretorius, H. Prillinger, J. Pronk, A. Rapoport, D. Rauhut, L. Scheffers, R. Sentandreu, G. Stewart, I. van der Klei, A. Vaughan-Martini and Ana Clara Schemberg.

**Report from the Chair**

Andriy Sibirny welcomed the delegates to the meeting and mentioned the apologies sent by those who could not attend. He thanked Prof. Patricia Lappe and other organizers of ISSY29 for a wonderful Symposium, with attractive scientific, cultural, and social programs. He then announced the agenda, which was adopted.

**Tribute to Alessandro Martini, former Chair of the International Commission on Yeasts**

Andriy Sibirny briefly recalled the sad news of the demise of the former Chair of the International Commission on Yeasts, Professor at Perugia University (Italy) Alessandro Martini. A full tribute to A. Martini was made during a plenary session of the ISSY29 meeting. At that special event, A. Sibirny proposed to remember this outstanding person. Prof. Pietro Buzzini, ICY Commissioner representing Italy, made a brief summary of the scientific and personal life of A. Martini. Short speeches were also made by two former Chairs of ICY, Dr. Sally Meyer (USA) and Prof. Leda Cristina Mendonça-Hagler (Brazil). The speakers stated that A. Martini was a distinguished scientist and mentor of young yeast researchers.

**New members of ICY (Commissioners)**

Andriy Sibirny recalled that during last year he obtained applications from five candidates for ICY membership, A. Ganga (Chile), J. Nielsen (Denmark), J. Tamang (India), J.-M. Francois (France), and D. Libkind (Argentina). Each candidate provided his/her CV with list of

publications, letters of recommendation from a National or International Society and one or two letters of recommendations from current members of ICY. These materials were distributed electronically among Commissioners so that they could express their assessment of the candidates. It should be noted that most ICY members expressed their opinions regarding all proposed candidates and that only enthusiastically supportive comments have been obtained.

A discussion of each proposed candidature took place next. Present as invited guest at the meeting, candidate for ICY membership Dr. Angelica Ganga was asked to leave the meeting during the discussion of her candidacy. A. Sibirny stated that A. Ganga, if elected, would become the first representative of Chile in the Commission. Amparo Querol, who had provided written support of A. Ganga, spoke highly of her research potential and achievements. The Commissioners unanimously supported Dr. Angelica Ganga's acceptance as a member of ICY.

The candidature of Prof. Jens Nielsen, Göteborg, Sweden, was discussed next. A. Sibirny reported that J. Nielsen is an outstanding yeast researcher who recently was awarded a prestigious ERC (European Research Council) large research grant in the field of yeast metabolic engineering. He is currently Chief Editor of FEMS Yeast Research, a journal which from its foundation has maintained close contacts with our Commission. It has been customary for meetings of the Editorial Board during ISSY and ICY Congresses, and Lex Scheffers and Teun Boekhout, two former Editors-in-Chief, are the members of ICY. The election of J. Nielsen as member of ICY is therefore expected to strengthen our Commission. This proposal was unanimously supported by Commissioners.

Prof. Jyoti Tamang, Sikkim, India, has applied for membership in ICY. A. Sibirny underlined that India, a huge country, is currently represented by only one Commissioner, Dr. Rajendra Prasad, who has not been active as a member. It follows that the election of a young and active yeast scientist from India would be important for further development of yeast science in this part of the world. This proposal was unanimously supported.

The candidature of Prof. Jean-Marie François, Toulouse, France, was discussed next. A. Sibirny informed members that currently, France is represented by the maximum allowed number of Commissioners (three): Monique Bolotin-Fukuhara, Claude Gaillardin and Sylvie Dequin. However, Prof. M. Bolotin-Fukuhara asked in 2009 in Paris, during ISSY27 (organized by her), to step down from active membership due to her approaching retirement. It was decided at the time not to accept her resignation in view of the

excellent Symposium organized by Monique. Two years later she again requested to step down from active membership in ICY. A. Sibirny decided to accept her resignation but, in recognition of her long-term contributions to yeast science and her outstanding organization of ISSY27 (Institut Pasteur, Paris), he proposed the election of Prof. Monique Bolotin-Fukuhara as Honorary member of ICY. A. Sibirny also proposed the election of Prof. J.-M. François. Commissioners supported both proposals and unanimously elected Prof. M. Bolotin-Fukuhara as Honorary member and Dr. Jean-Marie François as new regular member of ICY.

Several days before the Symposium, an application for membership in ICY was received from Dr. Diego Libkind, Bariloche, Argentina. A. Sibirny reported that the documentation provided by Dr. D. Libkind was distributed among Commissioners a few days before ISSY29. Argentina was currently represented by only one member, Dr. Lucia de Figueroa, following the death of Prof. Frank Spencer. Dr. Libkind has a very good publication record and has been invited to be a speaker at the 13<sup>th</sup> International Congress on Yeasts to be held in 2012 in Madison, Wisconsin, USA. Prof. Andre Lachance spoke in strong support of Dr. Libkind's candidature and the Commissioners unanimously elected Dr. Diego Libkind as new member of ICY.

Prof. Leda Mendonca-Hagler proposed the nomination of Dr. Rosane Freitas Schwan to represent Brazil. Dr. Schwan excused herself for the duration of this discussion. Prof. Mendonca-Hagler presented the highlights of Dr. Schwan's biography and distributed copies of her CV to the Commissioners. Dr. Schwan is Professor of Microbiology at the Federal University of Lavras, Minas Gerais (Brazil), where she is strongly engaged in Graduate Programs and relevant research projects. She is recognized in Brazil as a distinguished researcher. The candidacy of Dr. Schwan was strongly supported by Prof. Graham Fleet who described her achievements in yeast research, with special emphasis on her contributions to the area of fermented foods. Dr. Schwan had been invited to speak in several ICY sponsored meetings. In addition, Dr. Rosane F. Schwan was recommended by the Brazilian Society for Microbiology (SBM), in a written letter from Prof. Adalberto Pessoa, the President of this major national society. ICY Commissioners supported this proposal and Dr. Rosane F. Schwan was elected unanimously.

Last, A. Sibirny introduced Dr. Anne Gschaedler, present at the ICY meeting, as invited guest and proposed her candidacy for membership. He thanked Anne for his efforts in the organization of an excellent Symposium. Dr. Gschaedler nomination had been proposed by the Mexican delegation during the ISSY meeting in Bangkok, Thailand (2010). Her substantial contribution to research on yeast diversity and agave fermentation processes was stressed by Dr. P. Lappe, Prof. P. Romano, and Prof. Mendonça-Hagler. Dr. Gschaedler's nomination as ICY Commissioner was supported unanimously by ICY members present at the Guadalajara meeting and her membership will become active after receiving the required documents (CV and Letters of Recommendation) in written form.

### **Minutes of the previous meeting**

Andriy Sibirny reported, on behalf of members that were unable to attend the Commission meeting, on ISSY28 held in 2010 in Bangkok, Thailand, and on forthcoming meetings: ISSY30 to be held in 2013 in Slovakia and ISSY31 to be held in Sweden in 2014. The relevant information was obtained from C. Charoenchai (Thailand), I. Hapala (Slovakia), and J. Piskur (Sweden) by electronic mails.

### **Reports on Meetings**

**ISSY 28 (2010) – Metabolic and Bioprocess Engineering for Sustainable Development. Sep 14-18<sup>th</sup>, Montien Riverside Hotel, Bangkok, Thailand** – Coordinator: Dr. Charoen Charoenchai. ISSY28 gathered attendance by nearly 100 participants, 60 of which representing 30 foreign countries. There were 40 oral communications and over a hundred posters. Dr. Charoenchai reported on the difficulties experienced in organizing the meeting due to political instability in Bangkok. In spite of this, the organizers succeeded in hosting a very good meeting. The main topics included in the program were: metabolic and evolutionary engineering of yeasts for ethanol production, yeast biodiversity and agriculture, traditional fermented foods and beverages, wine from fruits and rice, yeast metabolites, and biopharmaceuticals. ICY Commissioners expressed their satisfaction and gratitude to Charoen for the excellent ISSY 28 meeting.

**ISSY 29 (2011) - The relevance of yeasts and microbial consortia in traditional and industrial fermentations. Aug., 29th-Sep.2nd. Guadalajara, Mexico** – Coordinator: Prof. Patricia Lappe. Patricia reported on the highlights of ISSY29. She explained the reasons for the decision to organize ISSY together with the 1<sup>st</sup> International Symposium on Agave. Altogether, there were nearly 280 participants, representing 26 countries, at the two Symposia. She mentioned support and funding from several institutions in Mexico, such as UNAM, CONACYT, CIATEJ, Universidad Iberoamericana, the Municipal Government of the City of Guadalajara, the Department of Tourism, the Convention and Visitors Bureau, the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA), the Consejo Regulador del Tequila, and eighteen industry sponsors (Casa Herradura, Lallemand, Heineken-Cuauhtemoc, Yacult, Waters, Springer, CLP, BioAgave, Rosh, Millipore Merck, Perkin Elmer, and 3M Food Safety, among others). The conference venue, Hotel Camino Real, located in the center of Guadalajara, provided excellent facilities for accommodation and the symposium. It also offered excellent international and national Mexican cuisine. P. Lappe Oliveras also expressed gratitude to Dr. Anne Gschaedler and Prof. Ruben Moreno for their enormous contributions in organizing ISSY29. In the discussion to follow, members of ICY expressed their gratitude to Patricia, other members of local Organizing Committee, and staff for their excellent work.

### **Future Meetings**

**ICY 2012 - 13<sup>th</sup> International Congress on Yeasts, USA.** A member of the Organizing Committee of 13<sup>th</sup>

International Congress on Yeasts, Prof. Charles Abbas, presented a progress report on Congress preparation, informing members of the facilities available at Madison, WI. He reported that the Congress website is active and that registration and abstract submission are opened. The preliminary estimates show that the Congress will be a large event, with an expected number of participants of 500 or more. Leading yeast researchers have been invited and most of them expressed their interest to attend the Congress and accepted the invitation. Currently, a fund raising campaign is underway. It is hope that support will be provided for leading invited speakers and young scientists.

**ISSY 30, 2013, Slovakia** – Organizer: Dr. Ivan Hapala. Dr. I. Hapala planned to attend ISSY29, but had to cancel because of illness. On his behalf, A. Sibirny presented the current status of the preparation of ISSY30 in 2013. Earlier, I. Hapala had proposed the following topic of this Specialized Symposium: “Membranes and Cell Surface in Yeast: from Basic Science to Applications“. However, this topic was questioned due to the fact that there are annual SMYTE meetings with similar topics. After discussion with I. Hapala, he proposed a more balanced and broader topic: "Cell Surface and Organelles in Yeasts: from Basics to Applications". This amended topic was accepted unanimously by members of ICY present at the meeting. A. Sibirny also reported that I. Hapala and his colleagues plan to organize the meeting in the Smolenice castle, located near Bratislava, the capital of Slovakia. The number of participants would be limited (about 120). Commissioners accepted and approved this report.

**ISSY 31, 2014, Sweden** – Coordinator: Prof. J. Piskur. A. Sibirny, on behalf of J. Piskur, presented the current concept of the specialized Symposium to be held in 2014 in Lund, Sweden. Lund is located only 30 min away from Copenhagen international airport. The Specialized Symposium will likely be focused on yeast biodiversity, comparative genomics, evolution, and yeast in food. Many key speakers will be from the Cornucopia consortium (J. Thevelein, T. Boekhout, A. Querol, L. Jespersen, C. Compagno, Carlsberg and Chr. Hansen people, etc). J. Piskur already has part of the required funding (from mentioned EU project). Nearly 30 participants (from the EU consortium) are already confirmed and *ca.* 30-40 will represent the local (Copenhagen-Lund) yeast community. The Commissioners unanimously approved this report.

**ISSY 32, 2015, Italy** – Prof. Pietro Buzzini (Italy) proposed to organize Specialized Symposium in Italy and to dedicate it to the memory of the former Chair of ICY Prof. Alessandro Martini. The size and location of the meeting will be disclosed later, pending ICY approval. Prof. Patrizia Romano, organizer of ISSY 26 (Sorrento, Italy), supported P. Buzzini’s proposal and assured members that she (as member of ICY) and other Italian yeast researchers will help with the organization of a very good meeting. This proposal was approved by Commissioners.

#### **ICY 14, 2016**

Prof. Hiroshi Takagi renewed to his proposal made in Bangkok and officially proposed to organize 14<sup>th</sup> International

Congress on Yeasts in Japan. This would be the second yeast meeting held in Japan under the auspices of ICY after the Specialized Symposium held in Kyoto in 1972. Prof. Takagi proposed to organize Congress in Nara, a city with a population near 700,000, where he lives and works, or in Kyoto. Both cities are located on the south of Japan and far from the destroyed nuclear reactor in Fukushima. As Japan is expensive country, Prof. Takagi promised to initiate a fund raising campaign to reduce the costs of participation in the Congress.

Prof. Peter Raspor (Slovenia) together with Prof. Vladimir Mrsa (Croatia) proposed to organize the 14<sup>th</sup> International Congress on Yeasts in Croatia through joint efforts of Slovenian and Croatian yeast researchers. In view of this new proposal, A. Sibirny suggested that the decision regarding the venue and organizer(s) of the 14<sup>th</sup> International Congress on Yeasts be made next year, during the ICY 13 meeting in Madison, Wisconsin, USA. This suggestion was accepted by Commissioners.

It was reported that Dr. Diego Libkind (Argentina) expressed the intention to organize ISSY32 in 2015 in Bariloche, Argentina, although a formal proposal has not made, as Dr. Libkind was not a member of ICY at the time of sending the proposal. A. Sibirny suggested that a Specialized Symposium be held in Argentina in 2017 or 2018, depending on competing proposals. This suggestion was approved by Commissioners.

A. Sibirny reported that during recent years (since 2008) researchers from several new countries have become the members of ICY, i.e., Bulgaria, China, Croatia, Estonia, Poland, S. Korea, and Turkey. New Commissioners were also elected representing the following countries: Austria, France, Hungary, Italy, Japan, The Netherlands, Russia and, the USA, in addition to the new members elected in this meeting, from France, India, Chile, Argentina, Brazil, and Mexico. However, several important countries are still not represented in ICY, such as Greece, Switzerland, Norway, Ireland, Lithuania, Indonesia, Chile, New Zealand, and others.

#### **Report on Yeast Newsletter**

André Lachance reported on the Yeast Newsletter. Commissioners expressed their gratitude to André for his dedication and excellent job. André took the opportunity to encourage colleagues to send information and announcements to the YNL.

#### **Other business and activities**

Prof. A. Sibirny stressed the need to review ICY statutes in compliance to IUMS Bylaws. ICY is a COMCOF (Committees, Commissions and Federations) of IUMS. This discussion was deferred to the ICY meeting to be held during 13<sup>th</sup> International Congress on Yeasts in August 2012 in Madison, Wisconsin, USA. A. Sibirny asked Peter Raspor to prepare a draft of the amended Statute and all Commissioners to send suggestions.

A. Sibirny also mentioned the forthcoming meeting: “Non-Conventional Yeasts in Postgenomic Era” to be held in few days in Lviv, Ukraine. He informed members that although there are no restrictions on the number of yeast meetings organized under the auspices of ICY, in practice,

only one meeting is organized each year. Simultaneously, there are small (delete) independent societies that have gathered researchers of particular non-conventional yeast species, e.g., *Hansenula polymorpha*, *Yarrowia lipolytica*, *Kluyveromyces lactis*, etc. A. Sibirny mentioned the possibility of congregating these small groups of researchers into one community of non-conventional yeast researchers and to organize corresponding meetings every other year. P. Raspor suggested that these small societies be invited to join ICY. However, A. Sibirny replied that it is difficult for ICY to attract these small, but independent societies, in view

of the fact that ICY is a COMCOF of the IUMS Mycological Division and not an independent society.

#### **Meeting Close**

On behalf of ICY, the Chair expressed his gratitude to Profs. Patricia Lappe Oliveras, Anne Gschaedler, and Ruben Moreno and their staff for the excellent meeting and the well-balanced scientific and cultural programs. The meeting provided an excellent opportunity to discover Mexican cuisine, beverages, and cultural traditions, which include a warm hospitality. The ICY meeting was called to a close.

Minutes prepared by:

Prof. Andrei Sibirny, ICY Chair, and Prof. Leda C. Mendonça-Hagler, ICY Vice-Chair

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## **Recent Meeting**

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### **39th Annual Conference on Yeasts of the Czech and Slovak Commission on Yeasts, Smolenice, Slovakia, May 3-6, 2011**

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The 39th Annual Conference on Yeasts, organized by the Czech and Slovak Commission on Yeasts, Institute of Chemistry, the Slovak Academy of Sciences, and the Department of Biochemical Technology, Slovak University of Technology, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, May 3-6, 2011. The time pressure felt during previous conferences caused the organizers to extend the duration of the conference, for the first time, from three to four days. Although the conference was attended mainly by scientists from Czech Republic and Slovakia, numerous participants from other countries like Austria, Greece, Hungary, Italy, Netherlands, Poland, Slovenia, Spain, and Thailand gave the conference an international character. Several foreign scientists were invited speakers, supported by the Commission or by the organization NATURA associated with the Faculty of Natural Sciences of the Comenius University.

Prof. Kenneth H. Wolfe from the Smurfit Institute of Genetics, Trinity College, Dublin, Ireland presented the lecture dedicated to memory of Dr. A. Kocková-Kratochvílová entitled “Yeast comparative genomics and the after math of polyploidization”.

The opening ceremony, attended by the representative of the Czechoslovak Society for Microbiology, Doc. Dr. Ivan Čižnár, DrSc, was dedicated to the 70<sup>th</sup> birthday of RNDr. Peter Biely, DrSc, the former long-time secretary and chair of the Czech and Slovak Commission on Yeasts and still a member of the International Commission for Yeasts. On this occasion Dr. Biely presented a lecture entitled “Metamorphosis of chemists into yeast researchers”, a story of interesting transformation of chemists from the Institute of Chemistry not just to yeast researchers but also to chairs of the national Yeast Commission. Peter Biely and Vladimír Farkaš, graduates from analytical and nuclear chemistry, chaired the Commission for ten and eight years, respectively, while another chemist, Mária Vršanská, functioned for ten years as its secretary.

The conference program consisted of four sessions dedicated to Biotechnology, Medical Mycology and Genetics and Molecular Biology, and Biochemistry and Cell Biology. Tenty-six oral presentations were complemented by 47 posters, 10 of which were selected for short 5 min presentations by young scientists (Poster Highlights). The interesting scientific program was interrupted by some relaxed moments, such as presentation of distillates and wine, and wine tasting by the companies Old Herold Distilleries, Malik & Sons, Hubert (Villa Vlna Rača), and Včelco from Smolenice.

During the Conference a minute of silence and one of the lectures in the Medical Mycology session were dedicated to the memory of Prof. MUDr. Alena Tomšíková, DrSc, a foremost Czech medical mycologist and great Czech patriot, a long year active member of the Yeast Commission, who passed away at the age of 81, shortly after the 38<sup>th</sup> Annual Conference on Yeast, which was the last one she attended.

During the meeting of the Czech and Slovak Commission on Yeasts it was decided that the 40<sup>th</sup> Annual Yeast Conference will be organized in the Smolenice Castle on May 8-11, 2012. Its program will cover again the main streams of yeast research in previous Czechoslovakia, Genetics and Molecular Biology, Cell Biology and Biochemistry, Medical Mycology and Biotechnology. Further information about the activities of the Czech and Slovak Commission for Yeasts can be found on the website [www.chem.sk/yeast](http://www.chem.sk/yeast). The titles of lectures and posters of the 39<sup>th</sup> Annual Yeast Conference are listed below:

#### **Lectures in the Biotechnology Session**

- 1 Makri A, Fakas S, Papanikolaou S, Aggelis, G (Greece): Conversion of raw substrates (glycerol) into single cell oil using *Yarrowia lipolytica*.
- 2 Pesti, M (Hungary): Some aspects of regulation of stress processes in yeast.
- 3 Hanusová V, Antalová M, Brlejšová M, Breierová E,

- Márová I, Čertík M (Slovak Republic/Czech Republic): Effect of iron on growth and metabolism of carotenogenic yeasts.
- 4 Siniša P, Márová I, Hároniková A, Kostovová I, Dvořáková T (Croatia/Czech Republic): Production of carotene-enriched biomass by red yeast strains cultivated on waste glycerol and lipids.
  - 5 Sigler K, Pichová A, Matouloková D, Gabriel P (Czech Republic): Some factors affecting beer quality.
  - 6 Bogacz-Radomska, L (Poland): Methods of automatic substrate dosing regulation in yeast cultivation.
  - 7 Kočar N, Košir I.J, Oset M, Raspor P (Slovenia): Impact of serial repitching on sugar utilisation by lager brewing yeast.

#### Lectures in the Medical Mycology Session

- 1 Invited lecture to the Memory Prof. A. Tomšíková - Piecková E (Slovakia): Health implications of indoor mycobiota and its toxins.
- 2 Zaragoza O (Spain): Role of morphological changes in the virulence of *Cryptococcus neoformans* and *Cryptococcus gattii*.
- 3 Raška M, Beláková J, Horynová M, Krupka M, Weigl E (Czech Republic): HSP90 protein during *Candida albicans* versus mammalian host interaction.
- 4 Rajkowska K, Rygała A (Poland): Characterization of *Candida* strains isolated from human faeces.

#### Lectures in the Session Genetics and Molecular Biology

- 1 Kielland-Brandt Morten C (Denmark): Gradient Sensing by transporter-like nutrient sensors.
- 2 Mániková D, Vlasáková D, Fidler K, Broznanová J, Chovanec M (Slovakia): Genomic approach in the study of toxicity of sodium selenite in *Saccharomyces cerevisiae*.
- 3 Drobná E, Šubík J (Slovak Republic): Oxidative stress resistance conferred by three genes on multicopy plasmids in *Saccharomyces cerevisiae*.
- 4 Dudáš A, Ahmad S, Gregaň J (Austria): *Sgo1* is required for co-segregation of sister chromatids during achiasmate meiosis I.
- 5 Gabaldon T (Spain): From sequences to biological insights: tracing genomic changes underlying evolutionary adaptations in yeasts.
- 6 Fričová D, Valach M, Tomáška E, Nosek J (Slovak Republic): Linear mitochondrial genomes in yeast: A paradigm for evolution of linear chromosomes and the telomeric structures.
- 7 Ondrejovičová G, Pevala V, Ambro V, Kutejová E (Slovak Republic): Lon protease and its role in mitochondrial nucleoids.
- 8 Gahura O, Puta F, Folk P (Czech Republic): Splicing of *Saccharomyces cerevisiae* introns with long branch point 3' splice site distance depends on secondary structure.

- 9 Pfliegler, W, Antunovics Z, Sipiczki M.(Hungaria): Interspecific *Saccharomyces* hybrids reveal a new way of breaking down the sterility barrier.

#### Lectures in the Session Biochemistry and Cell Biology

- 1 Jazwinski SM (USA): A systems approach to yeast aging.
- 2 Volejníková A, Hlousková J, Sigler K, Pichová A (Czech Republic): Aging-related changes in yeast mitochondrial morphology and respiration.
- 3 De Smet C, de Kroon A (Netherlands): The yeast glycerol-3-phosphate acyltransferase Sct1p as novel regulator of membrane fluidity.
- 4 Mazáň M, Ragni E, Popolo L, Farkaš V (Slovak Republic/Italy): A fluorescent assay of yeast beta-(1,3)-glucanosyltransferases.

#### List of Posters

- 1 Dzięgielewska E, Marek Adamczak, Włodzimierz Bednarski (Poland): Analysis of biosurfactant synthesis by yeast in a medium with hydrophilic or hydrophobic waste product.
- 2 Stasiewicz K, Marek Adamczak, Włodzimierz Bednarski (Poland): Synthesis of intracellular lipids by yeast cultivated in a medium supplemented with waste glycerol.
- 3 Lakatošová J, Kornélia Nemcová, Emília Breierová, Jaroslava Kaňuchová Pátková (Slovakia): The effect of appiculate yeasts on fermentation aroma of wine.
- 4 Zoltán G, Belágyi J, Fujs S, Raspor P, Kálmán N, Kőszegi B, Máté G, Emri T, Pócsi I, Pesti M (Hungary, Slovenia): Unbalanced oxidoreduction state of a respiratory deficient mutant *Schizosaccharomyces pombe*.
- 5 Zoltán G, Kőszegi B, Čertík M, Máté G, Thürmer K, Belágyi J, Pesti M (Hungary): Examination of oxidative stress sensitivity and oxido-reduction state of *Saccharomyces cerevisiae* erg6 ergosterol mutant.
- 6 Horváth E, Papp G, Gazdag Z, Belágyi J, László Hornok N.M, Vágvolgyi C, Pesti M (Hungary): Regulation of patulin-induced oxidative stress processes in *Schizosaccharomyces pombe*.
- 7 Ilková, K, Zemková, Z, Molnárová, J, Omelková, J, Vadkertiová, R.; Stratilová, E (Czech Republic/Slovakia): Polygalacturonases produced by *Geotrichum candidum* under solid state fermentation on grape pomace.
- 8 Molnárová J, Vadkertiová R, Sláviková E, Stratilová E, Ilková K (Slovakia/Czech Republic): The enzymatic activity of yeasts isolated from plant material.
- 9 Janczar-Smuga M, Pietkiewicz J.J, Górska K (Poland): Measurement of respiration activity during yeast cultivation.

- 10 Khongto B, Gajdoš P, Laoteng K, Tongta A, Hapala I, Čertík M (Thailand/Slovakia): The influence of magnesium ion on growth and lipid synthesis of *Yarrowia lipolytica*.
- 11 Kregiel D, Ambroziak W (Poland): Co-immobilization of industrial yeast strains encapsulated in foamed alginate beads for fermentation of starchy materials
- 12 Kregiel D, Rygala A, Libudzisz Z (Poland): Yeasts and *Asaia* sp. in mixed populations as spoilage of fruit-flavoured bottled waters
- 13 Kregiel D (Poland): Monitoring ATP status in cells of different yeast strains from LOCK105 Collection
- 14 Hároniková A, Dvořáková T, Kubáčková M, Jankeje K, Breierová E, Márová I (Czech Republic/Slovakia): Use of dggc and pfgc to identification of red yeast strains
- 15 Márová I, Matoušková P, Hároniková A, Pospíšilová A, Čertík M, Obruča S, Lichnová A., Duroňová K (Czech Republic/Slovakia): Use of *A. pullulans* exoenzyme complex to waste substrate processing for carotenoid production by red yeasts
- 16 Nemcová K, Breierová E (Slovakia): The occurrence and identification of yeasts and yeasts-like microorganisms on the grape during three various climatic years.
- 17 Nemcová K, Breierová E, Lakatošová J, Kaňuchová –Pátková J (Slovakia): Red yeasts and their contribution on the aroma of varietal Slovak wine.
- 18 Paulovičová E, Kertys P, Hrubíško M, Pilišiová R, Hudáková T., Karelín A.A, Tsvetkov Y.E, Nifantiev N.E (Slovakia/Russia): *Candida vulvovaginitis* in allergic patients.
- 19 Raclavský V, Rusková L, Mallátová N, Hamal P (Czech Republic): *Candida tropicalis* in clinical samples: characterization of a dominant clone.
- 20 Paulovičová L, Paulovičová E, Pericolini E, Gabrielli E, Karelín A.A, Tsvetkov Y.E, Nifantiev N.E, Vecchiarelli A (Slovakia/Italy/Russia): Immunobiological properties of pathogenic *Candida* species natural cell wall derived polysaccharides and synthetically prepared oligosaccharides – protein conjugates.
- 21 Pesti M, Horváth E, Nagy G, Turáni M, Balogh E, Papp G, Mike N, Pollák E, Gyöngyi Z, Bánfalvi G (Hungary): Patulin-induced cytological alterations and chromatin changes in fission yeast.
- 22 Makri A, Bellou S, Mastoridou M, Mystrioti P, Onjaro G, Aggelis G (Greece): Biotechnological conversion of raw glycerol into single cell oils
- 23 Palovičová V, Bardelčíková A, Obernauerová M (Slovakia): Biochemical basis for viability of *Kluyveromyces lactis* yeast lacking anionic phospholipids.
- 24 Volejníková A, Hlousková J, Sigler K, Pichová A (Czech Republic): Aging-related changes in yeast mitochondrial morphology and respiration.
- 25 Dobáková E, Laco J, Gavurníková G (Slovakia): Preparation of deletion mutant strain in SAL1 gene in yeast *Schizosaccharomyces pombe*. Its genetic and biochemical characterization.
- 26 Pfeifenberger S, Amring M, Angerer T, Hlouskova J, Brandl C, Haslinger D, Wimmer B, Bösch M, Zhou J, Wang Y, Karl T, Bauer J, Hintner H, Pichova A, Breitenbach M, Breitenbach-Koller H (Austria/Czech Republic): Modulation of longevity and differential protein expression by gene dosage effects of ribosomal proteins.
- 27 Garaiová M, Nahálková V, Slezáková Z, Hapala I (Slovakia): Lipid droplets and protection of yeast cells from lipotoxicity.
- 28 Balážfyová Z, Hodúrová Z, Tóth Hervay N, Gbelská Y (Slovakia): KLYap1p in the control of KIPDR1 gene expression.
- 29 Bičanová V, Tahotná D, Griač P (Slovakia): Copper ions regulate yeast phospholipid biosynthesis by inhibiting choline transport.
- 30 Kulková N, Černáková L, Šoltýsová A, Drahovská H, Bujdáková H (Slovakia): Expression of genes participating in adhesion during biofilm formed by *Candida albicans* and *Candida parapsilosis*.
- 31 Dobiašová Z, Hegedúsová E, Nosek J, Vlčková V (Slovakia): Functional analysis of the OGG1 gene from the yeast *Candida parapsilosis*.
- 32 Elicharová H, Sychrová H (Czech Republic): Four pathogenic *Candida* species differ in salt tolerance and resistance to azole antimycotics.
- 33 Gérecová G, Fertál'ová J, Behalová L, Bhatia I, Drobcová B, Mentel M, Polčic P (Slovakia): Proteins bik, bid, bmf and noxa activate apoptosis by inhibiting anti-apoptotic proteins bcl-xl and bcl-2.
- 34 Goffa E, Bialková A, Džugasová V, Šubík J (Slovakia): The use of heterologous CgPDR1 expression for isolation of loss-of-function pdr1 mutants.
- 35 Gregan J, Zhang L, Rumpf C, Cimini D, Tolić-Nørrelykke IM (Austria/USA/Germany): Molecular architecture and mechanical properties of the kinetochore: a biophysical approach.
- 36 Kovacikova I, Cipak L, Miadokova E, Gregan J (Slovakia/Austria): Identification of novel protein kinases required for meiosis in *Schizosaccharomyces pombe*.
- 37 Andersen R.M, Jøck-Ramberg D, Merico, A, Poláková S, Piškur J, Laurenčík M, Procházka E, Sulo P (Slovakia/Denmark/Sweden): Phylogenetic analysis within the genus *Dekkera/Brettanomyces* revealed novel yeast species *Dekkera pseudobruxelensis* sp. nov.
- 38 Valach M, Pryszcz L.P, Tomaska L, Gabaldon T, Nosek J (Slovakia/Spain): Mitochondrial genome variability within the *Candida parapsilosis* species group.

- 39 Pevala V, Fričová D, Chovanec M, Tomáška L, Nosek J, Krejčí L, Kutejová E (Slovakia): DNA-binding properties of the yeast mitochondrial protein Mgm101
- 40 Polakova S, Gregan J (Austria): molecular mechanisms of chromosome segregation during meiosis, a genome-wide approach.
- 41 Procházka E, Laurenčík M, Sulo P (Slovakia): Experimental evolution of good brewer: Nucleo-mitochondrial relationship in *Kluyveromyces-Lachancea* interspecific hybrids.
- 42 Stříbný J, Sychrová H (Czech Republic): Potassium transporter *trk1* in the osmotolerant yeast *Zygosaccharomyces rouxii*.
- 43 Kyjácová L, Nosek, J, Tomáška E (Slovakia): Functional analysis of telomerase RNA of *Candida parapsilosis*.
- 44 Višacká K, Hofr, C, Kramara, J, Sepšiová, R, Eichler, T, Fajkus, J, Nosek, J, Tomáška E (Slovakia): Biochemical analysis of the telomere-binding protein Tay1 from the yeast *Yarrowia lipolytica*.
- 45 Vránová D, Šuranská H, Omelková J, Vadkertiová R, Augustová K (Czech Republic/Slovakia): PCR-fingerprinting as a method for identification of *Saccharomyces* yeasts isolated from various Moravian musts.
- 46 Zahrádka J, Sychrová H (Czech Republic): Yeast 14-3-3 proteins regulate the activity of plasma-membrane Na<sup>+</sup>/H<sup>+</sup> antiporter.
- 47 Centárová I, Zeman I, Nosek J (Slovakia): ODC1, an oxodicarboxylate carrier from the yeast *Candida parapsilosis*.

Communicated by Peter Biely

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## Forthcoming Meetings

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### 40<sup>th</sup> Czech and Slovak Annual Conference on Yeasts

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The 40<sup>th</sup> Czech and Slovak Annual Conference on Yeasts is still being planned for 8 -11 May 2012 at Smolenice Castle, Slovakia. On-line registration will be opened in December. The information will be updated on the following website:

<http://www.chem.sk/yeast>

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## International symposium - Yeast: A Model Organism for Biomedical Research Oviedo, Spain - May 23-24, 2012

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Participants and topics:

Casadevall A, New York, USA Thoughts on the origin of virulence for pathogenic yeasts

Devin A, Bordeaux, France. The Warburg and Crabtree effects: On the origin of tumor cell energy metabolism and its common features with yeast metabolism

Giorgini F, Leicester, UK. Identifying candidate therapeutic targets for Huntington's disease in yeast

Hidalgo E, Barcelona, Spain. Regulation of gene expression programs by the transcription factor Pap1 and the stress-dependent MAP kinase Sty1 of *Schizosaccharomyces pombe*

Langer T, Cologne, Germany. Mitochondrial quality control and neurodegeneration

Madeo F, Graz, Austria. Die harder: From apoptosis to necrosis in yeast

Moreno S, Salamanca, Spain. Cell cycle exit, cell differentiation and cancer

Posas F, Spain. Control of adaptive responses to stress by SAPKs

Prado F, Sevilla, Spain. Mechanisms and cell cycle regulation of replicative DNA damage repair by homologous

recombination

Riera A, Oviedo, Spain. *Mig2* at the hub of a regulatory network connecting glucose repression signaling and mitochondrial morphology

Sanz P, Valencia, Spain. Regulation of AMP-activated protein kinase by glucose: from yeast to humans

Sesaki H, Baltimore, USA. Mitochondrial dynamics in yeast and mammals

Sole R, Barcelona, Spain. Cellular computation: lessons from synthetic biology

Westermann B, Bayreuth, Germany. Mitochondrial motility, fusion, and fission in yeast

Thiele D, North Carolina, USA. Using yeast to understand and modulate protein chaperone expression for therapeutic development in neurodegenerative disease

Zaragoza O, Madrid, Spain. Non conventional morphogenesis in *Cryptococcus* influences virulence in different types of hosts

Participation is free of charge, but a registration form will be needed. Please contact Dr. Fernando Moreno at: [fmoreno@uniovi.es](mailto:fmoreno@uniovi.es)

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## Brief News Items: Address Changes

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### Jorgen Stenderup

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I am in the process of changing employment. Please note my new email and a temporary correspondence address.

Jorgen Stenderup  
17 A Hjortholms Alle  
DK 2400 Copenhagen NV  
Denmark.

Email: <[j\\_stenderup@hotmail.com](mailto:j_stenderup@hotmail.com)>

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### Sakkie Pretorius

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I am to commence in my new role as Vice President: Research & Innovation at the University of South Australia on Thursday, the 1st of December. My new contact details are given below. I will continue to be involved with yeast research (yeast omics to be more specific).

Postal address:

Sakkie Pretorius  
University of South Australia  
Chancellery, GPO Box 2471  
Adelaide, South Australia 5001

Phone: 8302 0060

Location:

Chancellery  
Hawke Building  
Level 4, 55 North Terrace  
Adelaide South Australia 5000

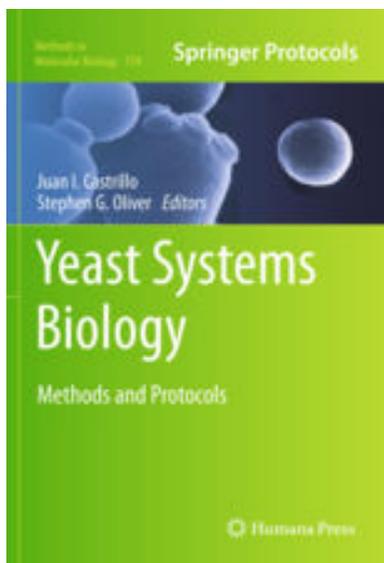
Email : <[Sakkie.Pretorius@unisa.edu.au](mailto:Sakkie.Pretorius@unisa.edu.au)>

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## Publications of Interest

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Castrillo JI, Oliver SG (Eds.) 2011 **Yeast Systems Biology: Methods and Protocols**. Series: Methods in Molecular Biology, Vol. 759. 1st Edition, XIV, 535 pp. A product of Humana Press (Springer). Hardcover - ISBN 978-1-61779-172-7.



Systems Biology aims at deciphering the genotype-phenotype relationships at the levels of genes, transcripts (RNAs), peptides, proteins, metabolites, and environmental factors participating in complex cellular networks in order to reveal the mechanisms and principles governing the behavior of complex biological systems. *Yeast Systems Biology: Methods and Protocols* presents an up-to-date view of the optimal characteristics of the yeast *Saccharomyces cerevisiae* as a model eukaryote, perspective on the latest experimental and computational techniques for systems biology studies, most of which were first designed for and validated in yeast, and selected examples of yeast systems biology studies and their applications in biotechnology and medicine. These experiments under controlled conditions can uncover the complexity and interplay of biological networks with their dynamics, basic principles of internal organization, and balanced orchestrated functions between organelles in direct interaction with the environment as well as the characterization of short and long-term effects of perturbations and dysregulation of networks that may illuminate the origin of complex human diseases. Written for the highly successful *Methods in Molecular Biology*<sup>TM</sup> series, this volume contains the kind of detailed description and implementation advice that is crucial for getting optimal results. Practical and cutting-edge, *Yeast Systems Biology: Methods and Protocols* serves researchers interested in comprehensive systems biology strategies in well-defined model systems with specific objectives as well as a better knowledge of the latest post-genomic strategies at all 'omic levels and computational approaches towards analysis, integration, and modeling of biological systems, from single-celled organisms to higher eukaryotes.

<http://www.springer.com/life+sciences/microbiology/book/978-1-61779-172-7>

<http://www.springerlink.com/content/978-1-61779-173-4>

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## The Yeasts: A Taxonomic Study, 5th Edition

Fully revised, updated and offered in a new three-volume format, *The Yeasts: A Taxonomic Study*, 5th Edition remains the most comprehensive presentation of yeast taxonomy and systematics available. Nearly 1500 species of ascomycete and basidiomycete yeasts are included, each description offering not only standard morphological and physiological characters, but also information on systematics, habitat, ecology, agricultural and biotechnological applications and clinical importance.

Extensive introductory chapters discuss clinical aspects of yeasts, their role in biotechnology, food and beverage spoilage, agriculture and ecology, while other chapters include methodology for isolation of species from various habitats, phenotypic characterization, chemotaxonomy, gene sequence analysis and phylogenetics, including whole genome analysis.

Additionally, easy-to-understand trees illustrate the phylogenetic placement of each species in its assigned genus as they have been determined from gene sequence analysis. This essential work, prepared by the leading experts in the field, is the most definitive treatment of taxonomy and systematics of yeasts on the market, and a necessary reference for any bookshelf or workbench.

- High-quality photomicrographs and line drawings
- Detailed phylogenetic trees
- Up-to-date, clearly presented yeast taxonomy and systematic, easy-to-use reference sequence accession numbers to allow for correct identification

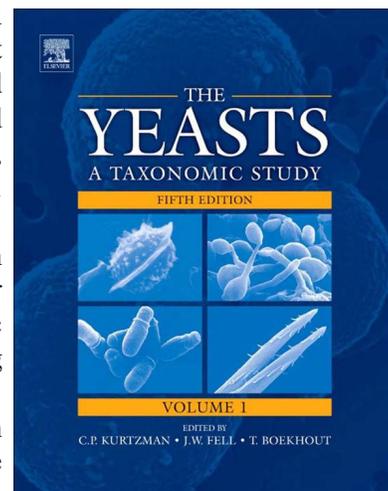
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### 50 Years Ago

#### Y E A S T

### A News Letter for Persons Interested in Yeast November 1961, Volume X, Number 2

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Dr. **Santa Maria**, Instituto Nacional de Investigaciones Agronomicas, Madrid, writes:

“We have isolated a sporogenous culture, LeAc218A-1 adenine-deficient which produced a pigment, that changes from red to brown-red, depending on the medium, and which does not diffuse in the agar.”

Dr. **Phaff**, University of California. Davis, writes:

“Our project dealing with the yeasts found in the crops of the various species of *Drosophila*, occurring in the Sacramento Valley and in the surrounding hills, is now completed.”

“It has been observed that some species or strains of *Sporobolomyces* and of *Bullera* do not discharge or form ballistospores on malt agar and similar rich media, but will do so on corn meal agar.”

Dr. **Lindregren**, Southern Illinois University, writes:

[Recent publication]

Lindregren, C. C.. A hypothesis concerning the mechanism of gene-action. *Nature* 189: 959 (1961).

Dr. **Mackenzie**, Queen's University of Belfast, writes:

“Formation of “germ tubes” identical to those produced in vivo [by Candida albicans] can be induced in the presence of 5-30% human, bovine, rabbit and guinea pig serum.”

Dr. **Siepmann**, University of Bonn, West Germany, writes:

“It was necessary to observe the sugar assimilation tests (done on a shaker according to the method of Ahearn et al.) for at least 140 days. Yeasts which contained adaptive enzymes (e.g. lactase in Deb. Subglobovirus) started to grow often after a long starvation period (up to 17 weeks) before utilizing the carbon source.”

Dr. **Kobayashi**, National Science Museum, Tokyo, writes:

“I am now engaged in monographic studies on the genera Endomyces and Endomycopsis. These are very difficult genera, because of the fact that so many species have been imperfectly described without details of asci and physiological characters.”

Dr. **di Menna**, Soil Bureau Experiment Station, New Zealand, writes:

“I was able to make very pleasant visits to a number of yeast workers in Europe and the United States. Beginning in Copenhagen, I saw Dr. C. Roberts, formerly of the Carlsberg Laboratories, and Dr. A. Lund of the Tuborg Breweries, and then went to Delft where I spent a day with Miss W. Sloof of the Centraalbureau voor Schimmelcultures. In England I met Dr. J. Barnett of the Low Temperature Research Station in Cambridge, and in the United States Dr. S. Hunter of the Haskins Laboratories, New York; Dr. M. Silva of the Dermatology Department, College of Physicians and Surgeons, Columbia University; Professor S. P. Meyer, Dr. F. Roth, Miss S. A. Meyers and Messrs. D. Ahearne [*original spellings*] and J. Fell, all of the University of Miami; Dr. L. J. Wickerham of the Northern Utilization Research Branch, Dept. of Agriculture, Peoria, and Professor H. J. Phaff and Dr. M. Miller of the University of California. Also at Davis I was pleased to meet Dr. L. do Carmo Sousa of the Botanical Institute at Lisbon, a laboratory which I had not had the opportunity of visiting. After Davis I leave for Hawaii where I want to collect some soil samples.”

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