

# Yeast

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Marc-André Lachance, Editor  
University of Western Ontario, London, Ontario, Canada N6A 5B7  
<[lachance@uwo.ca](mailto:lachance@uwo.ca)>

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

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#### Associate Editors

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

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

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

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## Editorial

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### Complete Archive of Yeast Newsletter Back Issues Available

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Thanks to Kyria Boundy-Mills, readers can now have access to all back issues of the Yeast Newsletter as PDF scans. The archive is available at the following link:

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

Addition of these large files made it necessary to move the YNL web site to a new server. Readers are therefore urged to update links on their own web pages the new URL:

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

I take this opportunity to thank the University of Western Ontario for making this server space available free of charge. The university has also, over the years, been most forthcoming in the matter of accounting, for which I am very grateful.

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### ICY 13 - Madison, Wisconsin, USA

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The 13<sup>th</sup> International Congress on Yeasts, held in Madison, Wisconsin, last August, was a resounding success. Members of the US Organizing Committee, namely Clete Kurtzman, Charles Abbas, Tom Jeffries, Kyria Boundy-Mills, and Jan Fassler, are to be congratulated, as are Patti Thompson and Deborah Curry who took care of local logistics. The excellent scientific program was well complemented by a superb venue on the shore of Lake Monona and the many culinary delights provided. Others who greatly contributed to this success include the staff at the Monona Terrace, as well as Martin Price, George Hornik, Tanya Long, Ellyn Lepinski, Houa Vang, Khaled Ali, and Chris Calvey.

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

M.A. Lachance, Editor

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

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Recent publications.

- 1 Farakas Z, Márki-zay J, Kucsera J, Vágvölgyi Cs, Golubev WI & Pfeiffer I 2012 Characterization of two different toxins of *Wickerhamomyces anomalus* (*Pichia anomala*) VKM Y-159. *Acta Biologica Hungarica* 63:277-287.

*Wickerhamomyces anomalus* VKM Y-159 strain produces two types of toxin designated as WAKT a and WAKT b, encoded by chromosomal genes. The WAKT a toxin is heat-labile, pronase sensitive acting in pH range 3–4 affecting on several yeasts including pathogenic *Candida*

species while the WAKT b toxin is protease- and thermo-resistant, acting in pH range 3–7 on two species, *Candida alai* and *Candida norvegica*. The rapid decrease of the number of viable cells after toxin treatment demonstrates that both toxins have cytotoxic effect.

- 2 Golubev WI & Tomashevskaya MA 2012 Mycocin sensitivity patterns of *Kluyveromyces* species: *Kluyveromyces* sensu lato vs. *Kluyveromyces* sensu stricto. *Biol Bull* 39:481-484.

The *Kluyveromyces* species reassigned to the genera *Lachancea* and *Vanderwaltozyma* are insensitive to five mycocins secreted by *Pichia membranifaciens*. The remaining *Kluyveromyces* species including species

transferred to the genera *Kazachstania*, *Nakaseomyces*, and *Tetrapisispora* are sensitive to them. Only the neotype strain is insensitive to mycocins among *Kluyveromyces lactis* cultures.

- 3 Golubev WI 2012 Distribution of the maximum temperature for growth among *Rhodotorula mucilaginosa* strains. *Problems Medical Mycol* 14:77.

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**II Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic. Communicated by Marie Kopecká.**  
<[mkopecka@med.muni.cz](mailto:mkopecka@med.muni.cz)> <http://www.med.muni.cz/~mkopecka/>

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Papers published in journals in 2012.

- 1 Marie Kopecká, Wladyslav Golubev, Vladimíra Ramíková, Dobromila Klemová & Ladislav Ilkovic 2012 [Ultrastructural characteristics and variability of vegetative reproduction in \*Fellomyces penicillatus\*](#). *J Basic Microbiol* 52:531-538. DOI:[10.1002/jobm.201100405](https://doi.org/10.1002/jobm.201100405) [PDF].

The yeast strains VKM Y-2977 and VKM Y-2978, derived from the isolate Pa-202, were examined for their physiological properties and mycocin sensitivities and studied by light, phase-contrast, fluorescence, transmission and scanning electron microscopy. The cells of the first strain produced long stalk-like conidiophores, whereas the cells of the second one had the appearance of typical budding yeast under the light microscope. Transmission and scanning electron microscopy showed the formation of stalk-like conidiophores and long necks in VKM Y-2977, similar in appearance to *Fellomyces fuzhouensis*. The actin cytoskeleton, microtubules and nuclei were similar as well,

but due to presence of a capsule, they were not clearly visible. The second isolate, VKM Y-2978, had very short stalk-like conidiophores, and the neck, microtubules and actin cables were shorter as well. The actin patches, actin cables, and microtubules were similar in VKM Y-2977 and VKM Y-2978 and not clearly visible. The physiological characteristics and mycocin sensitivity patterns, together with the microscopic structures and ultrastructures, led us to conclude that both strains belong to *Fellomyces penicillatus*, even though they differ in the lengths of their stalk-like conidiophores and necks.

- 2 Marie Kopecká, Soichi Yoshida & Masashi Yamaguchi 2012 [Actin ring formation around the cell nucleus of long-neck yeast](#). *J Electron Microsc* (Tokyo) 61:249-55. DOI: [10.1093/jmicro/dfs049](https://doi.org/10.1093/jmicro/dfs049) [PDF].

The unique long-neck yeast *Fellomyces fuzhouensis* has F-actin cables and cortical patches. Here, we describe a new F-actin structure present in fungi, a perinuclear F-actin

collar ring around the cell nucleus. This F-actin structure can be visualized by fluorescent microscopic imaging of rhodamine-phalloidin-stained F-actin in cells treated with

the mitotic drug isopropyl N-(3-chlorophenyl) carbamate or the microtubule inhibitor thiabendazol or when cells were grown in cut dried radish medium or yeast extract peptone

dextrose (YEPD) medium. In contrast, these structures were absent in cells treated with Latrunculin A. The hypothetical functions of the F-actin ring are discussed.

- 3 Marie Kopecká, Susumu Kawamoto & Masashi Yamaguchi 2012 [A new F-actin structure in fungi: actin ring formation around the cell nucleus of \*Cryptococcus neoformans\*](#). J Electron Microsc (Tokyo). DOI: [10.1093/jmicro/dfs074](#) [PDF]; [Epub ahead of print].
- 4 Marie Kopecká 2012 [Yeast and fungal cell-wall polysaccharides can self-assemble in vitro into an ultrastructure resembling in vivo yeast cell walls](#). J Electron Microsc (Tokyo). DOI: [10.1093/jmicro/dfs076](#) [PDF] [Epub ahead of print].

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**III State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <[gnaumov@yahoo.com](mailto:gnaumov@yahoo.com)>.**

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We are grateful to Dr. Feng-Yan Bai (Institute of Microbiology, Chinese Academy of Sciences, Beijing) and Dr. Ching-Fu Lee (National Hsinchu University, Taiwan) for visiting their labs in October-November 2012.

The following are papers for 2012 or in press.

- 1 Naumov GI, Lee CF & Naumova ES 2012 Molecular genetic diversity of the *Saccharomyces* yeasts in Taiwan: *S. arboricola*, *S. cerevisiae* and *S. kudriavzevii*. Antonie van Leeuwenhoek (online 1 Sept. 2012).

Genetic hybridization, sequence and karyotypic analyses of natural *Saccharomyces* yeasts isolated in different regions of Taiwan revealed three biological species: *S. arboricola*, *S. cerevisiae* and *S. kudriavzevii*. Intraspecific variability of the D1/D2 and ITS1 rDNA sequences was detected among *S. cerevisiae* and *S. kudriavzevii* isolates. According to molecular and genetic

analyses, the cosmopolitan species *S. cerevisiae* and *S. kudriavzevii* contain local divergent populations in Taiwan, Malaysia and Japan. Six of the seven known *Saccharomyces* species are documented in East Asia: *S. arboricola*, *S. bayanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, and *S. paradoxus*.

- 2 Naumov GI, Naumova ES, Kondratieva VI, Chen GY & Lee CF 2012 Killer activity of *Williopsis Zender* yeasts: study of Taiwanese populations. Mikologiya i Fitopatologiya. 46 (4):264-268 (in Russian).
- 3 Naumov GI, Naumova ES, Martynenko NN & Korhola M 2013 Reidentification of chromosomal *CUP1* translocations in wine yeasts *Saccharomyces cerevisiae*. Microbiology (Moscow). 82 (1) (in press).
- 4 Naumova ES, Sadykova AZh, Martynenko NN & Naumov GI 2013 Molecular and genetic characterization of distillers' yeasts *Saccharomyces cerevisiae*. Microbiology (Moscow). 82 (2) (in press).
- 5 Naumova ES, Dmytruk KV, Kshanovska BV, Sibirny AA & Naumov GI 2013 Molecular identification of industrially important strain of *Ogataea parapolymorpha*. Microbiology (Moscow). 82 (3) (in press).
- 6 Naumov GI 2013 Ecological and biogeographical peculiarities of the yeasts *Saccharomyces paradoxus* Batschinskaya and related species: earlier days research. Microbiology (Moscow). 82 (in press).

- 7 Naumova ES, Michailova YuV & Naumov GI Sympatric speciation in *Saccharomyces* yeasts: *S. cariocanus* and *S. paradoxus*. 13th International Congress on Yeasts, 26-30 August 2012, Madison, WI, USA, p 9.
- 8 Naumov GI 2012 Genetic species and genus concepts of yeast organisms. 13th International Congress on Yeasts, 26-30 August 2012, Madison, WI, USA, p 202.
- 9 Naumov GI, Martynenko NN & Naumova ES 2012 Chromosomal polymorphism of *SUC* genes for  $\beta$ -fructoside fermentation in *Saccharomyces cerevisiae*. 13th International Congress on Yeasts, 26-30 August 2012, Madison, WI, USA, p 237.

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**IV Department of Microbial, Biochemical & Food Biotechnology, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa - <http://www.ufs.ac.za/biotech>. Communicated by James du Preez <[dpreezjc@ufs.ac.za](mailto:dpreezjc@ufs.ac.za)>.**

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Recent publications.

- 1 Swart CW, Ditsebe K, Pohl CH, Swart HC, Coetsee E, Van Wyk PWJ, Swarts JC, Lodolo EJ & Kock JLF 2012 Gas bubble formation in the cytoplasm of a fermenting yeast. *FEMS Yeast Research* 12:867–869.

Current paradigms assume that gas bubbles cannot be formed within yeasts although these workhorses of the baking and brewing industries vigorously produce and release CO<sub>2</sub> gas. We show that yeasts produce gas bubbles that fill a significant part of the cell. The missing link between intracellular CO<sub>2</sub> production by glycolysis and eventual CO<sub>2</sub> release from cells has therefore been resolved.

Yeasts may serve as model to study CO<sub>2</sub> behavior under pressurized conditions that may impact on fermentation biotechnology (This discovery, utilizing Auger nanotechnology, visualizes cellular gas exchange which is a basic phenomenon in biology. This may serve as a model to all prokaryotic and eukaryotic cell types).

- 2 Swart CW, Olivier AP, Ditsebe K, Pohl CH, Van Wyk PWJ, Swart HC, Coetsee E & Kock JLF 2012 Yeast sensors for novel drugs: chloroquine and others revealed. *Sensors* 12:13058-13074.

In this study the mitochondrion is regarded as a target to reveal compounds that may be used to combat various diseases. Consequently, the sexual structures of yeasts (with high mitochondrial activity) were identified as sensors to screen for various anti-mitochondrial drugs that may be toxic to humans and that are directed, amongst others, against fungal diseases and cancer. Strikingly, these sensors indicated that chloroquine is a potent pro-mitochondrial drug which stimulated yeast sexual reproduction. In

addition, these sensors also showed that some Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), anti-malarial drugs, antifungal and anticancer drugs are anti-mitochondrial. These yeast sensor bio-assays may fast track studies aimed at discovering new drugs as well as their mechanisms and should now be further evaluated for selectivity towards anti- / pro-mitochondrials, fertility drugs and contraceptives, using in vitro, in vivo, in silico and omics research.

- 3 Motaung TE, Albertyn J, Kock JLF & Pohl CH 2012 *Cryptococcus cyanovorans* sp. nov. a basidiomycetous yeast isolated from cyanide contaminated soil. *Int J Syst Evol Microbiol* 62:1209-1215.

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 found to be identical and represent a novel species. Phylogenetic analysis based on the combined dataset 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 were 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% (w/v) NaCl and 5% (w/v) 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 novel species for which the name *Cryptococcus cyanovorans* sp. nov. is given (type strain CBS 11948<sup>T</sup> (=NRRL Y-48730<sup>T</sup>).

- 4 Thibane VS, Ells R, Hugo A, Albertyn J, Janse van Rensburg WJ, Van Wyk PWJ, Kock JLF & Pohl CH 2012 Polyunsaturated fatty acids cause apoptosis in *C. albicans* and *C. dubliniensis* biofilms. *Biochimica et Biophysica Acta - General Subjects* 1820:1463-14683.

Background: Polyunsaturated fatty acids (PUFAs) have antifungal properties, but the mode by which they induce their action is not always clear. The aim of the study was to investigate apoptosis as a mode of action of antifungal PUFAs (stearidonic acid, eicosapentaenoic acid and docosapentaenoic acid) which are inhibitory towards biofilm formation of *C. albicans* and *C. dubliniensis*. Methods: *Candida* biofilms were grown in the absence or presence of 1 mM PUFAs (linoleic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid) for 48 h at 37 °C. The effect of these PUFAs on the membrane fatty acid profile and unsaturation index, oxidative stress, mitochondrial transmembrane potential and apoptosis was evaluated. Results: When biofilms of *C. albicans* and *C. dubliniensis* were exposed to certain PUFAs there was an

increase in unsaturation index of the cellular membranes and accumulation of intracellular reactive oxygen species (ROS). This resulted in apoptosis, evidenced by reduced mitochondrial membrane potential and nuclear condensation and fragmentation. The most effective PUFA was stearidonic acid. Conclusions: The resultant cell death of both *C. albicans* and *C. dubliniensis* is due to apoptosis. General significance: Due to the increase in drug resistance, alternative antifungal drugs are needed. A group of natural antifungal compounds is PUFAs. However, understanding their mechanisms of action is important for further use and development of these compounds as antifungal drugs. This paper provides insight into a possible mode of action of antifungal PUFAs.

- 5 Ells R, Kock JLF, Albertyn J & Pohl CH 2012 Arachidonic acid metabolites in pathogenic yeasts. *Lipids in Health and Disease* 11:100. DOI: 10.1186/1476-511X-11-100.

Although most of what is known about the biology and function of arachidonic acid metabolites comes from the study of mammalian biology, these compounds can also be produced by lower eukaryotes, including yeasts and other fungi. It is also in this group of organisms that the least is known about the metabolic pathways leading to the production of these compounds as well as the functions of these compounds in the biology of fungi and yeasts. This review will deal with the discovery of oxylipins from

polyunsaturated fatty acids, and more specifically the arachidonic acid derived eicosanoids, such as 3-hydroxy eicosatetraenoic acid, prostaglandin F2 $\alpha$  and prostaglandin E2, in yeasts starting in the early 1990s. This review also focuses on what is known about the metabolic pathways and/or proteins involved in the production of these compounds in pathogenic yeasts. The possible roles of these compounds in the biology, including the pathology, of these organisms is discussed.

- 6 Motaung TE, Albertyn J, Kock JLF, Lee C-F, Suh S-O, Blackwell M & Pohl CH 2012 *Trichosporon vanderwaltii* sp. nov., an asexual basidiomycetous yeast isolated from soil and beetles. *Antonie van Leeuwenhoek*. DOI: 10.1007/s10482-012-9811-2. In press

During a survey of unidentified yeast isolates deposited in the UNESCO-MIRCEN Biotechnological Yeast Culture Collection housed at the Department of Microbial, Biochemical and Food Biotechnology of the University of the Free State, one isolate obtained from soil in South Africa showed 100% identity in D1/D2 rDNA sequence with undescribed basidiomycetous yeasts isolated from the gut of beetles from the United States of America and forest soil from Taiwan in the NCBI sequence database. Phylogenetic analyses using sequences of the D1/D2 rDNA and ITS regions indicated that all these isolates form a well-

supported sub-clade that is the sister clade to the Brassicae plus Porosum clades of *Trichosporon* in the order Trichosporonales. Subsequent phenotypic tests revealed that asexual reproduction by budding is rare but dominated by arthroconidia resulting from segmentation of hyphae and that fusiform giant cells are characterized by budding from a broad base. These findings further suggest that these isolates belong to a single tremellomycetous yeast species for which the name *Trichosporon vanderwaltii* CBS 12124<sup>T</sup> (=NRRL Y-48732<sup>T</sup>, =UOFS Y-1920<sup>T</sup>) is proposed.

- 7 Ezekiel OO, Aworh OC, du Preez JC & Steyn L 2012 Cultivation of *Candida utilis* on cassava peel hydrolysates for single-cell protein production. *J Food Sci Engin* 2:452-461.

The growth of *Candida utilis* NRRL Y-1084 in acid and enzymatic hydrolysates of cassava peel and on glucose in a mineral salts medium was investigated in aerobic submerged cultivation. Kinetic and stoichiometric parameters for growth were determined. The cardinal

temperatures of this yeast strain were 14 °C, 33 °C and 41 °C. *C. utilis* exhibited no absolute requirement for growth factors, although its maximum specific growth rate ( $\mu_{max}$ ) was higher in the mineral salts medium with yeast extract than without, but its biomass yield coefficient ( $Y_{x/s}$ )

did not differ much in these two media. In the enzymatic hydrolysate, its  $Y_{x/s}$  value on sugar was 0.44 with a  $\mu_{max}$  of  $0.35 \text{ h}^{-1}$ , whereas the corresponding values were 0.52 and  $0.48 \text{ h}^{-1}$  in the acid hydrolysate and 0.50 and  $0.37 \text{ h}^{-1}$  in the mineral salts medium without yeast extract. The crude protein content of biomass grown in the glucose medium

and the acid and enzymatic hydrolysates were 47.5%, 49.1% and 56.7%, respectively. The amino acid profile of the yeast biomass compared favourably with the FAO standard. Cassava peel hydrolysate has potential as a cheap carbohydrate feedstock for the production of yeast single cell protein by using *C. utilis*.

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**VI Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Wolczanska 171/173, 90-924 Lodz, Poland. Communicated by Dorota Kregiel <[dorota.kregiel@p.lodz.pl](mailto:dorota.kregiel@p.lodz.pl)>.**

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The following posters were presented recently.

- 1 Kregiel D 2012 Volatile profiles of different yeast strains cultivated on apple pomace. COST Action FA0907 BIOFLAVOUR , 3<sup>rd</sup> European Yeast Flavour Workshop, Vevey, Switzerland.
- 2 Ambroziak W 2012 The role of yeast in aroma formation from its non-volatile flavourless glycoside precursors. COST Action FA0907 BIOFLAVOUR , 3<sup>rd</sup> European Yeast Flavour Workshop, Vevey, Switzerland.
- 3 Berłowska J, Kregiel D & Dziugan P 2012 Monitoring vitality and ATP status in yeast cells in malt broth supplemented by thick beet juice. IBS2012 15<sup>th</sup> International Biotechnology Symposium and Exhibition, Daegu, Korea.
- 4 Dziugan P, Berłowska J & Kregiel D 2012 The thick juice from sugar beet as valuable source in yeast fermentation processes. IBS2012 15<sup>th</sup> International Biotechnology Symposium and Exhibition, Daegu, Korea.

The following papers were published in journals.

- 5 Kregiel D, Berłowska J & Szubzda B - Novel permittivity test for determination of yeast surface charge and flocculation abilities. J Indust Microbiol Biotechnol DOI: 10.1007/s10295-012-1193-y.
- 6 Kręgiel D, Berłowska J & Ambroziak W 2012 Adhesion of yeast cells to different porous supports, stability of cell-carrier systems and formation of volatile by-products. World J Microbiol Biotechnol 28:3399-3408. DOI: 10.1007/s11274-012-1151-x.
- 7 Kunicka-Styczyńska A & Rajkowska K 2012 Phenotypic and genotypic diversity of wine yeasts used for acidic musts. World J Microbiol Biotechnol 28:1929-1940. DOI: 10.1007/s11274-011-0994-x
- 8 Kunicka-Styczyńska A & Rajkowska K 2012 Fermentative stability of wine yeasts *Saccharomyces sensu stricto* complex and their hybrids. Food Technol Biotechnol 50:222-229.
- 9 Kunicka-Styczyńska A & Rajkowska K 2012 Fermentative diversity of yeast selected for acidic musts fermentation. African J Microbiol Res 6:3768-3773. DOI: 10.5897/AJMR12.355
- 10 Rajkowska K, Kunicka-Styczyńska A & Rygała A 2012 Probiotic action of *Saccharomyces cerevisiae* var. *boulardii* against human pathogens. Food Technol Biotechnol 50:230-236.
- 11 Rajkowska K 2012 Application of conventional and molecular methods to identify microorganisms of natural environment. Corrosion Protection 9s/A:233-238.

Recent publications.

- 1 Jouhten P, Wiebe M & Penttilä M 2012 Dynamic flux balance analysis of the metabolism of *Saccharomyces cerevisiae* during the shift from fully respirative or respirofermentative metabolic states to anaerobiosis. FEBS Journal 279:3338–3354. doi: 10.1111/j.1742-4658.2012.08649.x

Dynamic flux balance analysis was utilized to simulate the metabolic behaviour of initially fully respirative and respirofermentative steady-state cultures of *Saccharomyces cerevisiae* during sudden oxygen depletion. The hybrid model for the dynamic flux balance analysis included a stoichiometric genome-scale metabolic model as a static part and dynamic equations for the uptake of glucose and the cessation of respirative metabolism. The yeast consensus genome-scale metabolic model [Herrgård MJ *et al.* (2008) *Nat Biotechnol* 26, 1155–1160; Dobson PD *et al.* (2010) *BMC Syst Biol* 4, 145] was refined with respect to oxygen-dependent energy metabolism and further modified to reflect *S. cerevisiae* anabolism in the absence of

oxygen. Dynamic flux balance analysis captured well the essential features of the dynamic metabolic behaviour of *S. cerevisiae* during adaptation to anaerobiosis. Modelling and simulation enabled the identification of short time-scale flux distribution dynamics under the transition to anaerobic metabolism, during which the specific growth rate was reduced, as well as longer time-scale process dynamics when the specific growth rate recovered. Expression of the metabolic genes was set into the context of the identified dynamics. Metabolic gene expression responses associated with the specific growth rate and with the cessation of respirative metabolism were distinguished.

- 2 Toivari M, Vehkomäki ML, Nygård Y, Penttilä M, Ruohonen L & Wiebe MG 2012 Development of processes for the conversion of xylose to xylitol using high formic acid containing hydrolysates. BIO Pacific Rim Summit on Industrial Biotechnology and Bioenergy 2012, Vancouver, Canada, October 9-12, 2012.

The five carbon polyol xylitol, derived from D-xylose, is currently used primarily as a sweetener, particularly in products which affect dental hygiene such as chewing gum and pastilles. Other applications (e.g. in copolymers) for xylitol are under development. Xylitol is a natural product, being found in some plants and as a by-product of D-xylose metabolism in many microorganisms. Currently xylitol is produced chemically by hydrogenation of pure D-xylose, but environmentally friendly, biotechnological production routes are considered desirable and various studies have considered the potential yields and production rates which could be attained, particularly using various yeast. Since D-xylose is derived from lignocellulosic biomass, a process for efficient xylitol conversion directly from biomass hydrolysates would be suitable for incorporation into a biorefinery in which six carbon sugars and lignin would be used for other

applications. To be economically viable, bio-production requires robust strains with high xylitol yields and production rates in these hydrolysates. Most investigations of xylitol production from plant biomass hydrolysates have focussed on the use of hydrolysates obtained by steam explosion or acid pre-treatments. Both 5 and 6 carbon sugars remain in the pre-treated material and further treatment is required to separate them. In contrast, organosolv pre-treatments, such as that developed at CIMV, France, generate C5-enriched hydrolysate fractions directly during the process. Formic and acetic acid concentrations in such fractions may be considerably higher than from other pre-treatment methods. Here we explore the possibilities of using high formic acid C5 hydrolysates in the production of xylitol and demonstrate that xylitol concentrations over 100 g l<sup>-1</sup> can be achieved with minimal detoxification of the hydrolysate.

- 3 Toivari MH, Nygård Y, Penttilä M, Ruohonen L & Wiebe MG 2012 Microbial D-xylonate production. *Appl Microbiol Biotechnol* 96(1):1-8.

D-Xylonic acid is a versatile platform chemical with reported applications as complexing agent or chelator, in dispersal of concrete, and as a precursor for compounds such as co-polyamides, polyesters, hydrogels and 1,2,4-butanetriol. With increasing glucose prices, D-xylonic acid may provide a cheap, non-food derived alternative for gluconic acid, which is widely used (about 80 kton/year) in pharmaceuticals, food products, solvents, adhesives, dyes,

paints and polishes. Large-scale production has not been developed, reflecting the current limited market for D-xylonate. D-Xylonic acid occurs naturally, being formed in the first step of oxidative metabolism of D-xylose by some archaea and bacteria via the action of D-xylose or D-glucose dehydrogenases. High extracellular concentrations of D-xylonate have been reported for various bacteria, in particular *Gluconobacter oxydans* and

*Pseudomonas putida*. High yields of D-xylonate from D-xylose make *G. oxydans* an attractive choice for biotechnical production. *G. oxydans* is able to produce D-xylonate directly from plant biomass hydrolysates, but rates and yields are reduced because of sensitivity to hydrolysate inhibitors. Recently, D-xylonate has been produced by the genetically modified bacterium *Escherichia coli* and yeast *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Expression of NAD(+)-dependent

D-xylose dehydrogenase of *Caulobacter crescentus* in either *E. coli* or in a robust, hydrolysate-tolerant, industrial *Saccharomyces cerevisiae* strain has resulted in D-xylonate titres, which are comparable to those seen with *G. oxydans*, at a volumetric rate approximately 30% of that observed with *G. oxydans*. With further development, genetically modified microbes may soon provide an alternative for production of D-xylonate at industrial scale.

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**VIII Yeast Molecular Genetics Laboratory, Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev str., 1113 Sofia, Bulgaria. Communicated by G. Miloshev <[miloshev@bio21.bas.bg](mailto:miloshev@bio21.bas.bg)>, <http://www.chromatinepigenetics.com>.**

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The following are abstracts of recently published papers and summaries of current projects of the group.

- 1 Miloshev G & Georgieva M 2011 The linker histone and chromatin of yeast *Saccharomyces cerevisiae*. In: Histones: Class, Structure and Function. Chang-hui Shen (Ed). Nova Science Publishers Inc; ISBN-13: 978-1621002741.

The advantages of using *Saccharomyces cerevisiae* as a model for chromatin studies imply its relatively small number of genes, about 6000, which enables uncomplicated genome-wide study. On the other hand, its short cell cycle (around two hours for genome duplication) is another benefit for research. It has been shown that *S. cerevisiae* cells possess about 28% homology of genes with human. Interestingly 20% of genes known to take part in the development and progression of certain human diseases have their homologs in yeast cells. Furthermore, yeast genome possesses a full complement of histones and other chromatin proteins which express vast homology with the respective proteins in human cells. These characteristics of *S. cerevisiae* allow obtained results on them to be easily approximated for human cells. Chromatin has always been a matter of extensive scientific research. A good part of our knowledge on its structure and function has been gathered

by experimenting on *S. cerevisiae*. Strangely enough, among the plethora of proteins which build up yeast chromatin the linker histone catches a specific attention with its odd features. *S. cerevisiae* linker histone, Hho1p, is the only histone known to possess two globular domains instead of one. Previous hypotheses suggested that it is responsible for the organization of yeast chromatin mostly in euchromatin. Nonetheless, recent data, including ours, have shown that Hho1p functions in yeast chromatin are far more diverse. It has been proved to be involved in the building and maintenance of the higher-order chromatin organization in yeast, from the 30 nm fiber, chromatin loop organization up to further levels of chromatin compaction. Altogether these data brings this histone protein closer to linker histones of higher eukaryotic cells and points out that linker histones and higher-order chromatin structures are more evolutionary conserved than it is generally believed.

- 2 Staneva D, Peycheva E, Georgieva M, Efremov T & Miloshev G 2012 Application of comet assay for the assessment of DNA damage caused by chemical genotoxins in the dairy yeast *Kluyveromyces lactis*. Antonie Van Leeuwenhoek. [Epub ahead of print]. DOI 10.1007/s10482-012-9793-0.

*Kluyveromyces lactis*, also known as dairy yeast, has numerous applications in scientific research and practice. It has been approved as a GRAS (Generally Recognized As Safe) organism, a probiotic, a biotechnological producer of important enzymes at industrial scale and a bioremediator of waste water from the dairy industry. Despite these important practical applications the sensitivity of this organism to genotoxic substances has not yet been assessed. In order to evaluate the response of *K. lactis* cells to genotoxic agents we have applied several compounds with well-known cyto- and genotoxic activity. The method of comet assay (CA) widely used for the assessment of DNA damages is presented here with new special modifications

appropriate for *K. lactis* cells. The comparison of the response of *K. lactis* to genotoxins with that of *Saccharomyces cerevisiae* showed that both yeasts, although considered close relatives, exhibit species-specific sensitivity toward the genotoxins examined.

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- 3 Georgieva M, Uzunova K, Staneva D & Miloshev G 2012 Higher-order chromatin structure as an epigenetic regulator of the processes of cellular aging. 9<sup>th</sup> IMYA, Rome, Italy, 16-20 Sept, 2012.

The processes of aging are of major interest nowadays especially when searching deeper at cellular and molecular level. Different model systems are used in order to shed more light on these mechanisms with the ultimate aim to slow cellular aging. Chromatin regulates tight compaction of the genetic material but on the other hand it rules its proper functioning. The indirect antagonism centered in these functions makes chromatin a field of constant research. Higher-order chromatin organization is the most complex and yet not quite easily palpable structure of the genetic material. At present it is not known how and how much this structure controls the activity of the genome. Here, we discuss our data about the role of the higher-order chromatin structure in the processes of cellular aging. The

attention is centered on a novel method, named Chromatin Comet Assay (ChCA) that allows easy studying of the higher-order chromatin organization at a single-cell level. Generally, yeast cells *Saccharomyces cerevisiae* which have impaired chromatin organization as a result of mutations in certain important chromatin proteins were used as a model organism. Our results were further confirmed on another commonly used experimental model – cells from *Drosophila melanogaster* fragile-X chromosome line.

Acknowledgments: This research is partly financed by the Bulgarian Science Fund, Grants' Numbers: DMU 02/8 and DID 02-35.

- 4 Georgieva M, Uzunova K, Staneva D, Efremov T, Balashev K, Harata M & Miloshev G 2012 The interaction between *S. cerevisiae* linker histone Hho1p and Arp4 protein affects chromatin remodeling. EMBO Conference “Gene Transcription in Yeast: from Mechanisms to Gene Regulatory Networks”, Girona, Spain, 16<sup>th</sup> – 21<sup>st</sup> June, 2012.

*S. cerevisiae* unusual and less abundant linker histone Hho1p and its roles in yeast chromatin are a subject of continuous debates. Although, it was shown that Hho1p binds linker DNA between two adjacent nucleosomes its involvement in the higher – order chromatin structure and hence, gene regulation was seriously questioned. On the other hand, Arp4p (Actin Related Protein 4) is an important subunit of several chromatin modifying complexes, such as INO80, NuA4 and SWR1. Here, we show that the linker histone Hho1p and Arp4p physically contact *in vivo*. Similarly *Arp4* mutants with single point mutations in the actin fold domain of Arp4 and *hho1Δ* cells express explicit abnormalities in the cellular phenotype, i.e. bigger cell size, irregular morphology and anomalous cell cycle progression.

Chromatin structure in the studied single mutants is also changed when compared to the wild type. Noticeable alterations in chromatin of both single mutants are manifested in chromatin loops size and organization as well as in chromatin compaction in “30-nm” fiber. Interestingly, in the *arp4 hho1Δ* double mutant the absence of Hho1p incompletely reverses *arp4* mutant phenotype, prompting partial suppression of *arp4* mutation when the gene for the linker histone is knocked-out.

Acknowledgements: Work is fully supported by the Bulgarian Science Fund, Grant number DMU 02/8 and by the World Federation of Scientists, National Scholarship Programme.

- 5 Georgieva M & Miloshev G 2012 The linker histone Hho1p is crucial for the proper organisation of *S. cerevisiae* higher - order chromatin structure *in vivo*. EMBO Conference “Gene Transcription in Yeast: from Mechanisms to Gene Regulatory Networks”, Girona, Spain, 16–21 June, 2012.

The existence of linker histone in *S. cerevisiae* was questioned for more than two decades. Its discovery after yeast genome sequencing though proved to possess several peculiar features raised new issues in the enigma. Here, we present evidence that Hho1p is involved in the organization and maintenance of the “30-nm” fiber and loop organization of *S. cerevisiae* chromatin. In a methodical search for possible effects of Hho1p on the global organization of chromatin, we have applied Chromatin Comet Assay (ChCA) on *HHO1 knock-out* yeast cells. The results showed that yeast cells without the linker histone exhibit highly distorted higher-order organization of chromatin. Characteristically, linker histone depleted chromatin is generally represented by longer chromatin loops than the

wild type. Moreover, according to atomic force microscopy data, the wild-type chromatin appeared regularly structured in a “30-nm” fiber in contrast to *HHO1 knock-out* yeast where we could not identify such structures. These results unambiguously confirm the role of *S. cerevisiae* linker histone, Hho1p in the formation and maintenance of the “30-nm” fiber and chromatin structures above it. How this obvious disruption of the higher-order chromatin structures influences the gene expression and overall cellular phenotype of the yeast will be discussed.

Acknowledgements: Work is fully supported by the Bulgarian Science Fund; Grant numbers DMU 02/8 and DID 02-35.

Papers:

- 1 Birkhofer K, Schöning I, Alt F, Herold N, Klärner B, Marhan S, Oelmann Y, Wubet T, Yurkov A, Begerow D, Berner D, Buscot F, Daniel R, Diekötter T, Ehnes RB, Erdmann G, Fischer C, Foessel B, Groh J, Gutknecht J, Kandeler E, Lang C, Lohaus G, Meyer A, Nacke H, Näther A, Overmann J, Polle A, Pollierer MM, Scheu S, Schloter M, Schulze ED, Schulze W, Weinert J, Weisser WW, Wolters V & Schrumpf M 2012 General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. PLoS ONE 7(8): e43292. DOI: 10.1371/journal.pone.0043292.

Very few principles have been unravelled that explain the relationship between soil properties and soil biota across large spatial scales and different land-use types. Here, we seek these general relationships using data from 52 differently managed grassland and forest soils in three study regions spanning a latitudinal gradient in Germany. We hypothesize that, after extraction of variation that is explained by location and land-use type, soil properties still explain significant proportions of variation in the abundance and diversity of soil biota. If the relationships between predictors and soil organisms were analyzed individually for each predictor group, soil properties explained the highest amount of variation in soil biota abundance and diversity, followed by land-use type and sampling location. After extraction of variation that originated from location or land-use, abiotic soil properties explained significant amounts of variation in fungal, meso- and macrofauna, but not in yeast or bacterial biomass or diversity. Nitrate or nitrogen

concentration and fungal biomass were positively related, but nitrate concentration was negatively related to the abundances of Collembola and mites and to the myriapod species richness across a range of forest and grassland soils. The species richness of earthworms was positively correlated with clay content of soils independent of sample location and land-use type. Our study indicates that after accounting for heterogeneity resulting from large scale differences among sampling locations and land-use types, soil properties still explain significant proportions of variation in fungal and soil fauna abundance or diversity. However, soil biota was also related to processes that act at larger spatial scales and bacteria or soil yeasts only showed weak relationships to soil properties. We therefore argue that more general relationships between soil properties and soil biota can only be derived from future studies that consider larger spatial scales and different land-use types.

- 2 Kemler M, Martín MP, Tellería TM, Schäfer AM, Yurkov AM & Begerow D In press Contrasting phylogenetic patterns of anther smuts (Pucciniomycotina: *Microbotryum*) reflect phylogenetic patterns of their caryophyllaceous hosts. *Organisms Divers Evol* DOI: 10.1007/s13127-012-0115-1.

Anther smuts in the genus *Microbotryum* often show very high host specificity toward their caryophyllaceous hosts, but some of the larger host groups such as *Dianthus* are crucially undersampled for these parasites so that the question of host specificity cannot be answered conclusively. In this study we sequenced the internal transcribed spacer (ITS) region of members of the *Microbotryum dianthorum* species complex as well as their *Dianthus* hosts. We compared phylogenetic trees of these parasites including sequences of anther smuts from other Caryophyllaceae, mainly *Silene*, with phylogenies of Caryophyllaceae that are known to harbor anther smuts. Additionally we tested whether observed patterns in parasites are due to shared ancestry or if geographic

separation is a factor that should be taken into consideration in delimitating species. Parasites on *Dianthus* showed mainly an arbitrary distribution on *Dianthus* hosts, whereas parasites on other Caryophyllaceae formed well-supported monophyletic clades that corresponded to restricted host groups. The same pattern was observed in the Caryophyllaceae studied: morphologically described *Dianthus* species did not correspond well with monophyletic clades based on molecular data, whereas other Caryophyllaceae mainly did. We suggest that these different patterns primarily result from different breeding systems and speciation times between different host groups as well as difficulties in species delimitations in the genus *Dianthus*.

- 3 Yurkov AM, Wehde T, Kahl T & Begerow D - In press - Aboveground dead wood deposition supports development of soil yeasts. Diversity.

Master's Thesis:

- 4 Christian Richter 2012 Investigation of antimicrobial metabolites from epiphyllic yeasts on maize. Ruhr-Universität Bochum, Bochum, May 2012.

The phyllosphere plays an important role in the ecosystem and especially yeasts that colonize this habitat are still poorly studied. For a better understanding of the processes in the phyllosphere, in this work epiphyllic yeasts that have been isolated from corn leaves were examined for the production of antimicrobial substances. In addition, the maize leaves were surveyed with the scanning electron microscope. The maize leaves were collected according to a scheme of nine positions in the field. Each leaf was sampled at three spots. After plating, a total of 516 colonies grew on the plates, more than half (271 colonies) of these could be transferred into pure culture. All pure cultures were grouped into 12 different morphological types. One type was represented very dominantly by more than one third of all colonies, less common types could also be isolated though. The overall abundance of yeast colonies was at  $10^3$  CFU/cm<sup>2</sup> and  $10^5$  CFU/g. Most yeasts were isolated from the leaf tip, the least from the center of the leaf. These results are supported by investigations with the scanning electron microscope. It also can be confirmed that fewer colonies occur on the bottom side of the leaves and the colonies prefer to settle at stomata, hairs and at the midrib. With antagonism tests all 271 cultures were tested for antimicrobial activity against a basidiomycete, an

ascomycete and a bacterium. Nineteen cultures were active against *Bacillus subtilis*, these cultures have been identified molecularly. Selected strains of these were cultivated in three different media. Over a period of 192 hours the cultures were sampled and processed to extracts. These extracts were analyzed by HPLC and tested with an agar diffusion assay against three organisms for antimicrobial activity. Two strains were active, especially in the HLX-medium. The more active strain of the two *Tilletiopsis washingtonensis* strains, were cultivated at a larger scale. The amount of obtained extract was separated into three phases. These were tested and the ethyl acetate phase of the mycelium showed activity. Thereafter this phase was twice separated preparatively and the fractions obtained were tested and analyzed by HPLC. The result was a pure substance that has an antimicrobial effect against the gram-positive bacterium *Bacillus subtilis*. According to analyzes by mass spectroscopy, gas chromatography and nuclear magnetic resonance spectroscopy the substance could be identified as a fatty acid. In addition, statements about the functional groups and chemical bonds could be made. Thus, it is a C18- fatty acid with one or two double bonds and two hydroxyl- or keto-groups.

Bachelor Theses:

- 5 Julian Federici 2012 Physiological profiling of soil yeasts. Ruhr-Universität Bochum, Bochum, May 2012.

Soil is the most diverse and complex habitat on the planet. This is reflected by the wide range of life-forms it supports with representatives from all three domains of life, archaea, eubacteria and eukarya. Although the importance of the soil ecosystem is known, it remains a largely unexplored field. Yeasts are members of the kingdom fungi and are ubiquitous in soils worldwide. They contribute to the mineralization process, affect plant growth and interact with soil animals. Nevertheless, we lack the full understanding soil yeasts have in ecosystem processes and further investigations are needed. The work in hand deals with the description of 18 strains of soil yeasts that belong to undescribed species. They were isolated from soils in Germany, Russia, Iceland and Spitsbergen. These strains are characterised using well-founded methods on a morphological, molecular and physiological basis. Morphological characterisation was accomplished on a macroscopic and microscopic level. Taxonomic placement was based on genetic marker regions from a multicopy rDNA repeat. Physiological capabilities were assessed on the basis of assimilation tests with 39 different compounds

that are used for the classification of yeasts since the middle of the 20th century. Results of the assimilation tests were processed in an UPGMA analysis in PAUP\* to detect underlying structures in the dataset. The 18 strains were assigned to six different genera of yeast species, *Candida*, *Cryptococcus*, *Dioszegia*, *Mrakia*, *Rhodotorula* and *Saccharomyces*. For every strain, assimilation profile and growth characteristics were determined and sequences of ribosomal DNA (ITS region and large subunit) amplified. In addition, microscopic pictures were taken, showing the most important features. The results of the physiological investigation showed that closely related species are not necessarily similar in their physiological profiles and intraspecific variability occurs. Furthermore it was demonstrated that all included yeast strains are able to exploit at least one substance, which is a typical carbon source in soil. The physiological data of the analysed species was further discussed in relation to their taxonomical affinity and ecological properties of the original habitat. Moreover several problematic aspects of the employed method were addressed.

6 Colette Kurth 2012 Occurrence and diversity of yeasts in floral nectar of *Helleborus viridis* L. Ruhr-Universität Bochum, Bochum, May 2012.

Yeasts are frequent inhabitants of floral nectar of animal-pollinated plants. According to several previous studies, they have to be considered as a third player in the plant-pollinator mutualism; however the influence they have on this interaction is not clarified, yet. Since nectar is hardly colonisable due to its composition, yeast species diversity was found to be very low with only few specialists being able to survive and reproduce well in this habitat. In this study, we investigated the occurrence and diversity of yeasts in floral nectar of *Helleborus viridis*, an early-blooming plant. To allow temporal, as well as spatial comparisons, four nectar samplings were carried out at four different points of time and two different plant populations. Yeasts were then identified using molecular methods. Yeasts were found to be frequent in floral nectar of *Helleborus viridis*. Their frequency of occurrence showed a clear temporal increase with under 10 % of nectar samples containing yeasts for the first sampling date (middle of March) to over 80 % for the last sampling date (beginning of April). Results of this study suggest that the occurrence of nectar yeasts is not associated to a certain nectar volume. However, it is probably linked to foraging patterns of

pollinating insects, since these act as vectors for nectar yeasts. For example, yeasts were more frequent in the upper nectaries than in the lower ones, and if a yeast species was present in several nectaries of a single flower, these usually were adjacent. Both findings are suggested to reflect foraging patterns of pollinators. The most frequent yeast species in collected nectar samples was *Metschnikowia reukaufii*. Its frequency of occurrence, as well as its high cell densities within nectar, are in agreement with several previous studies denoting *Metschnikowia reukaufii* as the dominating and most specialised nectar yeast. *Starmerella bombicola* came second in this study. Species of the genus *Cryptococcus* and other basidiomycetes were frequent as well, but since they did not show as high cell densities, nor the typical distribution patterns (e.g. occurrence in several nectaries per flower) mentioned above, it is suggested that these (mostly ubiquitous) species occur in floral nectar rather "accidentally". In total, the low yeast species richness reported by several previous studies for nectar of different plant species, could also be demonstrated for floral nectar of *Helleborus viridis*.

7 Katharina Gorges. Optimization of in-vitro cultivation methods of endophytic fungi. Ruhr-Universität Bochum, Bochum, May 2012.

Endophytic fungi can be found in the internal tissue of every healthy or asymptomatic higher plant. Host plants provide nutrients and steady environmental conditions for the fungal development. In return for this ideal living conditions fungi excrete secondary metabolites to the plant tissue that protect plants from phytopathogens and phytophages. Only few is known about the interaction between endophytes and their host plant. Furthermore their interaction with plant pathogens and their capability as biocontrol agents is poorly understood and insufficiently studied. In the future biocontrol agents could be alternative to chemical pesticides in agriculture, with great advantages for farmers, consumers and our environment. However, some endophytes may turn out to be pathogens, if the environmental factors change, or at the onset of natural senescence of the host tissue. The aim of this bachelor thesis was to improve the existing methods of in vitro cultivation of endophytic fungi and to establish some new techniques, such as the enzymatic decomposition of plant tissue. This objective was accomplished by isolating

endophytic fungi from two perennial herbaceous plants, namely *Ranunculus ficaria* and *Oxalis acetosella*, and testing several techniques of probe extraction (using mortar and pestle, blender, enzymatic breakdown and razor blade) and in vitro culturing of two different parts of plant tissue, leaf tissue and root tissue. Our data show that for some plants approved methods like cutting plant tissues in small fragments and placing them on potato dextrose agar seems to be the best choice. But especially for extremely thin leaves and plant tissue enzymatic digestion of these plant components showed better results in cultivation. A comparison of cultivation media showed significant differences: YMA and PDA-medium showed good results in fungal growth and diversity. In most cases culture medium which has been added plant extract displayed an increase of outcomes in fungal diversity and fungal growth. However, our dataset was limited by the time-consuming planting and cultivation and therefore statistic support is sometimes weak. Additional studies using more replicates for less variants might improve the results.

I have recently changed my research position and am now starting as curator for Fungi and Yeasts at the DSMZ. As one of the largest biological resource collections, the DSMZ has been accredited with the worldwide valid ISO 9001:2008 quality standard. Aside from the scientific service, the collection-related research represents the second pillar of the DSMZ. The collection with its place of business in Braunschweig has existed for 42 years and accommodates more than 32,000 cultures and

biomaterials. The DSMZ is the world's most diversified collection: aside from fungi, yeasts, bacteria and Archaea, it has also performed research and archived human and animal cell cultures as well as plant viruses and plant cell cultures. DSMZ offers diverse deposition options and identification services. Currently, DSMZ keeps around 3,000 strains of fungi including human- and animal pathogenic strains. We would like to encourage scientists to deposit their yeast strains in DSMZ.

Papers.

- 1 Yurkov AM & Golubev WI - In press - Phylogenetic study of *Cryptococcus laurentii* mycocinogenic strains. Mycological Progress. DOI: 10.1007/s11557-012-0873-3.

Previous studies reported *Cryptococcus laurentii* strains to secrete mycocins of different inhibition range. These mycocinogenic strains were studied by molecular methods to verify their phylogenetic relationships. Based on the combined analysis of LSU and ITS sequence data, these strains were confirmed to belong to the species

*C. laurentii*. Therefore, different strains of the same basidiomycetous yeast *C. laurentii* secrete a distinct mycocin. This trait has not been demonstrated for basidiomycetous yeasts before. Additionally, this study provides several DNA-barcodes (LSU, ITS and *RPB1*) and reports their variability for this species.

- 2 Yurkov AM, Wehde T, Kahl T & Begerow D - In press - Aboveground dead wood deposition supports development of soil yeasts. Diversity.

Unicellular saprobic fungi (yeasts) inhabit soils worldwide. Although yeasts species typically occupy defined areas on biomes scale, their distribution patterns within a single type of vegetation, like forests are more complex. In order to understand factors that shape soil yeast communities, soils collected underneath decaying wood logs and under forest litter were analyzed. We isolated and identified molecularly a total of 25 yeast species including three new species. Occurrence and distribution of yeasts isolated from these soils provide new insights into ecology and niche specialization of several soil-borne species. Although abundance of typical soil yeast species varied among experimental plots, the analysis of species

abundance and community composition revealed a strong influence of wood log deposition and leakage of organic carbon. Unlike soils underneath logs, yeast communities in adjacent areas harbored a considerable number of transient (phylloplane-related) yeasts reaching 30% of the total yeast quantity. We showed that distinguishing autochthonous community members and species transient in soils is essential to estimate appropriate effects of environmental factors on soil fungi. Furthermore, a better understanding of species niches is crucial for analyses of culture-independent data and might hint to the discovery of unifying patterns of microbial species distribution.

- 3 Kemler M, Martín MP, Tellería TM, Schäfer AM, Yurkov AM & Begerow D - In press - Contrasting phylogenetic patterns of anther smuts (Pucciniomycotina: *Microbotryum*) reflect phylogenetic patterns of their caryophyllaceous hosts. Organisms Diversity and Evolution. DOI: 10.1007/s13127-012-0115-1.

- 1 Kurtzman CP 2011 A new methanol assimilating yeast, *Ogataea parapolyomorpha*, the ascosporic state of *Candida parapolyomorpha*. Antonie van Leeuwenhoek 100:455–462. DOI: 10.1007/s10482-011-9603-0.

*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. *O. parapolyomorpha* is closely related to *Ogataea angusta* and *Ogataea 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, *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 Hawksworth DL et al. (Kurtzman CP) 2011 The Amsterdam declaration on fungal nomenclature. IMA Fungus. 2:105-112.

The Amsterdam Declaration on Fungal Nomenclature was agreed at an international symposium convened in Amsterdam on 19–20 April 2011 under the auspices of the International Commission on the Taxonomy of Fungi (ICTF). The purpose of the symposium was to address the issue of whether or how the current system of naming pleomorphic fungi should be maintained or changed now that molecular data are routinely available. The issue is urgent as mycologists currently follow different practices, and no consensus was achieved by a Special Committee appointed in 2005 by the International Botanical Congress to advise on the problem. The Declaration recognizes the need for an orderly transition to a single-name nomenclatural system for all fungi, and to provide mechanisms to protect names that otherwise then become endangered. That is, meaning that priority should be given

to the first described name, except where that is a younger name in general use when the first author to select a name of a pleomorphic monophyletic genus is to be followed, and suggests controversial cases are referred to a body, such as the ICTF, which will report to the Committee for Fungi. If appropriate, the ICTF could be mandated to promote the implementation of the Declaration. In addition, but not forming part of the Declaration, are reports of discussions held during the symposium on the governance of the nomenclature of fungi, and the naming of fungi known only from an environmental nucleic acid sequence in particular. Possible amendments to the Draft BioCode (2011) to allow for the needs of mycologists are suggested for further consideration, and a possible example of how a fungus only known from the environment might be described is presented.

- 2 Kurtzman CP 2012 *Citeromyces hawaiiensis* sp. nov., an ascosporic yeast associated with *Myoporium sandwicense*. Int J Syst Evol Microbiol 62:1215–1219. DOI: 0.1099/ijms.0.035360-0.

*Citeromyces hawaiiensis* sp. nov. (NRRL Y-11581<sup>T</sup> 5CBS 12303T, type strain) is described from 12 strains isolated from flux of the sandalwood (*Myoporium sandwicense*) and adjacent soil in Hawaii, USA. Analyses of gene sequences from the D1/D2 domains of nuclear large subunit rRNA, internal transcribed spacer (ITS), mitochondrial small-subunit rRNA and translation elongation factor-1 $\alpha$  each separated the proposed novel

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

- 3 Kurtzman CP 2012 *Candida kuoi* sp. nov., an anamorphic species of the *Starmerella* yeast clade that synthesizes sphorolipids. Int J Syst Evol Microbiol 62:2307–2311. DOI: 10.1099/ijms.0.039479-0.

A novel strain of anamorphic yeast, designated strain NRRL Y-27208T, was isolated from concentrated grape juice in Cape Province, South Africa. Analysis of nuclear large subunit rRNA gene sequences from the D1/D2

domains separated the novel isolate from strains of *Starmerella bombicola* and *Starmerella meliponinorum*, as well as from species of the genus *Candida* that are members of the *Starmerella* clade. Compared to previously described

species, strain NRRL Y-27208T is most closely related to *S. bombicola* but can be separated from this species by its ability to grow on D-ribose and erythritol. Strain NRRL Y-27208T produced sophorolipids that have an open chain structure similar to *Candida batistae*, *Candida riocensis* and *Candida stellata*, which is in contrast to the closed

chain sophorolipids produced by *S. bombicola* and *Candida apicola*. The analyses showed that NRRL Y-27208T (CBS 7267T) represents a novel species distinct from previously described species, for which the name *Candida kuoi* sp. nov. is proposed.

- 4 Kurtzman CP 2012 *Komagataella populi* sp. nov. and *Komagataella ulmi* sp. nov., two new methanol-assimilating yeasts from exudates of deciduous trees. *Antonie van Leeuwenhoek* 101:859-868.

Two new species of the methanol assimilating ascosporic yeast genus *Komagataella* are described. *Komagataella populi* sp. nov. (NRRL YB-455, CBS 12362, type strain, MycoBank accession number = 564110) was isolated from an exudate on a cottonwood tree (*Populus deltoides*), Peoria, Illinois, USA, and *Komagataella ulmi* sp. nov. (NRRL YB-407, CBS 12361, type strain, MycoBank accession number = 564111) was isolated from the exudate on an elm tree (*Ulmus americana*), also growing in Peoria,

Illinois. The species were resolved from divergence in gene sequences for domains D1/D2 LSU rRNA, ITS1-5.8S-ITS2, mitochondrial small subunit rRNA, RNA polymerase subunit 1 and translation elongation factor-1 $\alpha$ . Species of *Komagataella* assimilate few carbon compounds and are unlikely to be resolved from differences in standard growth and fermentation tests. For this reason, separation of species is dependent on gene sequence analysis.

- 5 Kurtzman CP & Robnett CJ 2012 Relationships among genera of the Saccharomycotina (Ascomycota) from multigene phylogenetic analysis of type species. *FEMS Yeast Res* (in press). DOI: 10.1111/1567-1364.12006.

Relationships among ascomycetous yeast genera (subphylum Saccharomycotina, phylum Ascomycota) have been uncertain. In the present study, type species of 70 currently recognized genera are compared from divergence in the nearly entire nuclear gene sequences for large subunit rRNA, small subunit rRNA, translation elongation factor-1 $\alpha$ , and RNA polymerase II, subunits 1 (RPB1) and 2 (RPB2). The analysis substantiates earlier proposals that all known ascomycetous yeast genera now assigned to the Saccharomycotina represent a single clade. Maximum likelihood analysis resolved the taxa into eight large multi-genus clades and four one and two genus clades. Maximum parsimony and neighbor-joining analyses gave similar results. Genera of the family Saccharomycetaceae remain

as one large clade as previously demonstrated to which the genus *Cynicomyces* is now assigned. *Pichia*, *Saturnispora*, *Kregervanrija*, *Dekkera*, *Ogataea* and *Ambrosiozyma* are members of a single large clade, which is separate from the clade that includes *Barnettozyma*, *Cyberlindnera*, *Phaffomyces*, *Starmera* and *Wickerhamomyces*. Other clades include *Kodamaea*, *Metschnikowia*, *Debaryomyces*, *Cephaloascus* and related genera, which are separate from the clade that includes *Zygoascus*, *Trichomonascus*, *Yarrowia* and others. This study once again demonstrates that there is limited congruence between a system of classification based on phenotype and a system determined from DNA sequences.

- 6 Kurtzman CP & Robnett CJ 2012 *Saitoella coloradoensis* sp. nov., a new species of the Ascomycota, subphylum Taphrinomycotina. *Antonie van Leeuwenhoek* 101:795-802. DOI: 10.1007/s10482-011-9694-7.

*Saitoella coloradoensis* sp. nov. (NRRL YB-2330, CBS 12360, type strain, MycoBank accession number 563858) is described. This new member of the phylum Ascomycota, subphylum Taphrinomycotina was isolated from insect frass occurring in an Engelmann spruce (*Picea engelmannii*) that was growing in Colorado, USA. Multigene sequence analysis showed that *S. coloradoensis* is distinct from *Saitoella complicata*, the only other known species of *Saitoella*. The two species may be separated

phenotypically from growth reactions on D-xylose, ribitol and methyl- $\alpha$ -D-glucoside. Asexual reproduction is by budding and both species produce thick-walled, spherical cells that appear morphologically similar to the ascogenous cells formed in plant host tissue by species of *Protomyces* and some species of *Taphrina*. The thick-walled cells did not form ascospores but did produce buds when placed on fresh growth media.

- 7 McCormick SP, Price NPJ & Kurtzman CP 2012 Glucosylation and other biotransformations of T-2 toxin by yeasts of the *Trichomonascus* clade. *Appl Environ Microbiol* (published ahead of print). doi: 10.1128/AEM.02391-12

Trichothecenes are sesquiterpenoid toxins produced by *Fusarium* species. Since these mycotoxins are very stable, there is interest in microbial transformations that can remove toxins from contaminated grain or cereal products. Twenty-three yeasts assigned to the *Trichomonascus* clade (Saccharomycotina, Ascomycota), including four *Trichomonascus* species and 19 anamorphic species presently classified in *Blastobotrys*, were tested for their ability to convert the trichothecene T-2 toxin to less toxic products. These species gave three types of biotransformations: acetylation to 3-acetyl T-2 toxin,

glycosylation to T-2 toxin 3-glucoside, and removal of the isovaleryl group to form neosolaniol. Some species gave more than one type of biotransformation. Three *Blastobotrys* species converted T-2 toxin into T-2 toxin 3-glucoside, a compound that has been identified as a masked mycotoxin in *Fusarium* infected grain. This is the first report of a microbial whole cell method for producing trichothecene glycosides, and the potential large-scale availability of T-2 toxin 3-glucoside will facilitate toxicity testing and development of methods for detection of this compound in agricultural and other products.

- 8 Price NJP, Ray KJ, Vermillion KE, Dunlap CA & Kurtzman CP 2012 Structural characterization of novel sophorolipid biosurfactants from a newly identified species of *Candida* yeast. *Carbohydr Res* 348:33-41. doi: 10.1016/j.carres.2011.07.016

The 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)  $\beta$ -O-glycosidically-linked to 17-L-hydroxy- $\delta$ -9-octadecenoic acid, where the acyl carboxyl often forms a 4"-lactone to the terminal glucosyl residue. In a recent MALDI-TOF/MS-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. Unlike those reported previously, the NRRL Y-27208 sophorolipids contain a terminal-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-TOF/MS from this strain. The surfactant-like properties of these sophorolipids have value as potential replacements for petroleum-based detergents and emulsifiers.

- 9 Schoch CL et al. (Kurtzman CP) 2012 The nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc Natl Acad Sci* 109:6241-6246.

Six DNA regions were evaluated as potential DNA barcodes for *Fungi*, the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium. The region of the mitochondrial cytochrome *c* oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to amplify in fungi, often includes large introns, and can be insufficiently variable. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein-coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein). Although the protein-coding gene regions often had a higher percent of correct identification compared with ribosomal markers, low PCR amplification and sequencing success eliminated them as candidates for a universal fungal barcode. Among

the regions of the ribosomal cistron, the internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecific variation. The nuclear ribosomal large subunit, a popular phylogenetic marker in certain groups, had superior species resolution in some taxonomic groups, such as the early diverging lineages and the ascomycete yeasts, but was otherwise slightly inferior to the ITS. The nuclear ribosomal small subunit has poor species-level resolution in fungi. ITS will be formally proposed for adoption as the primary fungal barcode marker to the Consortium for the Barcode of Life, with the possibility that supplementary barcodes may be developed for particular narrowly circumscribed taxonomic groups.

- 10 Witzgall P, Proffitt M, Rozpedowska E, Becher PG, Andreadis S, Coracini M, Lindblom TU, Ream LJ, Hagman A, Bengtsson M, Kurtzman CP, Piskur J & Knight A 2012 "This is not an Apple" - Yeast Mutualism in Codling Moth. *J Chem Ecol* 38:949-57. doi: 10.1007/s10886-012-0158-y.

The larva of codling moth *Cydia pomonella* (Tortricidae, Lepidoptera) is known as the worm in the apple, mining the fruit for food. We here show that codling moth larvae are closely associated with yeasts of the genus *Metschnikowia*. Yeast is an essential part of the larval diet and further promotes larval survival by reducing the incidence of fungal infestations in the apple. Larval feeding, on the other hand, enables yeast proliferation on unripe fruit. Chemical, physiological and behavioral analyses demonstrate that codling moth senses and responds to yeast aroma. Female moths are attracted to fermenting yeast and lay more eggs on yeast-inoculated than on yeast-free apples. An olfactory response to yeast volatiles

strongly suggests a contributing role of yeast in host finding, in addition to plant volatiles. Codling moth is a widely studied insect of worldwide economic importance, and it is noteworthy that its association with yeasts has gone unnoticed. Tripartite relationships between moths, plants, and microorganisms may, accordingly, be more widespread than previously thought. It, therefore, is important to study the impact of microorganisms on host plant ecology and their contribution to the signals that mediate host plant finding and recognition. A better comprehension of host volatile signatures also will facilitate further development of semiochemicals for sustainable insect control.

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**XI Dipartimento di Scienze e Tecnologie Agro-Forestali e Ambientali, University of Reggio Calabria, Via Feo di Vito, I-89122 Reggio Calabria, Italy. Communicated by A Caridi <[acaridi@unirc.it](mailto:acaridi@unirc.it)>.**

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Recently accepted papers.

- 1 Caridi A, Sidari R, Pulvirenti A, Meca G & Ritieni A 2012 Ochratoxin A adsorption phenotype: An inheritable yeast trait. *J Gen Appl Microbiol* 58:225-233.

This study aimed to evaluate the inheritance of the trait *ochratoxin A adsorption* in two wine strains of *Saccharomyces cerevisiae* and their 46 descendants. Each strain was inoculated in triplicate in test tubes containing 10 ml of must obtained from the Calabrian *Zibibbo* white grape variety, artificially contaminated with ochratoxin A to reach a total content of 4.10 ng/ml. The microvinification trials were performed at 25°C. After 30 days, ochratoxin A values ranged from 0.74 to 3.18 ng/ml, from 0.01 to 2.69 ng/ml, and from 0.60 to 2.95 ng/ml respectively in wines, in lees after washing, and in the saline solution used to wash the lees. The analysis of OTA in wines was performed to find the residual toxin content after yeast activity, thus obtaining technological evidence of yeast influence on wine detoxification. The analysis of OTA in lees after washing was performed to distinguish the OTA linked to cells. The analysis of OTA in the saline solution used to wash the lees

was performed to distinguish the OTA adsorbed on yeast cell walls and removed by washing, thus focusing on the adsorption activity of wine yeast through electrostatic and ionic interactions between parietal mannoproteins and OTA. Ploidy of the two parental strains was controlled by flow cytometry. Results demonstrated that the *ochratoxin A adsorption* is genetically controlled and is a polygenic inheritable trait of wine yeasts. The majority of the descendants are characterized by a great and significant diversity compared to their parents. Both the parental strains had genome sizes consistent with their being diploid, so validating the observed results. These findings constitute an initial step to demonstrate the mechanisms of inheritance and establish breeding strategies to improve the *ochratoxin A adsorption* trait in wine yeasts. This will allow a decrease in the ochratoxin A content of contaminated musts during winemaking, by using genetically improved wine yeasts.

- 2 Caridi A In press Improved screening method to select yeast for pigment adsorption activity. *Food Technol Biotech* (accepted July 13, 2012).

The aim of this research is to improve an existing low-cost and simple but consistent culturing technique for measuring the adsorption of grape skin pigments by yeasts, comprising: (a) growing yeasts in Petri dishes on chromogenic grape-skin-based medium, (b) photographing the yeast biomass, (c) measuring its red, green, and blue colour components, and (d) performing the statistical analysis of the data. Twenty strains of *Saccharomyces cerevisiae* were grown on different lots of the chromogenic medium, prepared using grape skins from the black cultivars

*Greco Nero*, *Magliocco*, and *Nero d'Avola*. Microscale wine fermentation trials were also performed. Wide and significant differences among wine yeasts were observed. The chromogenic grape-skin-based medium can be prepared using any grape cultivar, thus allowing the specific selection of the most suitable strain of *Saccharomyces cerevisiae* for each grape must, mainly for red winemaking. The research provides a useful tool to characterize wine yeasts in relation to pigment adsorption, allowing the improvement of wine colour.

The following papers were read at recent conferences.

- 3 Caridi A & Sidari R 2012 Optimal use of *Hanseniaspora* peculiarities in winemaking. 13th International Congress on Yeasts, Madison, U.S.A.

The genus *Hanseniaspora* includes apiculate yeasts that not only produce ethanol during fermentation, but also high amounts of acetic acid, which, although varying from strain to strain, prevent their use as single wine starters (Caridi *et al.*, 1991). Several studies show that the unacceptably high volatile acid production by some *Hanseniaspora* species does not occur when these yeasts are grown in mixed culture with *Saccharomyces cerevisiae* (Fleet 2008). This research aims at verifying the possibility to employ selected strains of the genus *Hanseniaspora* as single wine starters valorizing their weak point - the high production of volatile acidity - to obtain citrus wine characterized by a very high content in flavored and volatile compounds, including acetic acid. Then the citrus wine may be oxidized by selected acetic acid bacteria to produce a much appreciated citrus vinegar (Caridi 2005). Twenty-nine Calabrian strains of *Hanseniaspora* sp. and 4 control

strains of *Saccharomyces cerevisiae* were selected using two different juice combinations: A) mix of bergamot (*Citrus bergamia* Risso) juice and grape must from dried grapes [ratio 1:2] - pH 2.94, °brix 20.80, and B) bergamot juice with the addition of saccharose - pH 2.94, °brix 20.80. The juices were prepared and immediately inoculated in duplicate with the yeast strains. The fermentations were studied determining the weight loss caused by CO<sub>2</sub> production. When the CO<sub>2</sub> production finished the wines were analyzed. On the whole, the results demonstrate the possibility to employ selected strains of the genus *Hanseniaspora* as single starter culture in winemaking. The excellent results obtained may be further implemented by genetic improvement: since *Hanseniaspora* produces ascospores, this research will continue by segregation and breeding of the best selected strains.

- 4 Caridi A *et al.* 1991 Oenological characteristics of *Hanseniaspora guilliermondii*. *Vini d'Italia*, 33, 51-57 (in Italian).
- 5 Caridi A 2005 Fruit vinegars based on citrus wines. International Symposium on Vinegars and Acetic Acid Bacteria, Reggio Emilia (Italy), 8-12 May 2005. Fleet G.H. Wine yeasts for the future. *FEMS Yeast Res*, 8, 979-995, 2008.
- 6 Sidari R & Caridi A 2012 Effect of nutrient depletion on parietal adsorption activity of *Saccharomyces cerevisiae* selected for winemaking. 23rd International ICFMH Symposium FoodMicro 2012, Istanbul, Turkey.

The aim of this work was to investigate the effect of carbon and nitrogen availability on colour adsorption activity of selected wine yeasts, testing them in poor and rich media in the presence of coloured phenolic compounds from grape skins. Eleven autochthonous Calabrian yeast strains of the species *Saccharomyces cerevisiae* - previously selected as wine starter based on their different aptitude to adsorb grape pigments on their cell-wall - and 2 control strains of *S. cerevisiae* BY4742 and  $\Sigma$ 1278b were grown on (a) grape skin agar,<sup>1</sup> containing black grape skin powder 4% and dextrose 5%, 2% or 0.1%, (b) synthetic low ammonium dextrose agar<sup>2</sup> supplemented with black grape skin powder 4% and containing dextrose 2% or 0.1%, and (c) yeast peptone dextrose agar as control medium. After incubation at 28°C for 10 days, strains were processed for Red-Green-Blue analysis by Photoshop CS, that gives low or high values for yeast able (dark biomass) or unable (light biomass) to adsorb coloured compounds, respectively. The effect of nutrient availability is not univocal for the 13 yeast strains that exhibit significant differences for the colour

parameters. Interestingly, depletion in (a) nitrogen or (b) carbon and nitrogen determines a decrease in the yeast adsorption activity, possibly due to yeast overcoming nutrient stress by phenolic compounds that seem to have a role as nutrient supplements. There is ample evidence for the effects of nutrient depletion on transcriptional activation or repression of specific genes in *Saccharomyces cerevisiae*, as those connected to pseudohyphal growth and flocculation. The relevant literature and the present results have been discussed considering, above all, the oenological consequences for wine starter selection.

<sup>1</sup>Caridi A, Sidari R, Solieri L, Cufari A, Giudici P 2007 Wine colour adsorption phenotype: an inheritable quantitative trait loci of yeasts. *J Appl Microbiol* 103:735-742.

<sup>2</sup>Zaragoza O, Gancedo JM 2000 Pseudohyphal growth is induced in *Saccharomyces cerevisiae* by a combination of stress and cAMP signalling. *Antonie van Leeuwenhoek* 78:187-194.

Recent publications.

- 1 Lucia Paulovičová, Ema Paulovičová, Alexander A. Karelin, Yury E. Tsvetkov, Nikolay E. Nifantiev & Slavomír Bystrický 2012 Humoral and cell-mediated immunity following vaccination with synthetic *Candida* cell wall mannan derived heptamannoside–protein conjugate Immunomodulatory properties of heptamannoside–BSA conjugate. *Int Immunopharmacol* 14:179–187.

Chemically defined glycoprotein conjugate composed of synthetically prepared mannan derived heptamannoside with terminal  $\beta$ -1,2-linked mannose residue attached to the  $\alpha$ -1,3-linked mannose residues and BSA as carrier protein (M7–BSA conjugate) was analysed for the capacity to induce protective humoral immunity and appropriate alteration cellular immunity. To identify protective antigenic structure of *Candida* cell wall mannan M7–BSA conjugate was used for BALB/c mice immunization. The obtained results were compared with placebo group and with heat inactivated *C. albicans* whole cells immunization. The administration route of M7–BSA conjugate secondary booster injection significantly affected the intensity of humoral immune response and the specificity of produced antibodies. All prepared sera were able to elevate candidacidal activity of polymorphonuclear leukocytes (PMN) in cooperation with complement. Moreover,

polyclonal sera obtained after secondary subcutaneous (s.c.) booster injection of M7–BSA conjugate were able to induce candidacidal activity of PMN also in complement independent manner. M7–BSA conjugate immunization induced increases of phagocytic activity and respiratory burst of granulocytes, caused a raise of the proportion of CD3+ T lymphocytes and increased the CD4+/CD8+ T lymphocyte ratio. We observed also an increasing proportion of CD4+CD25+ T cells compared to immunization with heat inactivated whole *C. albicans* cells, which in turn promoted an increase of the CD8+CD25+ cell proportion. Immunization with M7–BSA conjugate induced Th1, Th2 and Th17 immune responses as indicated by the elevation of relevant cytokines levels. These data provide some insights on the immunomodulatory properties of oligomannosides and contribute to the development of synthetic oligosaccharide vaccines against fungal diseases.

- 2 Raj Kumar Salar, Milan Čertík, Vlasta Brezova, Marta Brlejova, Vladimira Hanusova & Emília Breierova - In press - Stress influenced increase in phenolic content and radical scavenging capacity of *Rhodotorula glutinis* CCY 20-2-26. *3 Biotech*.

*Rhodotorula glutinis* CCY 20-2-26 when grown under controlled stress of either NaCl (1–5 %) or H<sub>2</sub>O<sub>2</sub> (1–5 mM) on basal media exhibited a twofold increase in its total phenolic contents. The radical scavenging capacities (RSCs) as determined by ABTS test were found to be highest in 4 mM H<sub>2</sub>O<sub>2</sub> (1.44 mM TEAC mg<sup>-1</sup>) and 4% NaCl (1.13 mM TEAC mg<sup>-1</sup>) as compared to control samples (0.41 mM TEAC mg<sup>-1</sup>). Similarly, the RSCs as determined by DPPH test were also highest in 4 %

NaCl (1.83 mM TEAC mg<sup>-1</sup>) and 4 mM H<sub>2</sub>O<sub>2</sub> (1.78 mM TEAC mg<sup>-1</sup>) compared to control (0.48 TEAC mg<sup>-1</sup>). The relative RSCs from EPR spin-trapping assay for H<sub>2</sub>O<sub>2</sub>-stressed cultures were highest in 1 mM H<sub>2</sub>O<sub>2</sub> (56.1 IM TEAC g<sup>-1</sup>) whereas in NaCl-stressed cultures it was highest in 5 % NaCl (44.6 IM TEAC g<sup>-1</sup>) as compared to control (30.9 IM TEAC g<sup>-1</sup>). Five phenolic compounds (gallic acid, benzoic acid, catechin, caffeic acid and ferulic acid) were detected for the first time in *R. glutinis* CCY 20-2-26.

- 3 Renáta Vadkertiová, Jana Molnárová, Dana Vránová & Elena Sláviková - In press - Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. *Can J Microbiol*.

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

*Metschnikowia pulcherrima*) occurred regularly in the blossom samples, whereas *Galactomyces candidus*, *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *M. pulcherrima*, *Pichia kluyveri*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae* were the most frequently isolated species from the fruit samples. The ratio of the number of samples where only individual species were present, to the number of samples where two or more species were found (consortium), was counted. The occurrence of individual species in comparison to consortia was much higher in

blossom samples than in fruit samples. In the latter, consortia predominated. *Aureobasidium pullulans*, *M. pulcherrima*, and *S. cerevisiae*, isolated from both the

fruits and blossoms, can be considered as resident yeast species of various fruit tree species cultivated in southwest Slovakia localities.

- 4 Kateřina Illková, Zuzana Zemková, Dana Flodrová, Jakub Jäger, Dagmar Benkovská, Jiřina Omelková, Renáta Vadkertiová, Janette Bobáľová & Eva Stratilová 2012 Production of *Geotrichum candidum* polygalacturonases via solid state fermentation on grape pomace. Chemical Papers 66 (9):852–860.

*Geotrichum candidum* CCY 16-1-29 (teleomorph *Galactomyces geotrichum*) is able to grow and produce polygalacturonase of remarkable activities on pectin or grape pomace as a sole carbon source. The highest activities of extracellular enzymes were found on the third and the seventh day of cultivation. After extraction and precipitation, polygalacturonases produced in these cultivation periods were characterized. Production of multiple forms of polygalacturonase was observed in both cultivation periods. Two major forms, polygalacturonase with random action pattern (endo-PGase, EC 3.2.1.15) and oligogalacturonate hydrolase (exoPGase, exopolygalacturonase preferring oligogalacturonides as substrates), as well as numerous minor forms were detected by IEF-PAGE using the print technique detection. EndoPGase was identified by mass spectrometry. The

major forms have similar isoelectric points (below pH 6.0) and pH optima (4.6 and 4.8, respectively). pH optimum of 4.6 was associated with exoPGase and that of 4.8 with endoPGase. Both enzymes were stable after freeze-drying and storage at 4 °C. EndoPGase had molecular mass of about 29 kDa (36 kDa by SDS-PAGE) as determined by gel filtration, temperature optimum of about 45 °C and it was stable only below 35 °C. Molecular mass of exoPGase was about 50 kDa, its temperature optimum was about 60 °C, and it was stable to 60 °C. Optimal substrate for exoPGase was a pentamer, for endoPGase it was a pectate. Values of  $K_m$  for optimal substrate reached the values of  $11.4 \times 10^{-5}$  M for exoPGase and  $6.6 \times 10^{-5}$  M for endoPGase. Pectin methylesterase as another pectolytic enzyme was also identified by mass spectrometry.

- 5 Hana Šuranská, Dana Vránová, Jiřina Omelková & Renáta Vadkertiová 2012 Monitoring of yeast population isolated during spontaneous fermentation of Moravian wine. Chemical Papers 66 (9): 861-868.

In enology, yeasts play an important role in the characteristics of the final product. They are predominant in the biochemical interaction with components of must. Rapid identification of the yeast population is necessary for fermentation process monitoring and for obtaining a good quality wine. The main goal of this study was the isolation and characterisation of the yeast microbial community naturally present on grape berries, leaves and occurring during the spontaneous fermentation process of the white wine Veltlin green from the South Moravian region, Czech Republic. The results, based on PCR-RFLP of the 5.8S-ITS region of rDNA, PCR-fingerprinting using microsatellite oligonucleotide primers (GAG)<sub>5</sub>, (GTG)<sub>5</sub>, (GAC)<sub>5</sub>, and

M13 primer, showed great diversity of the yeast population. Including grape berries and fermented must, the following yeast species were identified: *Hanseniaspora uvarum*, *Aureobasidium pullulans*, *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, a number of *Pichia* species such as *P. fermentans*, *P. membranifaciens*, *P. kluyveri*, also *Sporidiobolus salmonicolor*, *Rhodospiridium toruloides*, *Rhodotorula mucilaginosa*, *Rhodotorula glutinis* as well as *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. Monitoring of the yeast strains during the wine fermentation process of traditional Moravian wine can contribute to the improvement of wine quality.

- 6 Čertík M, Brlejšová M, Breierová E, Guothová L, Adamechová Z & Klemková T 2012 Biotechnological production of useful compounds by carotenogenic microorganisms. Proceedings of 8<sup>th</sup> International Symposium on Biocatalysis and Agricultural Biotechnology (Invited Lecture), Sonoma Valley, California, USA, October 28-31, 2012, 45. Organized by Randall Weselake (University of Alberta, Edmonton, Canada) and Tomas McKeon (USDA, Albany, California, USA).

Recent publications.

- 1 Goretto M, Branda E, Turchetti B, Cramarossa MR, Onofri A, Forti L & Buzzini P 2012 Response surface methodology as optimization strategy for asymmetric bioreduction of (4S)-(+)-carvone by *Cryptococcus gastricus*. *Biores Technol* 121: 290-297.
- 2 Jagielski T, Buzzini P, Lassa H, Malinowski E, Branda E, Turchetti B, Polleichtner A, Roesler U, Lagneau PE, Marques S, Silva E, Thompson G, Stachowiak R & Bielecki J 2012 Multicenter etest evaluation of in vitro activity of conventional antifungal drugs against European bovine mastitis *Prototheca* sp. isolates. *J Antimicrob Chemother* 67:1945-1947.
- 3 Buzzini P, Branda E, Goretto M & Turchetti B 2012 Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. *FEMS Microbiol Ecol* 82:217-241.
- 4 Nativi C, Francesconi O, Gabrielli G, De Simone I, Turchetti B, Mello T, Di Cesare Mannelli L, Ghelardini C, Buzzini P & Roelens S 2012 Aminopyrrolic synthetic receptors for monosaccharides: a class of carbohydrate-binding agents endowed with antibiotic activity vs. pathogenic yeasts. *Chem. Eur J* 18:5064-5072.
- 5 Zuconci L, Selbmann L, Buzzini P, Turchetti B, Guglielmin M, Frisvad JC & Onofri S 2012 Searching for eukaryotic life preserved in Antarctic permafrost. *Polar Biol* 35:749-757.
- 6 Buzzini P, Goretto M, Ponzoni C, Caselli E, Branda E, Cramarossa MR, Turchetti B & Forti L 2012 Asymmetric reduction of (4S)-(+)-carvone catalyzed by enoate reductases (ERs) expressed by non-conventional yeast (NCY) whole-cells. In: *Practical Methods for Biocatalysis and Biotransformations* (Whittall J & Sutton P eds.), Wiley, New York, 104-108.
- 7 Turchetti B, Thomas Hall SR, Connell LB, Branda E, Buzzini P, Theelen B, Müller WH & Boekhout T 2011 Psychrophilic yeasts from Antarctica and European glaciers. Description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov and *Glaciozyma watsonii* sp. nov. *Extremophiles* 15:573-586.
- 8 Goretto M, Ponzoni C, Caselli E, Marchegiani E, Cramarossa MR, Turchetti B, Forti L & Buzzini P 2011 Bioreduction of alpha,beta-unsaturated ketones and aldehydes by non-conventional yeast (NCY) whole-cells. *Biores Technol* 102:3993-3998.
- 9 Ricchi M, Cammi G, Garbarino CA, Buzzini P, Belletti GL & Arrigoni N 2011 A rapid real time-PCR/DNA melting resolution method to identify *Prototheca* species. *J Appl Microbiol* 110:27-34.

Recent publications.

- 1 Calderon J, Ragni E, Fascio U, Sipiczki M, Fonzi WA & Popolo L 2011 Phr1 protein localization to apical growth sites and septum contributes to *Candida albicans* morphogenesis. *Fungal Genet Biol* 48:793-805.

Cell wall biogenesis is a dynamic process relying on the coordinated activity of several extracellular enzymes. *PHR1* is a pH-regulated gene of *Candida albicans* encoding

a glycosylphosphatidylinositol-anchored  $\beta(1,3)$ -glucanoyl-transferase of family GH72 which acts as a cell wall remodelling enzyme and is crucial for morphogenesis and

virulence. In order to explore the function of Phr1p, we obtained a green fluorescent protein (GFP) fusion to determine its localization. During induction of vegetative growth, Phr1p-GFP was concentrated in the plasma membrane of the growing bud, in the mother-bud neck, and in the septum. Phr1p-GFP was recovered in the detergent-resistant membranes indicating its association with the lipid rafts as the wild type Phr1p. Upon induction of hyphal growth, Phr1p-GFP highly concentrated at the apex of the germ tubes and progressively distributed along the lateral

sides of the hyphae. Phr1p-GFP also labelled the hyphal septa, where it colocalized with chitin. Localization to the hyphal septa was perturbed in nocodazole-treated cells, whereas inhibition of actin polymerization hindered the apical localization. Electron microscopy analysis of the hyphal wall ultrastructure of a *PHR1* null mutant showed loss of compactness and irregular organization of the surface layer. These observations indicate that Phr1p plays a crucial role in hyphal wall formation, a highly regulated process on which morphogenesis and virulence rely.

## 2 Balazs A, Batta G, Miklos I, Acs-Szabo L, Vazquez de Aldana CR & Sipiczki M 2012 Conserved regulators of the cell separation process in *Schizosaccharomyces*. Fungal Genet Biol 49:235-249.

The fission yeasts (*Schizosaccharomyces*) representing a highly divergent phylogenetic branch of Fungi evolved from filamentous ancestors by gradual transition from mycelial growth to yeast morphology. For the transition, a mechanism had been developed that separates the sister cells after the completion of cytokinesis. Numerous components of the separation mechanism have been characterised in *Schizosaccharomyces pombe*, including the zinc-finger transcription factor Ace2p and the fork-head transcription factor Sep1p. Here we show that both regulators have regions conserved within the genus. The most conserved parts contain the DNA-binding domains whose amino-acid sequences perfectly reflect the phylogenetic positions of the species. The less conserved parts of the proteins contain sequence blocks specific for the whole genus or only for the species propagating predominantly or exclusively as yeasts. Inactivation of

either gene in the dimorphic species *Schizosaccharomyces japonicus* abolished cell separation in the yeast phase conferring hypha-like morphology but did not change the growth pattern to unipolar and did not cause extensive polar vacuolation characteristic of the true mycelium. Neither mutation affected the mycelial phase, but both mutations hampered the hyphal fragmentation at the mycelium-to-yeast transition. Ace2p(Sj) acts downstream of Sep1p(Sj) and regulates the orthologues of the Ace2p-dependent *S. pombe* genes *agn1(+)* (1,3- $\alpha$ -glucanase) and *eng1(+)* (1,3- $\beta$ -glucanase) but does not regulate the orthologue of *cfh4(+)* (chitin synthase regulatory factor). These results and the complementation of the cell separation defects of the *ace2(-)* and *sep1(-)* mutations of *S. pombe* by heterologously expressed *ace2(Sj)* and *sep1(Sj)* indicate that the cell separation mechanism is conserved in the *Schizosaccharomyces* genus.

## 3 Sipiczki M 2012 *Candida borneonana* sp. nov., a new methanol-assimilating anamorphic yeast species isolated from decaying fruit in Borneo. Int J Syst Evol Microbiol 62:2303-2306.

Five strains of a previously uncharacterized anamorphic, methanol-assimilating yeast species are described here, for which the name *Candida borneonana* is proposed. The strains were isolated from fruit waste collected in markets in Brunei, Borneo. The sequences of the D1/D2 domains of the large subunit rRNA genes, the internal transcribed spacer (ITS) regions and the 18S rRNA genes were identical between the isolates and differed from the corresponding sequences of all previously described yeast species. Phylogenetic analysis of these sequences

showed that the new species formed a cluster with species of the genus *Kuraishia*, the closest related species being *K. capsulata* (6 % nucleotide substitutions in the D1/D2 domain). The type strain, 11-487(T), has been deposited in the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) as CBS 12507(T), the Culture Collection of Yeasts (Bratislava, Slovakia) as CCY 29-182-1(T) and the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary) as NCAIM Y.02008. Mycobank no. MB563710.

## 4 Siesto G, Capece A, Sipiczki M, Csoma H & Romano P 2012 Polymorphism detection among wild *Saccharomyces cerevisiae* strains of different wine origin. Annals Microbiol 2012 (available online) DOI: 10.1007/s13213-012-0516-6.

In this study, wild *Saccharomyces cerevisiae* strains, isolated from spontaneously fermenting grapes of different varieties and origin, were submitted to genetic analysis using different molecular techniques, such as amplification of genes coding for cell wall proteins and containing minisatellite-like sequences, karyotyping, mtDNA-RFLP, and analysis of the  $\delta$  region. The lowest discriminative

power was obtained by minisatellite analysis, in particular the amplification of *AGAI* genes. Karyotyping and mtDNA-RFLP analysis yielded the same differentiation among the strains, whereas the PCR amplification of  $\delta$  sequences resulted the best method as it was fast and it showed a very high discriminative power. In any case, it has to be underlined that some strains, showing the same

delta profiles, exhibited a different mtDNA restriction profile and electrophoretic karyotype, suggesting that more than one molecular marker is required for reliable strain discrimination. Although the techniques used revealed a different resolution power, they all revealed a genetic

relationship among strains isolated from spontaneous fermentation of grapes of different origins. In fact, none of the typing methods was able to discriminate some strains isolated from different areas.

- 5 Pfliegler WP, Antunovics Z & Sipiczki M 2012 Double sterility barrier between *Saccharomyces* species and its breakdown in allopolyploid hybrids by chromosome loss. *FEMS Yeast Res* 12:703-718.

The analysis of 57 synthetic interspecies hybrids revealed that *Saccharomyces cerevisiae* and *Saccharomyces uvarum* (*Saccharomyces bayanus* var. *uvarum*) are isolated by a double sterility barrier: by hybrid sterility (hybrid cells cannot produce viable spores) operating in allodiploids and by F1 sterility (F1 cells cannot produce viable spores) operating in allopolyploids. F1-sterility is caused by mating-type heterozygosity. It can be overcome by eliminating chromosome 2 of the *S. uvarum* subgenome that carries a *MAT* locus. The loss of this *MAT* gene abolishes the repression of mating activity. In cultures of the resulting fertile alloaneuploid F1 segregants, the cells can conjugate with each other like haploids and form zygotes capable of

performing meiotic divisions producing viable and fertile F2 spores. To the best of our knowledge, this is the first report on breaking down interspecies hybrid sterility by chromosome loss in eukaryotic organisms. The filial generations are genetically unstable and can undergo additional changes mainly in the *S. uvarum* subgenome (directional changes). It is proposed that regaining fertility and subsequent preferential reduction in one of the subgenomes may account for the formation of chimerical ('natural hybrid') genomes found among wine and brewery strains and may also play roles in speciation of hybrid taxa in the *Saccharomyces* genus.

- 6 Sipiczki M 2012 *Pichia bruneiensis* sp. nov., a novel biofilm producing dimorphic yeast species isolated from flowers in Borneo. *Int J System Evol Microbiol* [Epub ahead of print].

Taxonomic analysis of five yeast strains isolated from *Hibiscus* flowers in Brunei (Borneo) is described. The strains represent a dimorphic, biofilm-producing, anamorphic budding yeast species for which the name *Pichia bruneiensis* is proposed. *P. bruneiensis* alternates between yeast and pseudohyphal modes of growth. Its pseudohyphae form biofilms on the surface of liquid media and penetrate into solid substrates. The sequences of the D1/D2 domains of the large subunit rRNA genes, the ITS (internal transcribed spacer) regions, and the 18S rRNA genes were identical in the strains and indicated close phylogenetic relationship with teleomorph species of *Pichia*. In a phylogenetic analysis of these sequences, the

closest relative of the new species was *Pichia fermentans* (6% nucleotide substitutions and indels in the D1/D2 domain). The type strain is 11-485<sup>T</sup>. It has been deposited in Centralbureau voor Schimmelcultures (Utrecht, the Netherlands) as CBS 12611<sup>T</sup>, the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary) as NCAIM Y.02019T and the Culture Collection of Yeasts (Bratislava, Slovakia) as CCY 29-189-1<sup>T</sup>. The GenBank accession numbers for nucleotide sequences of *P. bruneiensis* are JQ692181 (D1/D2 domain of the 26S rRNA gene) and JX112045 (18S rRNA gene and ITS1-5.8S-ITS2). Mycobank: MB 800537.

- 7 Sipiczki M 2012 Detection of yeast species also occurring in substrates associated with animals and identification of a novel dimorphic species in *Verbascum* flowers from Georgia. Antonie van Leeuwenhoek [Epub ahead of print].

The molecular taxonomic analysis of yeasts isolated from *Verbascum* flowers collected in central Georgia identified strains that could be assigned to the species *Cryptococcus adeliensis*, *Cryptococcus magnus* and *Moniliella megachiliensis* detected previously also in substrates associated with insects and other animals and a hitherto undescribed species for which the name *Candida verbasci* is proposed. The new species forms slightly pink colonies, propagates by mostly unipolar budding, forms invasive pseudomycelium, and the sequences of its D1/D2 LSU rRNA genes and ITS1-5.8S-ITS2 regions indicate close phylogenetic relationship with a group of species that

form a cluster basal to the *Candida albicans*/*Lodderomyces elongisporus* clade. The type strain is 11-1055(T). It has been deposited in Centralbureau voor Schimmelcultures (Utrecht, the Netherlands) as CBS 12699(T), the National Collection of Agricultural and Industrial Microorganisms (Budapest, Hungary) as NCAIM Y.02048(T) and the Culture Collection of Yeasts (Bratislava, Slovakia) as CCY 29-185-1(T). The GenBank accession numbers for nucleotide sequences of the *C. verbasci* type strain are: JX515981 (D1/D2 domain of the 26S rRNA gene) and JX515982 (ITS1-5.8S-ITS2). Mycobank: MB 801391.

The following papers were published during 2011.

- 1 Jeyaram, K, Tamang, JP, Capece A & Romano P 2011 Geographical markers for *Saccharomyces cerevisiae* strains with similar technological origins domesticated for rice-based ethnic fermented beverages production in North East India. *Antonie van Leeuwenhoek* 100:569-578.

Autochthonous strains of *Saccharomyces cerevisiae* from traditional starters used for the production of rice-based ethnic fermented beverage in North East India were examined for their genetic polymorphism using mitochondrial DNA-RFLP and electrophoretic karyotyping. Mitochondrial DNA-RFLP analysis of *S. cerevisiae* strains with similar technological origins from *hamei* starter of Manipur and *marcha* starter of Sikkim revealed widely separated clusters based on their geographical origin. Electrophoretic karyotyping showed high polymorphism amongst the *hamei* strains within similar mitochondrial DNA-RFLP cluster and one unique karyotype of *marcha* strain was widely distributed in the Sikkim-Himalayan region. We conceptualized the possibility of separate

domestication events for *hamei* strains in Manipur (located in the Indo-Burma biodiversity hotspot) and *marcha* strains in Sikkim (located in Himalayan biodiversity hotspot), as a consequence of less homogeneity in the genomic structure between these two groups, their clear separation being based on geographical origin, but not on technological origin and low strain level diversity within each group. The molecular markers developed based on *Hinf*I-mtDNA-RFLP profile and the chromosomal doubles in chromosome VIII position of Sikkim-Himalayan strains could be effectively used as geographical markers for authenticating the above starter strains and differentiating them from other commercial strains.

- 2 Tamang JP, Tamang N, Thapa S, Dewan S, Tamang BM, Yonzan H, Rai AK, Chettri R, Chakrabarty J & Kharel N 2012 Microorganisms and nutritional value of ethnic fermented foods and alcoholic beverages of North East India. *Indian J Traditional Knowledge* 11:7-25.

Very few have realized that the North East India is the centre of the diverse food culture comprising fermented and non-fermented ethnic foods and alcoholic beverages. More than 250 different types of familiar and less-familiar ethnic fermented foods and alcoholic beverages are prepared and consumed by the different ethnic people of North East India, which include milk, vegetable, bamboo, soybean, meat, fish, cereal and alcoholic beverages. Diverse microorganisms ranging from filamentous fungi to enzyme and alcohol producing yeasts, lactic acid bacteria, bacilli and micrococci are associated with fermentation and production of ethnic foods and alcoholic drinks. Ethnic

foods are fermented naturally, except the alcoholic beverages which are produced by using consortia of microorganisms in the form of dry, cereal-based starter. Diversity within the species of lactic acid bacteria and bacilli has created the ethnic foods with different sensory characteristics. We have demonstrated that functional microorganisms present in the ethnic fermented foods of North East have many biological functions enhancing the health-promoting benefits, bio-preservation of perishable foods, bio-enrichment of nutritional value, protective properties and therapeutic values.

Presentations originating in my laboratory.

- 1 Burke C 2012 Migration of the sexual nuclei of *Metschnikowia hawaiiensis*. Ontario Biology Day. Laurentian University, Sudbury, Ont (March 2012).
- 2 Perri A 2012 Population structure and genetic diversity of the yeast species *Kurtzmaniella cleridarum*. Ontario Biology Day. Laurentian University, Sudbury, Ont (March 2012).
- 3 Burke C 2012 Migration of the sexual nuclei of *Metschnikowia hawaiiensis*. Great Lakes St.Lawrence Mycology Workshop, UWO, London, Ontario (April 2012).

- 4 Perri A, Farahbakhsh AS & Lachance MA 2012 Biogeography of *Kurtzmaniella cleridarum*, a yeast of the Sonoran Desert. Great Lakes St. Lawrence Mycology Workshop, UWO, London, Ontario (April 2012).
- 5 Lachance MA 2012 *Metschnikowia*: a model yeast for biogeography and speciation. 1st Joint Congress on Evolutionary Biology, Ottawa, Ontario (June 2012).
- 6 Lachance MA 2012 Circumscription of species and genera. Workshop on Yeast Systematics and Classification, 13th International Congress on Yeasts, Madison, Wisconsin.
- 7 Lachance MA, JD Collens, AS Farahbakhsh, AM Perri & AM Wardlaw 2012 Biogeography of endemic yeasts: towards a landscape genetics approach. 13th International Congress on Yeasts, Madison, Wisconsin.

Recently accepted papers.

- 8 Lachance MA 2011 Yeasts. In: eLS [based in part on the previous version of this Encyclopedia of Life Sciences (LS) article, Yeasts by Herman J Phaff]. John Wiley & Sons, Ltd, Chichester, pp. 1-12. DOI: 10.1002/9780470015902.a0000380.pub2
- 9 Cadete RM, Melo MA, Dussán KJ, Rodrigues RCLB, Silva SS, Zilli JE, Vital MJS, Gomes FCO, Lachance MA & Rosa CA 2012. Diversity and physiological characterization of D-xylose-fermenting yeasts isolated from the Brazilian Amazonian forest. PLoS One 7(8):e43135.
- 10 Thanh VN, DA Hai, DDuc Hien, M Takashima & MA Lachance 2012 *Moniliella carnis* and *Moniliella dehoogii*, two new species of black yeasts isolated from meat processing environments. Int J Syst Evol Microbiol 62:3089-3095.
- 11 Cadete RM, Melo MA, Zilli JE, Vital MJS, Mouro A, Prompt AH, Gomes FCO, Stambuk BU, Lachance MA & Rosa CA - *Spathaspora brasiliensis* sp. nov., *Spathaspora suhii* sp. nov., *Spathaspora roraimanensis* sp. nov. and *Spathaspora xylofermentans*, sp. nov., four novel D-xylose-fermenting yeast species from Brazilian Amazonian Forest. Antonie van Leeuwenhoek (Epub ahead of print).
- 12 Maciel NOP, Pilo FB, Freitas LFD, Gomes FCO, Johann S, Nardi RMD, Lachance MA & Rosa CA 2012 The diversity and antifungal susceptibility of the yeasts isolated from coconut water and reconstituted fruit juices in Brazil. Int J Food Microbiol 160:201-205.
- 13 Safar SVB, Gomes FCO, Guimarães ARM, Lachance MA & Rosa CA - In press - *Kazachstania rupicola* sp. nov., a yeast species isolated from water tanks of a bromeliad in Brazil. Int J Syst Evol Microbiol (Epub ahead of print).
- 14 Badotti F, Silva PAB, Mendonça MC, Gomes FCO, Morais PB, Lachance MA & Rosa CA - In press - *Wickerhamiella dulcicola* sp. nov. and *Wickerhamiella cachassae* sp. nov., two yeasts isolated from cachaça fermentation in Brazil. Int J Syst Evol Microbiol (Epub ahead of print).

The following papers, whose abstract were given in the last issue, have now appeared in print.

- 15 Cadete RM, Melo MA, Lopes MR, Pereira GM, Zilli JE, Vital MJ, Gomes FC, Lachance MA & Rosa CA 2012 *Candida amazonensis* sp. nov., an ascomycetous yeast isolated from rotting wood in Amazonian Forest, Brazil. Int J Syst Evol Microbiol 62:1438-1440.
- 16 de Vega C, Guzmán B, Lachance MA, Steenhuisen SL, Johnson SD & Herrera CM 2012. *Metschnikowia proteae* sp. nov., a nectarivorous insect-associated yeast species from Africa. Int J Syst Evol Microbiol 62:2538-2545.
- 17 Lachance MA, Rosa CA, Carvajal EJ, Freitas LFD & Bowles JM 2012 *Saccharomycopsis fodiens* sp. nov., a rare predacious yeast from three distant localities. Int J Syst Evol Microbiol 62:2793-2798.

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## Forthcoming Meeting

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ISSY30 - Cell Surface and Organelles in Yeasts: from Basics to Applications

High Tatras - Stará Lesná, Slovakia

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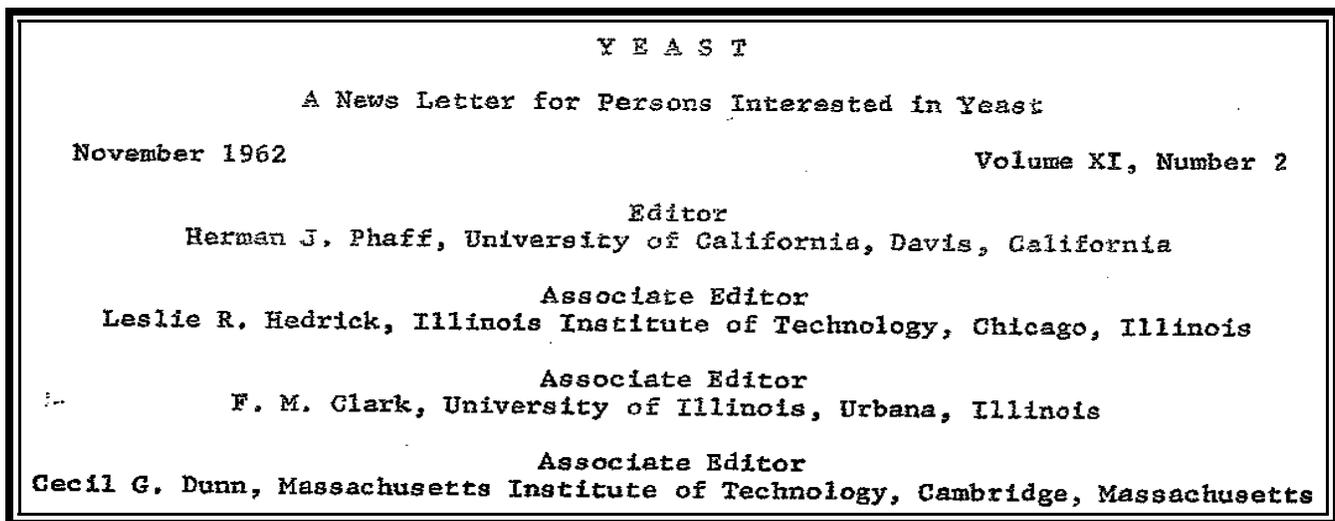
The 30th International Specialized Symposium on Yeast will be held June 18-22, 2013 in Stará Lesná (High Tatras), Slovakia. The theme of the symposium will be: "Cell Surface and Organelles in Yeasts: from Basics to Applications". The conference center of the Slovak Academy of Sciences in Stará Lesná is located at the foothills of the magnificent High Tatras, an area of natural beauty and the rich cultural heritage.

Ivan Hapala and Peter Griac (co-chairs of the 30th ISSY)

[www.issy2013.org](http://www.issy2013.org)

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## 50 Years Ago



### [Yeast Newsletter - November 1962 - Vol. XI, No. 2](#)

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

Mrs. N. J. W. Kreger-van Rij of the Centraalbureau voor Schimmelcultures, Yeast Division, Delft, Holland reported receipt of type strains of 13 new yeast species whose descriptions were published in 1962.

Dr. Juan Santa Maria of Instituto Nacional de Investigaciones Agronomicas, Madrid, Spain reported publication of the description of *Saccharomyces hienipiensis*, and forthcoming publication of the description of *Torulopsis salmanticensis*.

Drs. I. Banno and T. Hasegawa of the Institute for Fermentation, Osaka, Japan, reported:

made. When yeast cell types of auxotrophic double mutants of *Rhodotorula gracilis* and *Rh. koishikawensis* were mixed together in glucose-yeast extract-salt medium, prototrophic colonies appeared on minimal medium only from the mixed culture. Furthermore, fusing cells in pair were observed among the mixed cells by microscopic examination. It was, therefore, manifest that prototrophic colonies resulted from an interaction of cells of the two parental strains. It is noteworthy that the prototrophic growth consists of mycelium with clamp connections at septa. The fact that the mycelium has clamp connections suggests that the mycelial growth of this organism probably corresponds to secondary, dikaryon mycelium observed generally in Basidiomycetes.

Dr. **Shoji Goto** of the R. Institute of Fermentation, Yamanashi University, Kofu, Japan described taxonomic studies of film-forming yeast strains from the institution's collection using the system of Lodder and Kreger-van Rij. Many strains were assigned new species names, and new species and varieties were proposed, published in the Bull. R. Inst. Of Fermentation, Yamanashi Univ., 1962.

Prof. **Akira Yuasa**, University of Tokyo, shared details of the Seminar of Yeast-Cell Studies, with monthly meetings attended by about 80 members. Subjects of speeches included cytological studies, nuclear structure, salt tolerance, spore formation, UV mutation, and sake and beer yeasts. The October 1961 annual meeting in Tokyo was attended by 100 investigators.

Drs. **M. Shifrine** and **A.G. Marr**, University of California, Davis presented a summary of a paper on the requirements of fatty acids by *Pityrosporum ovale*, submitted to the Journal of General Microbiology.

Dr. **A. H. Rose**, University of Durham, England listed recently published and ongoing research in several areas including psychrophilic *Cryptococcus*, the role of biotin in the regulation of enzyme synthesis in *Saccharomyces cerevisiae*, and genetic analyses of adenine-deficient mutants of *Schizosaccharomyces pombe*.

Dr. **J. O. Lampen**, Insittute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey, USA listed several publications on the mechanism of action of polyene antifungals, and localization of sucrose and maltose fermenting systems.

Dr. **F. M. Clark**, University of Illinois described ongoing analysis of inositol compounds in the yeast *Schizosaccharomyces pombe*, and ascospore formation.

Mr. **J. Wynants** of Brasserie St. Josse, Bruxelles, Belgium observed that when revived, freeze-dried brewery yeast cultures generated a surprising number of abnormally small colonies. The dwarf colonies had lost their respiration abilities, and were morphologically and physiologically similar to the Ephrussi respiration-deficient mutants. They proposed that the lyophil process induces formation of these "Petites Colonies".

Dr. **John Kleyn** of the Sicks' Rainier Brewing Company, Seattle, WA, USA announced the following publication:

Dr. **A. Demain** of Merck Sharp and Dohme, Rahway, New Jersey, USA compared utilization of D and L isomers of

1. **Published: J. Kleyn, R. Mildner, and W. Riggs. Yeast viability as determined by methylene blue staining. The Brewers Digest 37, 6, 42, 1962.**

alpha-aminoadipic acid by *S. cerevisia* and *Neurospora crassa*.

Dr. **H. J. Phaff**, University of California, Davis, CA, USA described ongoing research in several areas. Dr. **Michael Lewis** continued work on release of nitrogenous substances from brewer's yeast during storage; Dr. **J. F. T. Spencer** studied taxonomy and ecology of yeasts isolated from flowers, shrimp, streams and lakes; and **H. Tanaka** isolated a *Bacillus circulans* strain from soil that could hydrolyze baker's yeast cell walls.

Dr. **K. Vas**, Institute of Food Technology and Microbiology, Budapest, studied the relation of yeast growth rate to gas production during fermentation, thermal decomposition of yeast cell mass, the effect of ionizing radiation on yeast cells, and microbiological stabilization of fruit juices.

Dr **C. Lindegren**, Biological Research Laboratory, Carbondale, Il, USA announced the following publication:

- Lindegren, C. C., Bang, Y. N. and Hirano, T. Progress report on the zymophage. Proc. N. Y. Academy of Science. 24: 540-566 (1962).**

<http://onlinelibrary.wiley.com/doi/10.1111/j.2164-0947.1962.tb01431.x/abstract>

<http://link.springer.com/article/10.1007%2F978-1-4939-9841-7>