Yeast

A Newsletter for Persons Interested in Yeast

ISSN 0513-5222

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

JUNE 2012 **Volume LXI, Number I**

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

http://publish.uwo.ca/~lachance/YeastNewsletter.html

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

Recent publication.

1 Golubev WI. 2012. Mycocinotyping. Mykologia Phytopathologia 46:1-13.

In this review the use of mycocinotyping as a taxonomic tool is considered. The determination of mycocin sensitivity patterns of cultures to a panel of selected mycocinogenic strains is useful in taxonomy and identification as well as in ecology of yeasts. The potential and limitations of mycocinotyping critically evaluated.

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

Invited lecture delivered at the 40th Annual Yeast Conference in Smolenice, Slovakia.

1 Kopecká M. On the nature, ultrastructure and assembly of the yeast cell wall.

Papers published in journals.

2 Marie Kopecká & Masashi Yamaguchi. 2011. Ultrastructural disorder of actin mutant suggests uncoupling of actin-dependent pathway from microtubule-dependent pathway in budding yeast. J Electron Microscopy 60:379-391 - doi: 10.1093/jmicro/dfr073

Temperature-sensitive actin mutant of *Saccharomyces cerevisiae* act1-1 was studied at a permissive temperature of 23°C by light, fluorescent and electron microscopy to elucidate the roles of actin cytoskeleton in the cycling eukaryotic cells. Mutant cells that grew slowly at the permissive temperature showed aberrations in the cytoskeleton and cell cycle. Mutant cells contained aberrant 'faint actin cables,' that failed in directing of mitochondria, vacuoles and secretory vesicles to the bud and the stray vesicles delivered their content to the mother wall instead of the bud. Bud growth was delayed. Spindle pole bodies and cytoplasmic microtubules did not direct to the bud, and nucleus failed to migrate to the bud. Repeated nuclear divisions produced multinucleated cells, indicating continued cycling of actin mutant cells that failed in the morphogenetic checkpoint, the spindle position checkpoint and cytokinesis. Thus, a single actin mutation appears to indicate uncoupling in space and time of the 'actin cytoskeleton-dependent cytoplasmic pathway of bud development and organelle positioning and inheritance' from the 'microtubule-dependent nuclear division pathway' in a budding yeast cell cycle.

3 Marie Kopecká, Wladyslav Golubev, Vladimíra Ramíková, Dobromila Klemová & Ladislav Ilkovics. 2011. Ultrastructural characteristics and variability of vegetative reproduction in *Fellomyces penicillatus*. J Basic Microbiol 51:1-8.

Papers sent to press.

4 Marie Kopecká, Soichi Yoshida &Masashi Yamaguchi - Actin rings around cell nucleus in long neck yeast. J Electron Microscopy.

5 Marie Kopecká - Self-assembly of fungal cell wall polysaccharides to the cell wall-like structure *in vitro*. J Electron Microscopy.

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>.

We are grateful to Dr. Ching-Fu Lee (National Hsinchu University, Taiwan) for collaboration during our stay in his lab and yeast expeditions in November 2011. Many thanks to Organizing Committee for the invitation to participate at the Symposium & Inauguration of CBC "Towards integrated biological control" on 19-20 April 2012, Uppsala, Sweden.

The following are papers for 2011–2012 or in press.

1 Naumoff DG. 2011. Hierarchical classification of glycoside hydrolases. Biochemistry (Moscow). 76:622–635. © Pleiades Publishing, Ltd.

2 Naumoff DG, Stepuschenko OO. 2011. Endo-α-1,4-polygalactosaminidases and their homologues: structure and evolution. Molecular Biology (Moscow). 45:647–657. © Pleiades Publishing, Ltd.

3 Naumoff DG. 2011. GHL1-GHL15: new families of the hypothetical glycoside hydrolases. Molecular Biology (Moscow). 45:983–992. © Pleiades Publishing, Ltd.

4 Naumoff DG. 2012. Furanosidase superfamily: search of homologues. Molecular Biology (Moscow). 46:322–327. © Pleiades Publishing, Ltd.

5 Naumova ES, Naumov GI, Nikitina TN, Sadykova AZh, Kondratieva VI. 2012. Molecular-genetic and physiological differentiation of *Kluyveromyces lactis* and *Kluyveromyces marxianus* yeasts: analysis of strains from All-Russian collection of microorganisms (VKM). Microbiology (Moscow). 81:216–223. © Pleiades Publishing, Ltd.

Molecular genetic identification of 52 *Kluyveromyces* strains from VKM, mainly of dairy origin, was carried out. Restriction analysis of 5.8S-ITS rDNA fragments was used to differentiate between *Kl. lactis* var. *lactis*, *Kl. lactis* var. *drosophilarum* (European population of "krassilnikovii"), and *Kl. marxianus*. *Kl. lactis* was shown to differ from *Kl. marxianus* in its ability to assimilate α-glucosides: maltose, melezitose, and α-methyl-glucoside.

5 Naumov GI. 2012. Genus assignment of small-spored aquatic and terrestrial species of the *Metschnikowia* yeasts. Microbiology (Moscow). 81:263–265. © Pleiades Publishing, Ltd.

6 Naumov G.I., Naumoff D.G. 2012. Molecular genetic differentiation of yeast α-glucosidases: maltases and isomaltases. Microbiology (Moscow). 81:278–282. © Pleiades Publishing, Ltd.

The review is dedicated to the molecular genetics of yeast α-glucosidases: the maltase and isomaltase isozymes. Comparative analysis of the genome sequence of the yeast *Saccharomyces cerevisiae* S288C using the isomaltase gene of *Saccharomyces cerevisiae* ATCC56960 revealed a new family of polymeric isomaltase genes *IMA1–IMA5* located in the telomeric regions of chromosomes VII, XV, IX, X and X, respectively. The isomaltase overexpression and substrate specificity are discussed.

7 Chang C-F, Liu Y-R, Chen S-F, Naumov G I, Naumova E S, Lee C-F. 2012. Five novel species of the anamorphic genus *Candida* in the *Cyberlindnera* clade isolated from natural substrates in Taiwan. Antonie van Leeuwenhoek. 101 (1; on line).

8 Daniel H.-M., Redhead S.A., Schnürer J., Naumov G.I., Kurtzman C.P. 2012. (2049–2050) Proposals to conserve the name *Wickerhamomyces* against *Hansenula* and to reject the name *Saccharomyces sphaericus* (*Ascomycota:Saccharomycotina*). Taxon (on line 16 Mar. 2012).

9 Naumov G.I., Naumova E.S., Kondratieva V.I., Chen G.-Y., Lee C.-F. 2012. Killer activity of *Williopsis* Zender yeasts: study of Taiwanese populations. Mikologiya i Fitopatologiya (in press).

Killer activity of 59 Taiwanese strains of the genus *Williopsis* has been studied. *Williopsis* strains from other world regions were used for comparison. The studied Taiwanese strains of *W. saturnus*, *W. mrakii*, *W. suaveolens* and *W. subsufficiens* were shown to have killer activity. The data obtained suggest ecological role of mycocins in soil yeasts.

10 Naumov GI, Naumova ES, Lee C-F. 2012. *Williopsis* Zender and *Zygowilliopsis* Kudriavzev are natural biocontrol yeasts. Symposium & Inauguration of CBC "Towards integrated biological control", 19-20 April 2012, Uppsala, Sweden, P. 59.

The yeasts *Williopsis* and *Zygowilliopsis* are associated with different types of soil. Strains of *Z. californica* are frequently isolated from the rhizosphere of cultured root crop plants (Vustin & Babjeva, 1981), whereas *W. suaveolens* and *W. saturnus* inhabit soils of Northern and Southern regions, respectively (Naumova et al., 2004). Species of both genera can be also isolated from plant surface. The genera *Williopsis* and *Zygowilliopsis* contain phenotypically similar sibling species (Naumov, 1987; Naumov et al., 2009). For instance, six biological species are identified in the genus *Williopsis* based on genetic hybridization analysis: *W. beijerinckii*, *W. mrakii*, *W. saturnus*, *W. suaveolens*, *W. sargentensis* and *W. subsufficiens*. The species of both genera are able to produce killer toxins (mycocines) with wide-spectrum activity toward, at least, different yeasts (Rosini, 1983; Nomoto et al., 1984; Vustin et al., 1988; Hotgon et al., 1995; Guyard et al., 2002). We study killer activity of *Williopsis* and *Zygowilliopsis* strains isolated from soil, leaves and mushrooms in different regions in Taiwan. Results of the killer experiments will be presented. Most of the 59 *Williopsis* strains under examination have been identified as *W. suoveolens*, *W. saturnus* and *W. mrakii*. Independently of species assignment, all strains were able to produce killer toxins. *Zygowilliopsis* strains studied were polymorphic on the ability to produce mycocines. The data obtained suggest ecological role of mycocins in natural populations of *Williopsis* and *Zygowilliopsi* yeasts. It is expedient to study intra- and interspecific evolution of killer toxins of *Williopsis* and *Zygowilliopsis* yeasts. It was shown that nucleotide sequences of the genes encoding killer proteins in *W. saturnus* and *W. mrakii* are very similar but not identical: 82% of similarity (Kimura et al., 1993, 1994). Killer toxin of *W. subsufficiens* is more divergent, while the toxins of *Williopsis* and *Zygowilliopsis* are not related. The yeasts *Williopsis* and *Zygowilliopsis* are of interest for evolutionary and population genetic investigations. The yeasts are also attractive for antimicrobial antagonism studies.

IV Food Science and Technology Program, Dalhousie University, D401-1360 Barrington St, PO Box 15000, Halifax, Nova Scotia, Canada B3H 2R2. Communicated by A Speers. <Alex.Speers@Dal.Ca>.

Peer reviewed contributions.

1 Speers RA (Editor). 2012. Yeast Flocculation, Vitality and Viability: Proceedings of the 2nd International Brewers Symposium. Master Brewers Association of the America's, St. Paul, MN. (~155 pages).

2 Lake JC & Speers R.A., 2012. Investigations of malt causing PYF. International Brewers Symposium: Yeast Flocculation, Vitality and Viability. R.A. Speers (Ed). Master Brewers Association of the America's, St. Paul, MN. (pp. 59-70).

3 Speers RA. 2012. Measurement of yeast flocculation. International Brewers Symposium: Yeast Flocculation, Vitality and Viability. R.A. Speers (Ed). Master Brewers Association of the America's, St. Paul, MN. (pp. 17-24).

4 Speers RA. 2012. A review of yeast flocculation. International Brewers Symposium: Yeast Flocculation, Vitality and Viability. R.A. Speers (Ed). Master Brewers Association of the America's, St. Paul, MN. (pp. 1-16).

5 Speers, RA & Eck E. 2012. Miniature fermentation assay. ASBC Methods of Analysis, Wort-22. American Society of Brewing Chemists, St. Paul, MN, In press.

6 MacIntosh AJ, Adler J, Eck E, Speers RA. 2012. Suitability of the miniature fermentability method to monitor industrial fermentations. Accepted J ASBC April 2012.

Presentations.

7 MacIntosh AJ & Speers RA. 2012. Mechanisms and measurement of yeast flocculation in the laboratory and production plant. Invited Presentation. The 13th International Congress on Yeasts, Madison, WI, Aug. 26-Aug. 30.

8 MacIntosh AJ, McKinnon M & Speers RA. 2012. Determination of fermentor shear through empirical and theoretical methods. Accepted for presentation at the World Brewing Congress, Portland, OR, July 28-Aug. 1.

9 Adler J & Speers RA. 2012. Threshold detection of premature yeast flocculation inducing malt using the miniature fermentation assay. Accepted for presentation at the World Brewing Congress, Portland, OR, July 28-Aug. 1.

10 Mishra A & Speers RA. 2012. Understanding and evaluating the effect of wort boil time and trub levels on malt fermentability with the miniature fermentation method. Accepted for presentation at the World Brewing Congress, Portland, OR, July 28-Aug. 1.

11 Bourque C & Speers RA. 2012. Fermentability of Canadian two row malting barley varieties: Wort turbidity, density, and sugar content as measures of fermentation potential. Accepted for presentation at the World Brewing Congress, Portland, OR, July 28-Aug. 1.

12 Speers RA. 2012. Fungally contaminated malting barley and its effect on beer fermentations, Invited Presentation. Post Harvest Management and Technology for Food Security. Jimma, ETH May 21 25, 2012

13 Webster G, Koutoulis A, Stewart D, Janusz A, Speers RA, Bowman J, Evans E, & Kaur M. 2012. Barley grain associated microbial community dynamics during malting and their impact on malt quality. Presented at the IBD Asia Pacific Sec. Melbourne, AUS Mar. 25-30.

V 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>.

Recent publications.

1 de Smidt O, du Preez JC. and Albertyn J. 2012. Molecular and physiological aspects of alcohol dehydrogenases in the ethanol metabolism of *Saccharomyces cerevisiae*. FEMS Yeast Research 12:33-47.

The physiological role and possible functional substitution of each of the five alcohol dehydrogenase (Adh) isozymes in Saccharomyces cerevisiae were investigated in five quadruple deletion mutants designated strains Q1–Q5, with the number indicating the sole intact *ADH* gene. Their growth in aerobic batch cultures was characterised in terms of kinetic and stoichiometric parameters. Cultivation with glucose or ethanol as carbon substrate revealed that Adh1 was the only alcohol dehydrogenase capable of efficiently catalysing the reduction of acetaldehyde to ethanol. The oxidation of produced or added ethanol

could also be attributed to Adh1. Growth of strains lacking the ADH1 gene resulted in the production of glycerol as a major fermentation product, concomitant with the production of a significant amount of acetaldehyde. Strains Q2 and Q3, expressing only *ADH2* or *ADH3*, respectively, produced ethanol from glucose, albeit less than strain Q1, and were also able to oxidise added ethanol. Strains Q4 and Q5 grew poorly on glucose and produced ethanol, but were neither able to utilise the produced ethanol nor grow on added ethanol. Transcription profiles of the *ADH4* and *ADH5* genes suggested that participation of these gene products in ethanol production from glucose was unlikely.

2 Ells, R., Kock, JLF, Albertyn J, Hugo A and Pohl CH. 2012. Sciadonic acid modulates prostaglandin E_2 production by epithelial cells during infection with *C. albicans* and *C. dubliniensis*. Prostaglandins and other lipid mediators. 97: 66-71.

Candida albicans is an important opportunistic pathogen in humans. During infection, arachidonic acid (w6) is released from host phospholipids, leading to the production of host and yeast derived prostaglandin E_2 (PGE_2). This stimulates yeast hyphal formation, is immunomodulatory and causes cell damage during infection. Although supplementation of mammalian cells with w3 fatty acids has received attention due to their immunomodulatory and anti-inflammatory activities, increased production of w3 fatty acid metabolites could lower the host's ability to combat infections. Since mammalian cells cannot produce PGE_2 from sciadonic acid (SA), a non-methylene interrupted w6 fatty acid (NMIFA), supplementation of cells with SA may decrease the production of PGE_2 without increasing levels of w3 fatty acid metabolites. Our study evaluated PGE_2 production by SA supplemented epithelial cells in response to *Candida albicans* and *C. dubliniensis*. We show that PGE_2 production during infection can be modulated by incorporation of SA into host lipids and that this does not influence the levels of w3 fatty acids in the epithelial cells.

3 Sebolai OM, Pohl CH, Kock JLF, Chaturvedi V, del Poeta M. 2012. The presence of 3-hydroxy oxylipins in pathogenic microbes. Prostaglandins and other lipid mediators. 97: 17-21.

There is a sufficient body of work documenting the distribution of 3-hydroxy oxylipins in microbes. However, there is limited information on the role of these compounds in microbial pathogenesis. When derived from mammalian cells, these compounds regulate patho-biological processes, thus an understanding of 3-hydroxy oxylipin function and metabolism could prove important in shedding light on how these compounds mediate cellular pathology and physiology. This could present 3-hydroxy oxylipin biosynthetic pathways as targets for drug development. In this minireview, we interrogate the relevant yeast and bacterial 3-hydroxy oxylipin literature in order to appreciate how these compounds may influence the inflammatory response leading to disease development.

VI Institute of Life Science, 4-8-15 Hagoromo, Takaishi-shi, Osaka 592-0002, Japan. Communicated by Akihiro Ota <akihirootago@yahoo.co.jp>.

Recent publications.

1 Ota A 2010 Prevention against infectious disease by *E. coli* O157:H7. Hypothesis of Life Science 1, 1:592-0002

2 Ota A 2012 Protection from disease by dysentery bacilli. in preparation.

Yoghurt containing the yeast *Saccharomyces florentinus* (Kefir) is effective against infection and in the cure of dysentery.

3 Ota A 2012 Effect of oxidative stress on heat, salt, acid and other substances in yeast. in preparation.

Given oxidative stress, the yeast will gain the tolerance of heat, salt, acid and other substances stress.

Paper presented at Biochemistry and Molecular Biology Congress held in Japan from December 7 to 10, 2010.

4 Tarumoto Y, Kanoh J & Ishihara F - 1T2-6: Cpc2/ Rack 1 facilitates adaptive response to environmental changes in fission yeast.

The fission yeast has obtained tolerance to heat after treatment under the condition of oxidative stress in no more than 60 min.

Recent activities.

A semiannual meeting of a yeast researchers' society was held at the institute of the sake maker, Kizakura Co., Kyoto, Japan on March 11, 2011. The presentations followed four subjects: application of yeast gene splicing and mapping; fragrance components of sake and the mechanism of their yields; control of gene expression; steady brewing of sake yeasts from bacteria contaminated culture. In the last subject, it was shown the process how to get rid of contaminated bacteria in the culture medium and to get the pure brewing sake yeast.

On March 6, 2012, a semiannual meeting of yeast researchers' society was held at the beer brewery, Kirin Co. Kobe, Japan. The presentations were following four subjects: chromosome mutation of *Candida albicans*; exclusion of damaged mitochondria of *Saccharomyces cerevisiae*; peptide uptake by the genes of yeast *Saccharomyces*; methods for making high quality beer using beer yeast.

VII Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. Communicated by B.A. Prior <bap@sun.ac.za>.

Recent publications.

1 Bester MC, Jacobson D, Bauer FF 2012 Many *Saccharomyces cerevisiae* cell wall protein encoding genes are coregulated by Mss11, but cellular adhesion phenotypes appear only Flo protein dependent. G3 (Bethesda) 2:131-41.

The outer cell wall of the yeast *Saccharomyces cerevisiae* serves as the interface with the surrounding environment and directly affects cell-cell and cell-surface interactions. Many of these interactions are facilitated by specific adhesins that belong to the Flo protein family. Flo mannoproteins have been implicated in phenotypes such as flocculation, substrate adhesion, biofilm formation, and pseudohyphal growth. Genetic data strongly suggest that individual Flo proteins are responsible for many specific cellular adhesion phenotypes. However, it remains unclear whether such phenotypes are determined solely by the nature of the expressed *FLO* genes or rather as the result of a combination of *FLO* gene expression and other cell wall properties and cell wall proteins. Mss11 has been shown to be a central element of *FLO1* and *FLO11* gene regulation and acts together with the cAMP-PKA-dependent transcription factor Flo8. Here we use genome-wide transcription analysis to identify genes that are directly or indirectly regulated by Mss11. Interestingly, many of these genes encode cell wall mannoproteins, in particular, members of the TIR and DAN families. To examine whether these genes play a role in the adhesion properties associated with Mss11 expression, we assessed deletion mutants of these genes in wild-type and *flo11Δ* genetic backgrounds. This analysis shows that only *FLO* genes, in particular *FLO1/10/11*, appear to significantly impact on such phenotypes. Thus adhesion-related phenotypes are primarily dependent on the balance of *FLO* gene expression.

2 Rossouw D, Jacobson D, Bauer FF 2012 Transcriptional regulation and the diversification of metabolism in wine yeast strains. Genetics 190:251-61.

Transcription factors and their binding sites have been proposed as primary targets of evolutionary adaptation because changes to single transcription factors can lead to far-reaching changes in gene expression patterns. Nevertheless, there is very little concrete evidence for such evolutionary changes. Industrial wine yeast strains, of the species *Saccharomyces cerevisiae*, are a genotypically and phenotypically diverse group of organisms that have adapted to the ecological niches of industrial winemaking environments and have been selected to produce specific styles of wine. Variation in transcriptional regulation among wine yeast strains may be responsible for many of the observed differences and specific adaptations to different fermentative conditions in the context of commercial winemaking. We analyzed gene expression profiles of wine yeast strains to assess the impact of transcription factor expression on metabolic networks. The data provide new insights into the molecular basis of variations in gene expression in industrial strains and their consequent effects on metabolic networks

important to wine fermentation. We show that the metabolic phenotype of a strain can be shifted in a relatively predictable manner by changing expression levels of individual transcription factors, opening opportunities to modify transcription networks to achieve desirable outcomes.

3 Rossouw D, Du Toit M, Bauer FF 2012 The impact of co-inoculation with *Oenococcus oeni* on the trancriptome of *Saccharomyces cerevisiae* and on the flavour-active metabolite profiles during fermentation in synthetic must. Food Microbiol. 29:121-31.

Co-inoculation of commercial yeast strains with a bacterial starter culture at the beginning of fermentation of certain varietal grape juices is rapidly becoming a preferred option in the global wine industry, and frequently replaces the previously dominant sequential inoculation strategy where bacterial strains, responsible for malolactic fermentation, are inoculated after alcoholic fermentation has been completed. However, while several studies have highlighted potential advantages of co-inoculation, such studies have mainly focused on broad fermentation properties of the mixed cultures, and no data exist regarding the impact of this strategy on many oenologically relevant attributes of specific wine yeast strains such as aroma production. Here we investigate the impact of co-inoculation on a commercial yeast strain during alcoholic fermentation by comparing the transcriptome of this strain in yeast-only and in co-inoculated fermentations of synthetic must. The data show that a significant number of genes are differentially expressed in this strain in these two conditions. Some of the differentially expressed genes appear to respond to chemical changes in the fermenting must that are linked to bacterial metabolic activities, whereas others might represent a direct response of the yeast to the presence of a competing organism.

4 Rossouw D, Jolly N, Jacobson D, Bauer FF 2012 The effect of scale on gene expression: commercial versus laboratory wine fermentations. Appl Microbiol Biotechnol. 93:1207-19.

Molecular and cellular processes that are responsible for industrially relevant phenotypes of fermenting microorganisms are a central focus of biotechnological research. Such research intends to generate insights and solutions for fermentation-based industries with regards to issues such as improving product yield or the quality of the final fermentation product. For logistical reasons, and to ensure data reproducibility, such research is mostly carried out in defined or synthetic media and in small-scale fermentation vessels. Two questions are frequently raised regarding the applicability of this approach to solve problems experienced in industrial fermentations: (1) Is synthetic medium a sufficiently accurate approximation of the generally more complex natural (and frequently highly variable) substrates that are employed in most fermentation-based industries, and (2) can results obtained in small-scale laboratory fermentations be extrapolated to large-scale industrial environments? Here, we address the second question through a comparative transcriptomic approach by assessing the response of an industrial wine yeast strain fermenting a natural grape juice in small-scale laboratory and large-scale industrial conditions. In yeast, transcriptome analysis is arguably the best available tool to holistically assess the physiological state of a population and its response to changing environmental conditions. The data suggest that scale does indeed impact on some environmental parameters such as oxygen availability. However, the data show that small-scale fermentations nevertheless accurately reflect general molecular processes and adaptations during large-scale fermentation and that extrapolation of laboratory datasets to real industrial processes can be justified.

5 Styger G, Prior B, Bauer FF 2011 Wine flavor and aroma. J Ind Microbiol Biotechnol. 38:1145-59.

The perception of wine flavor and aroma is the result of a multitude of interactions between a large number of chemical compounds and sensory receptors. Compounds interact and combine and show synergistic (i.e., the presence of one compound enhances the perception of another) and antagonistic (a compound suppresses the perception of another) interactions. The chemical profile of a wine is derived from the grape, the fermentation microflora (in particular the yeast *Saccharomyces cerevisiae*), secondary microbial fermentations that may occur, and the aging and storage conditions. Grape composition depends on the varietal and clonal genotype of the vine and on the interaction of the genotype and its phenotype with many environmental factors which, in wine terms, are usually grouped under the concept of

"terroir" (macro, meso and microclimate, soil, topography). The microflora, and in particular the yeast responsible for fermentation, contributes to wine aroma by several mechanisms: firstly by utilizing grape juice constituents and biotransforming them into aroma- or flavor-impacting components, secondly by producing enzymes that transform neutral grape compounds into flavor-active compounds, and lastly by the de novo synthesis of many flavor-active primary (e.g., ethanol, glycerol, acetic acid, and acetaldehyde) and secondary metabolites (e.g., esters, higher alcohols, fatty acids). This review aims to present an overview of the formation of wine flavor and aroma-active components, including the varietal precursor molecules present in grapes and the chemical compounds produced during alcoholic fermentation by yeast, including compounds directly related to ethanol production or secondary metabolites. The contribution of malolactic fermentation, ageing, and maturation on the aroma and flavor of wine is also discussed.

6 Jain VK, Divol B, Prior BA, Bauer FF 2012 Effect of alternative NAD^+-regenerating pathways on the formation of primary and secondary aroma compounds in a *Saccharomyces cerevisiae* glycerol-defective mutant. Appl Microbiol Biotechnol. 93:131-41.

Glycerol is a major by-product of ethanol fermentation by *Saccharomyces cerevisiae* and typically 2-3% of the sugar fermented is converted to glycerol. Replacing the NAD(+)-regenerating glycerol pathway in *S. cerevisiae* with alternative NADH reoxidation pathways may be useful to produce metabolites of biotechnological relevance. Under fermentative conditions yeast reoxidizes excess NADH through glycerol production which involves NADH-dependent glycerol-3-phosphate dehydrogenases (Gpd1p and Gpd2p). Deletion of these two genes limits fermentative activity under anaerobic conditions due to accumulation of NADH. We investigated the possibility of converting this excess NADH to NAD(+) by transforming a double mutant (*gpd1 gpd2)* with alternative oxidoreductase genes that might restore the redox balance and produce either sorbitol or propane-1,2-diol. All of the modifications improved fermentative ability and/or growth of the double mutant strain in a self-generated anaerobic high sugar medium. However, these strain properties were not restored to the level of the parental wild-type strain. The results indicate an apparent partial NAD(+) regeneration ability and formation of significant amounts of the commodity chemicals like sorbitol or propane-1,2-diol. The ethanol yields were maintained between 46 and 48% of the sugar mixture. Other factors apart from the maintenance of the redox balance appeared to influence the growth and production of the alternative products by the genetically manipulated strains.

7 Eschstruth A, Divol B 2011 Comparative characterization of endo-polygalacturonase (Pgu1) from *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* under winemaking conditions. Appl Microbiol Biotechnol. 91:623-34.

Wine strains of *Saccharomyces cerevisiae* have no to weak natural pectinase activity, despite their genetic ability to secrete an endo-polygalacturonase. The addition of external pectinase of fungal origin has therefore become a common step of winemaking in order to enhance the extraction of compounds located in the grape berry skins during maceration and to ease wine clarification after maturation. Recently, the strong pectinase activity of a wine strain of *Saccharomyces paradoxus* has been reported. In this study, the endo-polygalacturonase-encoding gene of *S. paradoxus* was sequenced and its activity was characterised, compared with that of *S. cerevisiae* and tested under winemaking conditions through overexpression of both genes individually in *S. cerevisiae*. A few differences in the amino acids sequences between the two proteins were revealed and the activity of the Pgu1 enzyme of *S. paradoxus* was shown to be weaker under winemaking conditions. Clear indicators of extracellular activity were observed in the wines made with both recombinant strains (i.e. enzyme activity in cell-free wine, higher methanol concentration and higher free-run wine), but the actual composition of the wines fermented with the mutants was only sparingly altered. Although unexpectedly found in lower concentrations in the latter wines, phenolic compounds were shown to be the most discriminatory components. Overexpressing the *PGU1* gene of *S. paradoxus* or that of *S. cerevisiae* did not make much difference, showing that the higher activity of *S. paradoxus* strains under laboratory conditions could be due to a different regulation mechanism rather than to a different sequence of *PGU1*.

8 Stone W, Jones BL, Wilsenach J, Botha A 2012 External ecological niche for *Candida albicans* within reducing, oxygen-limited zones of wetlands. Appl Environ Microbiol. 78:2443-2445.

Candida albicans within the human host is well studied; however, identifying environmental reservoirs of pathogens is epidemiologically valuable for disease management. Oxygen-limited, carbohydrate-rich zones of wetlands, to which sewage-borne *C. albicans* is often exposed, are characteristically similar to the gastrointestinal reservoir. Consequently, using quantitative real-time PCR (qRT-PCR) and gas chromatography-mass spectrometry (GC-MS), we demonstrated that oxygen-limited zones in polluted wetlands may act as potential reservoirs of *C. albicans*.

9 van Heerden A, van Zyl WH, Cruywagen CW, Mouton M, Botha A 2012 The lignicolous fungus *Coniochaeta pulveracea* and its interactions with syntrophic yeasts from the woody phylloplane. Microb Ecol 62:609-619.

The yeast-like fungus *Coniochaeta pulveracea* was studied with regard to its novel lignocellulolytic activities and the possible effect thereof on yeasts from the woody phylloplane. An enrichment procedure was used to isolate *C. pulveracea* from a decaying Acacia tree, and the identity of the isolate was confirmed using morphology, as well as molecular and phylogenetic techniques. This isolate, as well as strains representing *C. pulveracea* from different geographical regions, were compared with regard to optimum growth temperature and enzyme activity to representatives of closely related species. These include strains of *Coniochaeta boothii, Coniochaeta rhopalochaeta*, and *Coniochaeta subcorticalis*. Plate assays for cellulase and xylanase activity indicated that all representatives of the above-mentioned species were able to produce extracellular hydrolytic enzymes and were also able to degrade birchwood toothpicks during a 50-day incubation period at 30°C. To test the ability of these fungi and their enzymes to release simple sugars from complex cellulosic substrates, filtrates obtained from liquid cultures of Coniochaeta, cultivated on carboxymethyl cellulose (CMC) as sole carbon source, were analyzed using high-performance liquid chromatography analysis. Consequently, the presence of mono- and disaccharides such as glucose and cellobiose was confirmed in these culture filtrates. Two subsequent experiments were conducted to determine whether these simple sugars released from woody material by *Coniochaeta* may enhance growth of phylloplane yeasts. In the first experiment, representatives of *Coniochaeta* were co-cultured with selected yeasts suspended in agar plates containing birchwood toothpicks, followed by examination of plates for colony formation. Results indicated that *Coniochaeta* growth on the toothpicks enhanced growth of nearby yeast colonies in the agar plates. In the second experiment, representatives of selected yeasts and *Coniochaeta* species were co-cultured on CMC and xylan-containing plates where after yeast colony formation was recorded on the plates. *Saccharomyces cerevisiae* strains, engineered to utilize specific wood degradation products, i.e., cellobiose or xylose, as sole carbon source were used as positive controls. While it was found that cellobiose released from CMC was assimilated by the yeasts, no evidence could be obtained that xylose released from xylan was used as carbon source by the yeasts. These ambiguous results could be ascribed to secretion of nutritious metabolic end products, other than the products of fungal xylanases.

VIII School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England. Communicated by J.A. Barnett <j.barnett@uea.ac.uk>.

Current publication.

1 Barnett JA. 2012. Boris Ephrussi and the discovery of mitochondrial genetics. Wellcome History 49:15-16.

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

The following lecture and posters were presented recently.

1 Kregiel D, Czyzowska A, Ambroziak W. 2012. Apple pomace as a valuable material in yeast biotechnology. Invited lecture. 40th Annual Conference on Yeasts, Smolenice, Slovakia, L12.

In Poland almost 2 million tones of fruits per year are processed into juices, wines and concentrates. Among this number, apples make 60% and apple pomace constitutes 90% of all fruit pomaces. This waste product is the heterogenous substrate consisting of peel, core, seed, calyx, stem and soft tissue. Since the material is rich in high-value components and is highly biodegradable, its utilization is essential from both economical and environmental point of view. The examples of utilization of apple pomace are biomass (SCP), ethanol and other valuable by-products.

Three Polish apple varieties (*Melarose, Champion* and *Gala*) were used to prepare fresh apple pomace, after the juice extraction (dry weight 17.8% and pH 3,7). Yeast cultivation was performed in 100 ml of AP medium [apple pomace (27,5%, w/v), $(NH_4)_2SO_4$ (0,3%, w/v), KH_2PO_4 (0,1%, w/v), $MgSO_4 \cdot 7H_2O$ (0,05%, w/v), yeast extract (0,05%, w/v) and pH 4,5]. The cultivation was conducted under (i) aerobic and (ii) in conditions of limited access of oxygen at 25°C for 4-7 days. Different yeast strains from (i) the Pure Culture Collection LOCK105 and (ii) yeast strains isolated from fresh local fruits were used in this study. The amount of inoculation was established as 5×10^6 CFU per 100 ml of AP medium. The composition of fresh and fermented pomaces was analyzed by HPLC method. The number of viable cells was determined by the plate count method using YGC agar (Merck). The crude protein was determined using the Kjeldahl method.

The fresh apple pomaces contain both high proportion of monosaccharides that can be utilized by yeast and high proportion of compounds that are not utilized by yeasts under tested conditions. After cultivation the number of yeast cells per gram of dry apple pomace increased by 2 to 3 log units. Yeasts isolated from fruits showed a better multiplication, but exhibited a much weaker ability to fermentation. In addition, some strains produced large amounts of acetic acid. The highest biomass was obtained in the case of yeasts belong to genera *Cryptococcus* and *Rhodotorula*. The strains from LOCK105 Collection: *Saccharomyces cerevisiae* 1, *S. cerevisiae* 2 and *Candida* sp. also multiplied in similar efficient way. After aerobic cultivation the crude protein level increased maximally to about 50%. Collection strain *Pichia jadinii* (*Candida utilis)* was very promised since cells demonstrated the ability to produce the highest amounts of biomass and ethanol in conditions of limited oxygen. However, the results for *Saccharomyces cerevisiae* strains are also satisfactory. The results of experiments confirm that the selection of the strains is the crucial step in the choosing of the proper yeasts for specific substrate and special destination.

2 Berlowska J, Kregiel D, Ambroziak W. 2012. Adhesion of the yeast cells to different porous supports and stability of cell - carrier systems. 40th Annual Conference on Yeasts, Smolenice, Slovakia, PH3.

Adhesion to solid surface is the initial stage of biofilm formation. Previous studies on yeast adhesion were mainly concentrated on the pathogenic species *Candida albicans.* Nowadays, the research is also focused on biotechnological application of immobilized industrial yeast strains in different biotechnological processes. Available literature presents numerous immobilization methods suitable for production of various beverages.The most important advantage of using adhered cells is a possibility of employing immobilized systems in continuous processes which retain high cell densities per unit of bioreactor volume and result in very high fermentation rates. The objective of our research was to study how the type of ceramic carrier (hydroxylapatite and chamotte) influence on cells attachment. The industrial fermentative *Saccharomyces* strains and unconventional amylolytic strain *Debaryomyces occidentalis* were used in this study. The immobilization of selected yeasts on hydroxylapatie carrier was rather weak. The adhesion on hydroxylapatite carriers in wort broth had reversible character and better results of adhesion were observed in the case of another ceramic carrier - more porous chamotte tablets. The number of immobilized cells was about 10^6,10^7 per tablet. The carriers with immobilized cells were transferred into the fermentation medium M_o with 12% of glucose. The

cell-carrier systems were characterized by a differential stability in the fermentation process. In a case of chamotte, changing the culture medium to fermentation medium did not cause destabilization of cell-carrier systems. However, the number of free cells in the medium was relatively big because the immobilized cells accounted approx. 20-70% of all cells in the fermentation samples. Selecting a suitable carrier and proper yeast strains for stabile cell-carrier system seems to be very important step to useful immobilized cell technology. The main efforts should be concentrated on cheap, abundant, non-destructive and stable carriers, which will not influence on aroma profiles and specific taste of the final product. According to our results we can formulate a conclusion that the construction of efficient cell-carrier systems is possible only experimentally.

3 Kunicka-Styczynska A, Rajkowska K. 2012. Acidic stress as a potential factor in adaptive evolution of wine yeasts. 40th Annual Conference on Yeasts, Smolenice, Slovakia, PH6.

Wine yeasts *Saccharomyces* sensu stricto are known for their genome plasticity. The waste changes in their chromosomal DNA profiles are attributed to a formation of populations best suited to the constantly changing conditions and are a result of yeast response to the environmental stresses. Sipiczki (2011) formulated the model of fast adaptive genome evolution (FAGE) of wine yeasts, indicating the possibility of inducing genotypic changes not only at the sexual stage but also during vegetative growth. Acidic stress is one of the main fermentative stresses that adversely affects wine yeasts and change the sensory properties of wines. In cold regions country the excess of musts acidity is a crucial problem in winery. Malic acid is one of the organic acids causing the musts acidity; so malate-decomposing yeasts are of the great value. The aim of the study was to check the genetic stability of selected commercial wine yeasts with extended biological deacidification ability under acidic stress. Three strains of *Saccharomyces cerevisiae* and two strains of *Saccharomyces bayanus* previously characterized as decomposing up to 68% of L-malic acid present (Rajkowska and Kunicka, 2005) were under research. The yeasts were cultivated in standard media under aerobic and anaerobic conditions, simultaneously being subjected to acidic stress. We assessed changes in the karyotypes and mitochondrial DNA (mtDNA) profiles of these industrial wine yeasts after up to 180 generations. The waste changes detected in yeast chromosomal and mitochondrial DNA profiles were rather strain dependent and cannot be directly attributed to the presence of L-malic acid. For all the tested yeasts, the range of the genome alterations was broader after anaerobic cultivation, which implies that fermentative stress is one of the main driving forces in wine yeast adaptive evolution. Undoubtedly, L-malic acid adversely affected the growth of yeasts as the generation times of all strains after subculturing in both aerobic and anaerobic conditions were even doubled at the acid presence. Moreover, the ploidy of yeasts subjected to acidic stress changed in both aerobic and anaerobic conditions. The techniques applied in this study for yeast genetic stability monitoring (karyotyping and restriction analysis of mtDNA) can be successfully used in routine assessment of wine yeasts deposited in pure culture collections.

4 Dziugan P, Berlowska J. 2012. Use of the thick juice from sugar beet as brewing adjunct. 40th Annual Conference on Yeasts, Smolenice, Slovakia, P1.

In recent years, the dynamic development of the brewing industry and increased competition in the market forcing manufacturers to seek views possibilities for reduce production costs. One way is to use unmalted adjuncts. The most commonly used in brewing raw materials are barley, wheat, rice, defatted corn, sugar, corn syrup. Aim of this study was to examine the influence of adjunct of sugar beet thick juice on the yeast vitality, dynamic of fermentation process and on the beer quality. For this purpose wort with different thick juice supplementations levels were prepared and fermented using *Saccharomyces pastorianus* W 34/70. Our researches included yeast vitality determination (fluorescent staining) and comparison of physicochemical parameters of obtained beers, and their sensory analysis. On the basis of the results of the study can be concluded that the addition of beet thick juice as a substitute for the barley malt has a positive impact on the finished dark beer. The presence of several non-sugar compounds from tested unmalted material did not reduce the vitality of used yeast. Sensory evaluation did not show the presence of defects in flavor and aroma of beers with this supplementation. The intense characteristic smell of thick juice was not observed it in the finished beer. Increasing dose of the syrup increases both taste and smell qualities. The highest overall score was received by beer with the highest substitution degree.

Higher amount of thick juice from sugar beet results also in increased alcohol content. This means that the juice has a positive effect on the fermentation process. Thick juice does not cause noticeable changes in color of tested dark beers. There were no changes in colloidal stability. The only drawback of prepared beers was rather low acidity.

5 Kregiel D, Berlowska J, Szubzda B. The fast permittivity test for determination of yeast surface charge and flocculation abilities. 40th Annual Conference on Yeasts, Smolenice, Slovakia, 2012, P3.

The yeast flocculation characteristic is a key parameter to select proper production strains. The clumping properties of cells are important in various biotechnological industries, when flocculation is a major method of cell separation. Forces that influence cell-cell binding include electrostatic interactions. Yeast cells, due to surface charge, act like dielectric materials, therefore the aim of these studies was to apply of special unique technique with a flat condenser that measures the capacitance (permittivity) of yeast cell suspensions for assess their surface charge. As the biological material three industrial yeast strains: *Saccharomyces cerevisiae* NCYC 1017 (brewery, ale), *S. pastourianus* NCYC 870 (brewery, lager) and *Debaryomyces occidentalis* LOCK 0251 (unconventional amylolytic yeast) were used. The values of electric permittivity ε were calculated from the proportion of the capacitance of the capacitor with the tested sample, containing in each case 1×10^9/mL of cell suspension of the tested strain, to the capacitance of the analogous capacitor without the yeast content. Additionally, the classic test for surface charge determination - alcian blue retention (ABR) was applied. The electrical permittivity of the suspension of the tested strains decreased with the frequency increase of the measurement current, reaching at 100 kHz the level of 20 for the flocculating lager strain NCBY 680 and 83 for the ale strain NCBY 1017. The highest permittivity values were noted for the non-flocculating ale strain NCBY 1017 which at 1 kHz reached the value of 3.08×10^4. These values for particular strains were correlated directly with ABR (r = 0.9). Therefore we can concluded that the fast permittivity test may be useful method for determination of flocculation abilities of industrial yeast strains.

6 Kunicka-Styczynska A, Rajkowska K. 2012. Technological stability of wine yeasts with extended deacidification ability. 40th Annual Conference on Yeasts, Smolenice, Slovakia, P4.

Industrial wine yeasts are usually sensitive to acids and are not able to conduct vigorous fermentation of acidic musts. Meeting the market demands for high quality wines, there is a need for valuable wine strains expressing high fermentative stability combined with deacidification activity (Volschenk et al, 2006). Excess acidity of musts can be overcome by biological deacidification. L-malic acid is the only one of organic acids metabolized by *Saccharomyces* yeasts simultaneously with fermentation (Volschenk et al, 2003), so a wide range of commercial industrial strains was screened and twelve yeast with elevated L-malic acid fermentation ability selected. These strains expressed decomposition of 12.5-27.4% L-malic acid in YG model medium with initial concentration of 7 g/L the acid and 150 g/L glucose. The aim of the study was to check their technological stability under fermentative conditions in apple musts. Fermentations were carried out in triplicate in 2000 ml apple musts with the addition of 190 g/L sucrose (total sucrose content 267.5 g/L), 7 g/L L-malic acid, pH=3.01 and incubated at 28 °C during 30 days. L-malic acid was estimated by commonly recognised enzymatic method (Boehringer Mannhaim). The other organic acids affected wine acidity (succinic acid, acetic acid and lactic acid) were examined by HPLC method. The results were statistically estimated using Newman-Keuls test ($p<0.05$) by means of Statistica® for Windows 5.5 software. Among all the tested yeasts only five strains (Chambertain, Karłowo, Traminer, Warna and Zeltinger) decomposed from 0.17 to 0.71 g/L malic acid. The highest demalication activity, metabolising 11.3 and 7.5% L-malic acid characterized Chambertain and Zeltinger yeasts, respectively. Anyway, the degree of malate used up was two and four times lower than recorded in model conditions in YG medium. The other yeasts tested did not eliminate the acid from the fermentation environment and even expressed its formation from 0.03 to 2.41 g/L. The organic acid profiles of apple wines differed depending on the strain. The fermentation activity of all strains was strain-dependent and from 5.4 to 10.7% v/v of ethanol was produced. The low ethanol level was correlated with high concentration of reducing sugars, indicating incomplete musts fermentation. The lower fermentation rate may be caused by sensitivity of some yeast to multidirectional fermentation stress (elevated glucose and ethanol concentration as well as high

acidity). During the study, two commercial wine yeasts (Chambertain and Zeltinger) were selected as stable and useful for biological deacidification of acidic musts. Wines produced by these yeasts met the Polish official standards for fruit wines.

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

Recent publications.

1 T. Pham, T. Wimalasena1, W. G. Box1, K. Koivuranta, E., Storgårds, K. A. Smart and B.R. Gibson. 2011. Evaluation of ITS PCR and RFLP for differentiation and identification of brewing yeast and brewery 'wild' yeast contaminants. J Inst Brew 117:556–568.

A reference library of ITS PCR/RFLP profiles was collated and augmented to evaluate its potential for routine identification of domestic brewing yeast and known 'wild' yeast contaminants associated with wort, beer and brewing processes. This library contains information on band sizes generated by restriction digestion of the ribosomal RNA-encoding DNA (rDNA) internal transcribed spacer (ITS) region consisting of the 5.8 rRNA gene and two flanking regions (ITS1 and ITS2) with the endonucleases C*fo*I, *HaeII*I, *Hinf*I and includes strains from 39 non-*Saccharomyces* yeast species as well as for brewing and non-brewing strains of *Saccharomyces*. The efficacy of the technique was assessed by isolation of 59 wild yeasts from industrial fermentation vessels and conditioning tanks and by matching their ITS amplicon sizes and RFLP profiles with those of the constructed library. Five separate, non-introduced yeast taxa were putatively identified. These included *Pichia* species, which were associated with conditioning tanks and *Saccharomyces* species isolated from fermentation vessels. Strains of the lager yeast *S. pastorianus* could be reliably identified as belonging to either the Saaz or Frohberg hybrid group by restriction digestion of the ITS amplicon with the enzyme *HaeII*I. Frohberg group strains could be further sub-grouped depending on restriction profiles generated with *Hinf*I.

2 Mervi Toivari, Yvonne Nygård, Esa-Pekka Kumpula, Maija-Leena Vehkomäki, Mojca Benčina, Mari Valkonen, Hannu Maaheimo, Martina Andberg, Anu Koivula, Laura Ruohonen, Merja Penttilä and Marilyn Wiebe. 2012. Metabolic engineering of *Saccharomyces cerevisiae* for bioconversion of D-xylose to D-xylonate. Metabolic Engineering doi:10.1016/j.ymben.2012.03.002.

An NAD^+-dependent D-xylose dehydrogenase, XylB, from *Caulobacter crescentus* was expressed in *Saccharomyces cerevisiae*, resulting in production of 17 ± 2 g D-xylonate l^{-1} at 0.23 g l^{-1} h^{-1} from 23 g D-xylose l^{-1} (with glucose and ethanol as co-substrates). D-Xylonate titre and production rate were increased and xylitol production decreased, compared to strains expressing genes encoding *T. reesei* or pig liver $NADP^+$-dependent D-xylose dehydrogenases. D-Xylonate accumulated intracellularly to ~70 mg g^{-1}; xylitol to ~18 mg g^{-1}. The aldose reductase encoding gene *GRE3* was deleted to reduce xylitol production. Cells expressing D-xylonolactone lactonase *xylC* from *C. crescentus* with *xylB* initially produced more extracellular D-xylonate than cells lacking *xylC* at both pH 5.5 and pH 3, and sustained higher production at pH 3. Cell vitality and viability decreased during D-xylonate production at pH 3.0. An industrial *S. cerevisiae* strain expressing *xylB* efficiently produced 43 g D-xylonate l^{-1} from 49 g D-xylose l^{-1}.

3 Virve Vidgren and John Londesborough. 2011. 125th Anniversary Review: yeast flocculation and sedimentation in brewing. J Inst Brew 117:475–487.

Flocculation is prerequisite for bulk sedimentation of yeast during brewery fermentation. Although single yeast cells gradually sediment in green beer, this sedimentation rate is too slow without formation of large yeast flocs. The present review concerns the major determinants of yeast flocculation and sedimentation in brewery fermentations. Flocculation characteristics of yeast are strongly strain-dependent and largely defined by which *FLO* genes are functional in each strain. In addition to the genetic background, several environmental factors affect flocculation. These can be, somewhat arbitrarily, classified as physiological factors, such as the calcium availability, pH, temperature and ethanol and oxygen concentrations in the medium or physical factors, such as cell surface hydrophobicity, cell surface charge and

the presence of appropriate hydrodynamic conditions for the formation of large flocs. Once yeast flocs are formed, their size, shape and density and the properties of the surrounding medium affect the rate at which the flocs sediment. Higher gravity worts usually result in green beers with higher viscosity and density, which both retard sedimentation. Moreover, environmental factors during yeast handling before fermentation, e.g., propagation, storage and cropping, influence the flocculation potential of yeast in subsequent fermentation. Premature yeast flocculation (PYF) and the role of PYF factors are discussed. In conclusion, some potential options available to adjust yeast flocculation are described.

4 Virve Vidgren and John Londesborough. 2012. Characterization of the *Saccharomyces bayanus*-type Agt1 transporter of lager yeast. J Inst Brew 118: in press.

Transport of maltose and maltotriose into the yeast cell is thought to be rate-limiting in the utilization of these sugars. The maltose and maltotriose transporters Malx1, Agt1, Mtt1 and Mphx are present in different combinations in brewer's yeast strains, conferring different maltose and maltotriose transport characteristics to the strains. A new putative maltose/maltotriose transporter ORF was identified during whole genome sequencing of the lager strain WS34/70[16]. Sequence comparisons suggested this putative α-glucoside transporter might be a *Saccharomyces bayanus* counterpart of the Agt1 (*Saccharomyces cerevisiae* type) transporter. In the present work, the transporter coded by a *SbAGT1* gene from a lager strain, A15 (and with the same sequence as the corresponding gene in WS34/70) was characterized. It is shown that this *SbAGT1* encodes a functional α-glucoside transporter with a wide-substrate range, including maltose and maltotriose and probably trehalose, α-methylglucoside and sucrose. The SbAgt1 transporter exhibited K_m values of 17 ± 7 and 22 ± 2 for maltose and maltotriose, respectively. V_{max} values were 21 ± 7 for maltose and 12 ± 2 for maltotriose transport.

5 Marilyn G. Wiebe, Kari Koivuranta, Merja Penttilä, Laura Ruohonen - Lipid production in batch and fed-batch cultures of *Rhodosporidium toruloides* from 5 and 6 carbon carbohydrates. BMC Biotechnology, in press.

Microbial lipids are a potential source of bio- or renewable diesel and the red yeast *Rhodosporidium toruloides* is interesting not only because it can accumulate over 50% of its dry biomass as lipid, but also because it utilises both five and six carbon carbohydrates, which are present in plant biomass hydrolysates. *R. toruloides* was grown on glucose, xylose, arabinose or mixtures of these carbohydrates in batch and fed-batch, nitrogen restricted conditions. Lipid production was most efficient with glucose (up to 25 g lipid L^{-1}, 48 to 75% lipid in the biomass, at up to 0.21 g lipid L^{-1} h^{-1}) as the sole carbon source, but high lipid concentrations were also produced from xylose (36 to 45% lipid in biomass). Lipid production was low (15-19% lipid in biomass) with arabinose as sole carbon source and was lower than expected (30% lipid in biomass) when glucose, xylose and arabinose were provided simultaneously. The presence of arabinose and/or xylose in the medium increased the proportion of palmitic and linoleic acid and reduced the proportion of oleic acid in the fatty acids, compared to glucose-grown cells. High cell densities were obtained in both batch (37 g L^{-1}, with 49% lipid in the biomass) and fed-batch (35 to 47 g L^{-1}, with 50 to 75% lipid in the biomass) cultures. The highest proportion of lipid in the biomass was observed in cultures given nitrogen during the batch phase but none with the feed. However, carbohydrate consumption was incomplete when the feed did not contain nitrogen and the highest total lipid and best substrate consumption were observed in cultures which received a constant low nitrogen supply. Lipid production in *R. toruloides* was lower from arabinose and mixed carbohydrates than from glucose or xylose. Although high biomass and lipid production were achieved in both batch and fed-batch cultures with glucose as carbon source, for lipid production from mixtures of carbohydrates fed-batch cultivation was preferable. Constant feeding was better than intermittent feeding. The feeding strategy did not affect the relative proportion of different fatty acids in the lipid, but the presence of C5 sugars did.

XI Laboratório de Microbiologia, Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomia, 1349-017 Lisboa, Portugal. Communicated by M. Malfeito-Ferreira <mmalfeito@isa.utl.pt>.

Book Chapter.

1 Loureiro V, Malfeito-Ferreira M, Monteiro S & Ferreira RB. 2011. The microbial community of grape berry. In: Gerós, H, Chaves, M, Delrot, S (eds.). The biochemistry of the grape berry. Bentham Sciences Publishers. ISBN 978-1-60805-360-5.

The microbial community of grape berry is composed of an array of species exhibiting differential physiological characteristics and relevance to vine growing and winemaking. The most important phytopathogens responsible for grapevine diseases worldwide are the oomycete *Plasmopara viticola* (downy mildew) and the ascomycete *Erysiphe necator* (powdery mildew). The causal agent of grey rot is the saprophytic mould *Botrytis cinerea*. A wide diversity of yeast species are also common contaminants of berry surfaces, but the key agent of wine fermentation, *Saccharomyces cerevisiae*, is rarely recovered from grapes. Bacterial groups include the spoiling acetic acid bacteria and lactic acid bacteria responsible for the malolactic fermentation. These microorganisms colonise grape surfaces from berry set to ripening following a repeatedly cyclic pattern year after year. Highly complex interactions and chemical signalling take place among grapevines themselves and with the intervening biota, which also include insects, birds and mammals. The fundamental role played by the nonmicrobial biota on grape berry microbiota range from their role (especially the insects) as microbial vectors to damage directly inflicted on the grapes, which pave the way to the entrance of the saprophytes. The precise biota and the resulting interactions depend fundamentally on the berry development stage, on the intactness of the grape skin and on the prevailing environmental conditions, and exert a profound effect on the fruit quality. Given the great ecological, technological and economical importance of studying the grape microbiota, it is somewhat surprising to find scarce and fragmented information available on these topics. Here we provide a balanced, highly multidisciplinary overview of the most relevant components of grape berry microbiota. Our proposal establishes four distinct groups of microorganisms - residents, adventitious, invaders and opportunists - which are defined on the basis of grape biochemical evolution, nutrient availability and ability to proliferate on berry surface. Their natural proliferation is particularly dependent on two main events: *véraison* and berry damage. The origin and the colonization sequence on berry surface by the several groups of microorganisms will be tentatively settled.

Articles.

2 Barata A, Malfeito-Ferreira M & Loureiro V. 2012. The microbial ecology of wine grape berries. Int J Food Microbiol 153:243-259.

The microbial ecology of grapes is complex including filamentous fungi, yeasts and bacteria with different physiological characteristics and significances concerning wine production. Some species are only found in grapes, such as parasitic fungi and environmental bacteria, while others have the ability to survive and grow in wines, constituting the wine microbial consortium. This consortium gathers yeast species, lactic acid bacteria and acetic acid bacteria. The proportion of these microorganisms depends on the grape ripening stage and on the availability of nutrients. Grape berries are susceptible to fungal parasites until *véraison* after which the microbiota of truly intact berries is similar to that of plant leaves, dominated by basidiomycetous yeasts (e.g. *Cryptococcus* spp., *Rhodotorula* spp. *Sporobolomyces* spp.) and the yeast-like fungi *Aureobasidium pullulans*. The cuticle of visually intact berries may bear microfissures and softens with ripening, increasing nutrient availability and explaining the possible dominance by the oxidative or weakly fermentative ascomycetous populations (e.g. *Candida* spp., *Hanseniaspora* spp., *Metschnikowia* spp., *Pichia* spp.) approaching harvest time. When grape skin is clearly damaged, the availability of high sugar concentrations on berry surface favours the increase of ascomycetes with higher fermentative activity like *Issatchenkia* spp. and *Zygoascus hellenicus*, including dangerous wine spoilage yeasts (e.g. *Zygosaccharomyces* spp., *Torulaspora* spp.), and of acetic acid bacteria (e.g. *Gluconobacter* spp., *Acetobacter* spp.). The sugar fermenting species *Saccharomyces cerevisiae* is rarely found on unblemished berries, being favoured by grape damage.

Lactic acid bacteria are minor partners of grape microbiota while the typical agent of malolactic fermentation, *Oenococcus oeni*, has been seldom isolated from grapes in the vineyard. Environmental ubiquitous bacteria of the genus *Enterobacter* spp., *Enterococcus* spp., *Bacillus* spp., *Burkholderia* spp., *Serratia* spp., *Staphylococcus* spp., among others, have been isolated from grapes but do not have the ability to grow in wines. Saprophytic moulds, like *Botrytis cinerea*, causing grey rot, or *Aspergillus* spp., possibly producing ochratoxin, are only active in the vineyard, although their metabolites may affect wine quality during grape processing. This review is mostly focussed on yeast species which are far more studied than bacteria. The impact of damaged grapes in yeast ecology has been underestimated mostly because of inaccurate grape sampling. Injured berries hidden in apparently sound bunches explain the recovery of a higher number of species when whole bunches are picked. Grape health status is the main factor affecting the microbial ecology of grapes, increasing both microbial numbers and species diversity. Therefore, the influence of abiotic (e.g. climate, rain, hail), biotic (e.g. insects, birds, phytopathogenic and saprophytic moulds) and viticultural (e.g. fungicides) factors is dependent on their primary damaging effect.

3 Barata A, Campo E, Malfeito-Ferreira M, Loureiro V, Cacho J. & Ferreira V. 2011. Analytical and sensorial characterization of the aroma of wines produced with sour rotten grapes using GC-O and GC-MS: identification of key aroma compounds. J Agric Food Chem 59:2543-2553.

In the present work, the aroma profiles of wines elaborated from sound and sour rot infected grapes as raw material have been studied by sensory analysis, gas chromatography–olfactometry (GC–O) and gas chromatography–mass spectrometry (GC–MS), with the aim of determining the odor volatiles most likely associated to this disease. The effect of sour rot was tested in monovarietal wines produced with the Portuguese red grape variety Trincadeira, and in blends of Cabernet Sauvignon and sour rotten Trincadeira grapes. Wines produced from damaged berries exhibited clear honey-like notes not evoked by healthy samples. Ethyl phenylacetate (EPhA), and phenylacetic acid (PAA), both exhibiting honey-sweet like aromas, emerged as key-aroma compounds of sour rotten wines. Their levels were one order of magnitude above those found in controls and reached 304 and 1668 μgL^{-1} of EPhA and PAA, respectively, well above the corresponding odor thresholds. Levels of -nonalactone also increased by a factor 3 in sour rot samples. Results also suggest that sour rot exerts a deep impact on the secondary metabolism of yeast, decreasing the levels of volatiles related to fatty acids and amino acids synthesis. Highest levels of decalactone of up to 405 μgL^{-1} were also found in all the samples, suggesting that this could be a relevant aroma compound in Trincadeira wine aroma.

4 Barata A, Pais A, Malfeito-Ferreira M & Loureiro V. 2011. Influence of sour rotten grapes on the chemical composition and quality of grape must and wine. Eur Food Res Technol 233:183–194.

This study evaluated the effect of grape sour rot on wine fermentation and characterized the chemical composition and the sensory changes in wines produced from rotten musts. Microvinifications were performed during two vintages using healthy Trincadeira and Cabernet Sauvignon red grape varieties to which were added grapes affected by sour rot. Increasing sour rot percentages, up to 50%, contributed to a clear decrease in free run must and final wine yields and induced significant changes on grape must chemical composition expressed by the increase in sugar content, total acidity, volatile acidity, anthocyanins, total phenols and color intensity. After malolactic fermentation, wines from rotten grapes showed higher values of alcohol content, dry extract, reducing sugar content, total and volatile acidity, anthocyanins, total phenols and color intensity. Despite the higher levels of reducing sugars, the microbial stability was similar to that of healthy wines. The sensorial evaluation, after malolactic fermentation, showed that both types of wine were not statistically different regarding color, aroma, taste and overall quality. During 6 to 8 month storage, wines from rotten grapes showed a significant higher percentage of color loss, suggesting that sour rot is responsible for the decrease in color stability. Nevertheless, the results of sensorial analysis demonstrated that the fermentation of grape musts containing up to 30% sour rot yields wines with similar or even higher scores than wines made with healthy grapes.

5 Barata A, Malfeito-Ferreira M, Loureiro V (2012) Changes in sour rotten grape berry microbiota during ripening and wine fermentation. Int J Food Microbiol. doi:10.1016/j.ijfoodmicro.2011.12.029.

This study investigated the microbiota of sour rotten wine grapes and its impact on wine fermentations. Yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) were enumerated and identified on sound and sour rot grapes during the ripening stage. The alteration of the ecological balance induced by sour rot was particularly evidenced by the unequivocal increase of yeast and AAB counts on rotten grapes, since the beginning of ripening. Yeast and AAB species diversity in rotten grape samples were much higher to those found in sound grapes. LAB populations were low detected from both healthy and sour rotten grapes. The yeast species *Issatchenkia occidentalis*, *Zygoascus hellenicus* and *Zygosaccharomyces bailii* and the AAB species *Gluconacetobacter hansenii, Gluconacetobacter intermedius and Acetobacter malorum,* were recovered from damaged grapes and resulting grape juices in the winery. *Acetobacter orleaniensis* and *Acetobacter syzygii* were only recovered from sour rotten grapes. *Dekkera bruxellensis* and *Oenococcus oeni* were only recovered after wine fermentation induced by starter inoculation, irrespective of grape health, probably originating from cellar environment. After malolactic fermentation, racking and sulphur dioxide addition the only remaining species were the yeast *Trigonopsis cantarelli* and *Saccharomyces cerevisiae*, independently of the grape health status.

XII 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>.

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

1 Georgieva M, Roguev A, Balashev K, Zlatanova J, Miloshev G. 2012. Hho1p, the linker histone of *Saccharomyces cerevisiae*, is important for the proper chromatin organization *in vivo*. Biochim Biophys Acta 1819:366–374.

Despite the existence of certain differences between yeast and higher eukaryotic cells a considerable part of our knowledge on chromatin structure and function has been obtained by experimenting on *Saccharomyces cerevisiae*. One of the peculiarities of *S. cerevisiae* cells is the unusual and less abundant linker histone, Hho1p. Sparse is the information about Hho1p involvement in yeast higher-order chromatin organization. In an attempt to 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 the mutant cells exhibited highly distorted higher-order chromatin organization. Characteristically, linker histone depleted chromatin generally exhibited longer chromatin loops than the wild-type. According to the Atomic force microscopy data the wild type chromatin appeared well organized in structures resembling quite a lot the "30-nm" fiber in contrast to *HHO1 knock-out* yeast.

Acknowledgements: This work is fully supported by the Bulgarian Science Fund, project number: DMU 02/8 and by the World Federation of Scientists - National Scholarship Programme. Jordanka Zlatanova is supported in part by NSF grant 0504239.

2 Georgieva M, Uzunova K, Balashev K, Genova G and Miloshev G. 2011. How important are the higher-order chromatin structures for the proper gene expression? Sci Technol 1:49-54.

In the confined area of the nucleus DNA is organized in chromatin. It maintains the genetic material and by its upper levels of compaction allows proper functioning of the genome. The processes that govern the higher-order chromatin organization are complex and yet not well defined. Importantly, some data show that changes in chromatin organization lead to serious diseases in human which could be a consequence of altered expression of genes localized in chromatin regions with changed structure. Here, we present the development of a method for higher-order chromatin structure studies in two model systems - *Saccharomyces cerevisiae* and *Drosophila melanogaster*.

Acknowledgments: M.G. and G.M. are supported by the Bulgarian Science fund; Grant numbers DMU 02/8 and DID 02-35. This collection has been compiled with the financial support of the "Human Resources Development" Operational Pro-gramme, co-financed by the European Union through the European Social Fund under grant number BG051PO 001-3.3.04/58.

3 Staneva D, Georgieva M, Peycheva E, Miloshev G. 2011. Is there a linker histone in the yeast *Kluyveromyces lactis*? Sci Technol 1:14-19 - ISSN 1314-4111.

In all eukaryotic cells nuclear DNA is organized in a highly-ordered nucleoprotein complex called chromatin. Along with DNA, essential structural and functional components of chromatin are histone proteins: core histones and linker histones. The latter are involved in both the maintenance of higher chromatin structures and together with core histones in regulation of gene expression. It has to be mentioned, however, that both functions of linker histones are more presumed than proved and therefore are subject of disputes. The aim of the current research is to go in more details of the functions of linker histones. We have explored the yeast *Kluyveromyces lactis* as a model organism. In silico analysis revealed a single open reading frame (ORF) in *K. lactis* genome, with homology (around 48%) to linker histone genes of different organisms. The predicted amino acid sequence of the putative *K. lactis* H1 protein (KlH1p) showed 40% identity. Interestingly, an expression of mRNA from the gene was not detected. Knockout of *KlH1* gene has not shown great impact on the cellular viability. In order to answer whether *KlH1* is a true gene coding for a linker histone or it is a pseudogene several phenotypic features of *KlH1* knockout cells were examined. Based on the obtained results we determined the significance of *KlH1* for *K. lactis*.

Acknowledgments: This work was supported by National Science Fund, Grant DMU 02/8. This collection has been compiled with the financial support of the "Human Resources Development" Operational Programme, co-financed by the EU through the European Social Fund (Grant BG051PO 001-3.3.04/58).

4 Uzunova K, Georgieva M & Miloshev G. Chromatin structure is decisive for chronological aging of *Saccharomyces cerevisiae*. 9th International Meeting on Yeast Apoptosis, Rome, Italy.

The brilliance of *Saccharomyces cerevisiae* as a model for studying of aging has long been proved. Easily handled and amenable to different genetic manipulations these cells allow detailed investigation of the process. Two different types of aging have been discovered in yeast - replicative and chronological both used as useful models for studying of dividing and non-dividing post mitotic higher eukaryotic cells, respectively. The linker histone of yeast cells, Hho1p is a key player in chromatin organization and its knock-out leads to disordered and altered higher-order chromatin structure. We present our recent results with *hho1Δ S. cerevisiae* cells studied during the process of chronological aging. In this light we followed survival of the mutant cells in a time course of 20 days in complete minimal media. Chromatin structure and cellular morphology of the mutant has been accordingly checked during the time course of the experiment and compared with the wild type progenitor cells, thus digging into potential connection between chromatin structure and the ability of cells to age properly. Some intriguing morphological and physiological features of yeast *hho1Δ* mutants will be discussed. These could lead to the bold conclusion that the yeast linker histone has significant role in cellular aging.

Acknowledgments: This research is partly financed by the Bulgarian Science Fund, Grant Number: DMU 02/8.

5 Georgieva M, Balashev K and Miloshev G. 2012. The linker histone of *Saccharomyces cerevisiae*, Hho1p, is a moderator of chromatin organization *in vivo*. EMBO conference "Nuclear Structure and Dynamics", L'Isle sur la Sorgue, France, 28th Sept – 02nd Oct, 2012.

S. cerevisiae cells have long been used as model organisms in chromatin structure and function studies. It has been believed that these yeasts do not possess a linker histone until the sequencing of its genome when the gene for a linker histone has been discovered. Well known is that in higher eukaryotes linker histones are important for building and maintaining of the higher-order chromatin organization. Accordingly the elucidation of yeast linker histone functions in yeast chromatin has become of high interest. Few data accumulated in the past ten years, revealing some curious traits of this linker histone. In fact its exact role in the global organization of chromatin yet remains unproved. In higher eukaryotes the deletion of the genes for three of the linker histone subtypes causes embryonic lethality. In this sense it is quite logical to think that yeast linker histone (Hho1p) may have important roles in *S. cerevisiae* cells that are not defined until now. Therefore, in methodical search for Hho1p possible roles in chromatin organization, we have applied a set of techniques especially designed for revealing the higher-order chromatin organization

of HHO1 knock-out mutants. The results from the method of Chromatin Comet Assay (ChCA), Atomic force microscopy and nuclease digestion experiments showed that the wild-type chromatin appeared regularly structured as "30-nm" fiber while the mutant cells possessed highly distorted higher-order chromatin organization. These data reveal unknown until now roles of S. cerevisiae linker histone in the formation and maintenance of the "30-nm" fiber and the chromatin structures above it.

Acknowledgements: Milena Georgieva is supported by the Bulgarian Science Fund, project number: DMU 02/8, by the World Federation of Scientists - Bulgarian National Scholarship Programme. This collection has been compiled also with the financial support of the "Human Resources Development" Operational Programme, co-financed by the European Union through the European Social Fund - grant number BG051PO001-3.3.04/58.

6 Georgieva M, Peycheva E & Miloshev G. 2011. The yeast *Saccharomyces cerevisiae* – a promising model for studying of human brain tumors. 4th International Congress of Molecular Medicine, 27-30 June, 2011, Istanbul, Turkey. In vivo 25:467-576.

Background: *Glioblastoma multiforme* is the most aggressive among human gliomas with exclusively bad prognosis. Recently, we have started research on some epigenetic characteristics of these tumor cells. The attention is focused on a very specific linker histone subtype - H1 zero, characteristic for highly differentiated cells. We have detected that its quantity is reduced to completely missing in human glial cells. Therefore, we started developing *Saccharomyces cerevisiae* as a model for studying of this cancer. Methods: The biochemical methods for specific linker histones isolation allowed quantification of H1 zero in normal and tumor brain cells. Gene cloning techniques were applied for yeast linker histone knock-out and subsequent cloning of human H1 zero in yeast cells. Standard methods for monitoring of cell growth have been further used. Chromatin structure was assessed by Chromatin Comet Assay and AFM. Results: Successful cloning of human H1 zero in *S. cerevisiae* cells was proved beyond doubt. Growth potential of cells transformed with H1 zero conveyed interesting characteristics that will be discussed. Conclusion: *Saccharomyces cerevisiae* proved that it could be used as a "clean room" for studying linker histones and chromatin structure as important epigenetic phenomena in tumor development.

Acknowledgments: M.G. is supported by a young scientists' research grant from the Bulgarian Science Fund; Grant number DMU 02/8 and by the World Federation of Scientists - Bulgarian National Scholarship Programme. G.M. is supported by grant from the Bulgarian Science Fund - DID 02-35.

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

The following paper was accepted recently.

1 Sung-Oui Suh, Dmitri A. Maslov, Robert E. Molestina and Jianlong J. Zhou 2012. *Microbotryozyma collariae* gen. nov., sp. nov., a basidiomycetous yeast isolated from a plant bug *Collaria oleosa* (Miridae). Antonie van Leeuwenhoek, in press.

Two strains of a basidiomycetous yeast were derived from an insect trypanosomatid culture isolated from the intestine of a plant bug, *Collaria oleosa* (Heteroptera: Miridae), collected in Costa Rica. The yeast did not form ballistoconidia but reproduced only by budding. Teliospores were not observed in individual and crossed cultures of each strain. Morphological and other taxonomic characteristics of the yeast were similar to those of the species in the polyphyletic genus *Rhodotorula*. However, molecular phylogeny inferred from the internal transcribed spacers and D1/D2 region of the large subunit rRNA gene showed that the strains represent a new species placed among the smut fungi in the family Ustilentylomataceae, which includes *Aurantiosporium subnitens*, *Fulvisporium restifaciens*, *Ustilentyloma uitans*, and *Rhodotorula hordea*. Given the well distinguished phylogenetic position of this novel species within the Ustilentylomataceae, we propose *Microbotryozyma collariae* gen. nov., sp. nov. to accommodate the yeast isolated from *C. oleosa*, with strain American Type Culture Collection MYA-4666^T (= PRA303-1S = CBS 12537) designated as the type strain.

XIV Laboratoire INRA, MICALIS UMR1319 Micalis, Domaine de Vilvert, F-78352 Jouy-en-Josas, and BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.-V <nguyenhv@grignon.inra.fr>.

Recently published:

1 Huu-Vang Nguyen, Jean-Luc Legras, Cécile Neuveglise & Claude Gaillardin. 2011. Deciphering the hybridisation history leading to the lager lineage based on the mosaic genomes of *Saccharomyces bayanus* strains NBRC1948 and CBS380^T. PLoS One 6(10):e25821. Epub 2011 Oct 5

Saccharomyces bayanus is a yeast species described as one of the two parents of the hybrid brewing yeast *S. pastorianus*. Strains CBS380^T and NBRC1948 have been retained successively as pure-line representatives of *S. bayanus.* In the present study, sequence analyses confirmed and upgraded our previous finding: *S. bayanus* type strain CBS380^T harbours a mosaic genome. The genome of strain NBRC1948 was also revealed to be mosaic. Both genomes were characterized by amplification and sequencing of different markers, including genes involved in maltotriose utilization or genes detected by array-CGH mapping. Sequence comparisons with public *Saccharomyces* spp. nucleotide sequences revealed that the CBS380^T and NBRC1948 genomes are composed of: a predominant non-*cerevisiae* genetic background belonging to *S. uvarum,* a second unidentified species provisionally named *S. lagerae,* and several introgressed *S. cerevisiae* fragments. The largest *cerevisiae*-introgressed DNA common to both genomes totals 70kb in length and is distributed in three contigs, cA, cB and cC. These vary in terms of length and presence of *MAL31* or *MTY1* (maltotriose-transporter gene). In NBRC1948, two additional *cerevisiae*-contigs, cD and cE, totaling 12kb in length, as well as several smaller *cerevisiae* fragments were identified. All of these contigs were partially detected in the genomes of *S. pastorianus* lager strains CBS1503 (*S. monacensis*) and CBS1513 (*S. carlsbergensis*) explaining the noticeable common ability of *S. bayanus* and *S. pastorianus* to metabolize maltotriose. NBRC1948 was shown to be inter-fertile with *S. uvarum* CBS7001. The cross involving these two strains produced F1 segregants resembling the strains CBS380^T or NRRLY-1551. This demonstrates that these *S. bayanus* strains were the offspring of a cross between *S. uvarum* and a strain similar to NBRC1948**.** Phylogenies established with selected *cerevisiae* and non-*cerevisiae* genes allowed us to decipher the complex hybridisation events linking *S. lagerae*/*S. uvarum*/*S. cerevisiae* with their hybrid species, *S. bayanus/pastorianus.*

The data presented confirmed that *S. bayanus* CBS 380^T and strain NBRC 1948, regarded as *S. bayanus* pure line, are hybrids. Thus the reinstatement of *S. uvarum* as a real species is fully validated. I recall here articles that designated the species *S. uvarum* and not a variety (1,2,3) and the recently published in which the authors have re-use the correct name for *S. uvarum* (4,5,6,7)

[1] Nguyen HV, Gaillardin C. 2005. Evolutionary relationships between the former species *Saccharomyces uvarum* and the hybrids *Saccharomyces bayanus* and *Saccharomyces pastorianus*; reinstatement of *Saccharomyces uvarum* (Beijerinck) as a distinct species. FEMS Yeast Res 5:471-83.

[2] Antunovics Z, Nguyen HV, Gaillardin C, Sipiczki M. 2005. Gradual genome stabilisation by progressive reduction of the *Saccharomyces uvarum* genome in an interspecific hybrid with *Saccharomyces cerevisiae*. FEMS Yeast Res 5:1141-50.

[3] Sampaio JP, Gonçalves P. 2008. Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. Appl Environ Microbiol 74:2144-52.

[4] Libkind D, Hittinger CT, Valério E, Gonçalves C, Dover J, Johnston M, Gonçalves P, Sampaio JP. 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA 108:14539-44.

[5] Dunn B, Richter C, Kvitek DJ, Pugh T, Sherlock G. 2012. Analysis of the *Saccharomyces cerevisiae* pan-genome reveals a pool of copy number variants distributed in diverse yeast strains from differing industrial environments. Genome Res 22:908-24.

[6] Schwartz K, Wenger JW, Dunn B, Sherlock G. 2012. *APJ1* and *GRE3* homologs work in concert to allow growth in xylose in a natural *Saccharomyces sensu stricto* hybrid yeast. Genetics. [Epub ahead of print]

[7] Piotrowski JS, Nagarajan S, Kroll E, Stanbery A, Chiotti KE, Kruckeberg AL, Dunn B, Sherlock G, Rosenzweig F. 2012. Different selective pressures lead to different genomic outcomes as newly-formed hybrid yeasts evolve. BMC Evol Biol 12:46.

XV Geobotany, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany - http://www.ruhr-uni-bochum.de/geobot/en/. Communicated by Dominik Begerow <dominik.begerow@rub.de> and Andrey Yurkov* <andrey.yurkov@rub.de>.

*Current address: Centro de Recursos Microbiologicos (CREM), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal.

Recent papers.

1 Fröhlich-Nowoisky J, Burrows SM, Xie Z, Engling G, Solomon P A, Fraser MP, Mayol-Bracero OL, Artaxo P, Begerow D, Conrad R, Andreae MO, Després VR & Pöschl U. 2012. Biogeography in the air: fungal diversity over land and oceans. Biogeosciences 9:1125-1136.

Biogenic aerosols are relevant for the Earth system, climate, and public health on local, regional, and global scales. Up to now, however, little is known about the diversity and biogeography of airborne microorganisms. We present the first DNA-based analysis of airborne fungi on global scales, showing pronounced geographic patterns and boundaries. In particular we find that the ratio of species richness between Basidiomycota and Ascomycota is much higher in continental air than in marine air. This may be an important difference between the "blue ocean" and "green ocean" regimes in the formation of clouds and precipitation, for which fungal spores can act as nuclei. Our findings also suggest that air flow patterns and the global atmospheric circulation are important for the understanding of global changes in biodiversity.

2 Kachalkin AV & Yurkov AM. 2012. Yeast communities in *Sphagnum* phyllosphere along the hydrothermal ecocline in the boreal forest-swamp ecosystem and description of the *Candida sphagnicola* sp. nov. Antonie van Leeuwenhoek 102:29-43.

The effects of the temperature-moisture factors on the phylloplane yeast communities inhabiting *Sphagnum* mosses were studied along the transition from a boreal forest to a swamp biotope at the Central Forest State Biosphere Reserve (Tver region, Russia). We tested the hypothesis that microclimatic parameters affect yeast community composition and structure even on a rather small spatial scale. Using a conventional plating technique we isolated and identified by molec- ular methods a total of 15 species of yeasts. Total yeast counts and species richness values did not depend on environmental factors, although yeast community com- position and structure did. On average, *Sphagnum* in the swamp biotope supported a more evenly structured yeast community. Relative abundance of ascomycetous yeasts was significantly higher on swamp moss. *Rhodotorula mucilaginosa* dominated in the spruce forest and *Cryptococcus magnus* was more abundant in the swamp. Our study confirmed the low occurrence of tremellaceous yeasts in the *Sphagnum* phyllosphere. Of the few isolated ascomycetous yeast and yeast-like species, some were differentiated from hitherto known species in physiological tests and phylogenetic analyses. We describe one of them as *Candida sphagnicola* and designate KBP Y-3887^T (=CBS 11774^T = VKPM Y-3566^T = MUCL 53590^T) as the type strain. The new species was registered in MycoBank under MB 563443.

3 Schoch C L, Keith A. Seifert K A, Huhndorf S, Robert V, Spouge J L, Levesque C A, Chen W, & Fungal Barcoding Consortium (149 collaborators). 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. PNAS 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.

The following articles, whose abstracts appeared in the June and Dec 2011 issue, have now appeared in print:

4 Kellner R, Vollmeister E, Feldbrügge M & Begerow D. 2011. Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. PLoS Genetics 7(12): e1002436.

5 Yurkov AM, Krüger D, Begerow D, Arnold N & Tarkka MT. 2012. Basidiomycetous yeasts from Boletales fruiting bodies and their interactions with the mycoparasite *Sepedonium chrysospermum* and the host fungus *Paxillus*. Microbial Ecology 63: 295-303.

6 Yurkov AM, Kemler M & Begerow D. 2012. Assessment of yeast diversity in soils under different management regimes. Fungal Ecology 5: 24-35.

7 Yurkov AM, Schäfer AM & Begerow D. 2012. *Leucosporidium drummii* sp. nov. a new microbotryomycete isolated from soil in Germany. Int J Syst Evol Microbiol 62:728-734.

PhD thesis.

8 Moritz Mittelbach has started his PhD thesis on diversity of flower inhabiting yeasts in relation to flower syndrome and smut infection.

The project is aimed to explore diversity of yeasts and yeast-like fungi colonizing flower nectar and to compare yeast communities' parameters associated with different pollination systems and flower traits (nectar amount and sugar concentration). In addition to the analysis of seasonal fluctuations and the importance of sugar concentration, Moritz is especially interested in the correlation of evolutionary changes of flower syndrome and their consequences on the yeast diversity. Finally, we are interested in competition between different yeast species in the nectar, especially, when plant parasitic species such as anther smut *Microbotryum* species are involved. Recently, Moritz Mittelbach received a grant to conduct parts of his project on three closely related *Echium* species (Angiospermae, Boraginaceae) on the Canary Islands.

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

Recently accepted papers.

1 Cadete RM, Melo MA, Lopes MR, Pereira GM, Zilli JE, Vital MJ, Gomes FC, Lachance MA, Rosa CA. In press. *Candida amazonensis* sp. nov., an ascomycetous yeast isolated from rotting wood in Amazonian Forest, Brazil. Int J Syst Evol Microbiol (accepted August 2011).

Five strains of a new yeast species were isolated from rotting-wood samples collected in an Amazonian Forest site of the state of Roraima, Northern Brazil. The sequences of the D1/D2 domains of the large subunit of the rRNA gene showed that this species belongs to the *Scheffersomyces* clade and is related to *Candida coipomoensis*, *C. lignicola* and *C. queiroziae*. The novel species, *Candida amazonensis* sp. nov, is proposed to accommodate these isolates. The type strain of *C. amazonensis* sp. nov. is UFMG-HMD-26.3^T (=CBS 12363^T = NRRL Y-48762^T).

2 Pozo M, Lachance MA, Herrera C 2012 Nectar yeasts of two southern Spanish plants: the roles of immigration and physiological traits in community assembly. FEMS Microbiol Ecol 80:281-293.

Recent studies have shown that dense yeast populations often occurring in floral nectar are numerically dominated by a few species from the flower–insect interface specialized genus *Metschnikowia*, while generalist yeast species commonly occurring on leaf surfaces, soil, freshwater, and air were rarely isolated from nectar samples. This study was designed to understand the main factors responsible for the assembly of nectar yeast communities, by combining field experiments with laboratory tests characterizing the physiological abilities of all yeast species forming the pool of potential colonizers for two Spanish flowering plants (*Digitalis obscura* and *Atropa baetica*). Yeast frequency and species richness were assessed in external sources (bee glossae, air, plant phylloplane) as well as in pollinator rewards (pollen, nectar). Yeasts were most frequent in external sources (air, flower-visiting insects), less so in the proximate floral environment (phylloplane), and least in pollen and nectar. Nectar communities appeared to be considerably impoverished versions of those in insect glossae and phylloplane. Nectar, pollen, and insect yeast assemblages differed in physiological characteristics from those in other substrates. Nectarivorous *Metschnikowia* were not more resistant than other yeast species to plant secondary compounds and high sugar concentrations typical of nectar, but their higher growth rates may be decisive for their dominance in ephemeral nectar communities.

3 de Vega C, Guzmán B, Lachance MA, Steenhuisen SL, Johnson SD, Herrera CM. In press. *Metschnikowia proteae* sp. nov., a nectarivorous insect–associated yeast species from Africa. Int J Syst Evol Microbiol (accepted January 2012).

A collection of yeasts isolated from nectar of flowers of *Protea caffra* (Proteaceae) and associated scarab beetles (*Atrichelaphinis tigrina*, *Cyrtothyrea marginalis*, *Trichostetha fascicularis*, and *Heterochelus* sp.) and drosophilid flies in South Africa, contained twenty-eight isolates that could not be assigned to known species. Comparisons of the D1/D2 domains of the large subunit ribosomal RNA gene demonstrated the existence of three separate phylotypes with an affinity to the genus *Metschnikowia* and more specifically to the beetle-associated large-spored *Metschnikowia* clade. Twenty-six strains that had similar D1/D2 sequences were mixed in all pairwise combinations. They were found to mate and give rise to large asci typical of those in the clade. The name *Metschnikowia proteae* sp. nov. (type = EBDT1Y1^T = CBS 12522^T = NRRL Y-48784^T; allotype = EBDC2Y2 = CBS 12521= NRRL Y-48785) is proposed to accommodate this new species. The ecology of this novel yeast species is discussed in relation to its potential plant and insect host species. The additional two single strains isolated from *Heterochelus* sp. represent two new undescribed species (*Candida* sp. 1 EBDM2Y3 and *Candida* sp.2 EBDM8Y1). As these single strains are probably haploid mating types of *Metschnikowia* species, their description is deferred until the species are sufficiently well sampled to permit meaningful descriptions.

4 Araujo FV, Rosa CA, Freitas LFD, Lachance MA, Vaughan-Martini A, Mendonça-Hagler LC, Hagler AN 2012 *Kazachstania bromeliacearum* sp. nov., a yeast species from water tanks of bromeliads. Int J Syst Evol Microbiol 62:1002–1006.

Cultures of a novel nutritionally specialized, fermentative yeast species were isolated from 34 water tanks of five bromeliad species, two mangrove sediment samples and one swamp water sample in Rio de Janeiro, Brazil. Sequence analysis of the D1/D2 domains of the large subunit of the rRNA gene showed that the novel species belongs to the genus *Kazachstania*. The novel species differs from *Kazachstania martiniae* by 11 substitutions and 2 gaps in the sequence of the domains D1/D2 of the LSU rRNA gene. The name *Kazachstania bromeliacearum* sp. nov. is proposed for the novel species. The type strain is IMUFRJ 51496^T (= CBS 7996^T = DBVPG 6864^T = UFMG BR-174^T).

5 Lachance MA, Rosa CA, Carvajal EJ, Freitas LFD, Bowles JM In press. *Saccharomycopsis fodiens* sp. nov., a rare predacious yeast from three distant localities. Int J Syst Evol Microbiol (accepted May 2012).

Three strains representing a new yeast species were recovered as part of independent collections from flower-associated nitidulid beetles in Australia, Costa Rica, and the Galapagos Islands, Ecuador. Analysis of the D1/D2 domains of the large subunit ribosomal RNA gene indicated that the species belongs to the genus *Saccharomycopsis*, although the formation of ascospores was not observed. The yeast is capable of necrotrophic parasitism by means of infection pegs when mixed with other yeasts or filamentous fungi. Of particular interest is the fact that despite the large distances separating the isolation sites of the three strains, other strains of the species have not been recovered in other samples of flower-associated nitidulids even though these habitats have been sampled extensively. We raise the possibility that the dispersal may be linked to human historical factors. We propose for this yeast the name *Saccharomycopsis fodiens* sp. nov. The type strain is UWOPS 95-697.4^T (=CBS 8332^T =NRRL Y-48786^T).

Forum

In defense of yeast sexual life cycles: the *forma asexualis*

An informal proposal - M. A. Lachance

The soon-to-be published Melbourne Code will have a profound impact on yeast systematics, as it will provide for the elimination of multiple names for different stages of the same fungus. The use of two names for the sexual and asexual stages of the same fungal species has forever puzzled animal and plant taxonomists and its elimination has been much anticipated by many practitioners of fungal systematics over the last decade (Hawksworth et al 2011). Although bathed in good intentions, this change in policy is not without its potential shortcomings and regardless of what yeast systematists think of the change, the reality is that we must now live with this approach. I personally have some concerns, fueled largely by my role in the peer review of articles dealing with the description of new yeast species.

The underlying premise of the single name approach is that we now have the tools (DNA sequencing) required to resolve anamorph-teleomorph connections and consequently that the very notion of anamorph and teleomorph has outlived its utility (Taylor 2011). For instance, the presence or absence of a sexual state is completely irrelevant in the case of fungi described solely on the basis of environmental DNA sequencing, where nothing is known about morphology, physiology, or indeed the fungus itself. I would not advocate, however, extending practices designed for loose bits of DNA to whole living organisms that possess knowable morphologies, physiologies, and ecologies.

As someone who is frequently put in the position of having to evaluate the quality of species descriptions, I rank in the top tier descriptions that are based on the whole biology of organisms, including their morphology, growth characteristics, genetics, biochemistry, biogeography, and phylogenetic relationships. As with any biological phenomenon, these attributes have a mean and a variance, and consequently, I regard descriptions based on several, demonstrably independent isolates as having greater scientific value than the mere report of a new variant sequence. In the now moribund two-name system, we placed a special premium on the discovery of the "perfect" cycle of yeasts and rewarded such knowledge with the assignment of sexual species to distinct genera. Now that this distinction is no longer afforded, I fear that we shall witness a further deterioration of taxonomic practices, whereby less conscientious α-taxonomists will feel no obligation to wipe the dust off the old microscope and to exercise due diligence in demonstrating sexuality in newly described species. To my dismay, a number of colleagues have indicated to me, more often overtly than covertly, that they have little more than a mild nostalgia towards yeast sexual life cycles, implying that such features have been superceded by strings of As, Gs, Cs, and Ts. Loosely paraphrasing the great L.J. Wickerham, lest we forget that sequence-based taxonomy is subsidiary to biology, and not the other way around.

In consideration of the above, I have informally proposed the use of the expression *forma asexualis* or its abbreviation *f.a.*, to be tacked onto "*sp. nov.*", in the title of future descriptions of species whose sexual state has not been observed, and possibly in other contexts as well. Articles 4 and 5 of the Botanical Code recognize the use of the form as a sub-specific rank and allows for its informal use, as exemplified by

the *forma specialis*, which allows for the classification of parasitic fungi as a function of their hosts. The *forma asexualis* would fulfill two roles. First, it would inform the reader that a particular set of yeast strains at the rank of species or below do not exhibit a sexual cycle, which would have been indicated in the past by names such as *Candida* or *Cryptococcus*. More importantly the practice would serve as an encouragement to practitioners of α-taxonomy to exercise due diligence in documenting the life cycles of new species.

I have presented the idea of *forma asexualis* to various colleagues, including Associate Editors of relevant journals and members of the subcommittee on yeast nomenclature. The response has been mixed but is best qualified as one of indifferent dismissal. Gábor Péter, Associate Editor for Antonie van Leeuwenhoek and valued reviewer for IJSEM, is a notable exception. I hope that the present discussion will trigger some interest in implementing such a practice.

1 Hawskworth DL 2011 The Amsterdam Declaration on Fungal Nomenclature. IMA Fungus 1:105–112.

2 Taylor J 2011 One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2:113–120.

In support of the *forma asexualis* - Gábor Péter

The new International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) contains very important new rules which will have sweeping effects on the taxonomy and nomenclature of yeasts. After the 1st of January 2013, one fungus can have only one name, *i.e.*, the dual nomenclature for anamorphs and teleomorphs will no longer be allowed ("One fungus = One name"). In addition the principle of priority will not discriminate between anamorphic and teleomorphic names. These new rules in many cases raise the question "One fungus = Which name?", a question that will not be easy to answer properly in some cases. A lot of discussion and a concerted change of ideas will be required to find "good" solutions.

André Lachance initiated an informal discussion on the forthcoming changes in the nomenclature of yeasts. He has made a number of suggestions. Here I wish to reflect on one point, namely his proposal to add the letters *f.a.*, (*forma asexualis*) after the name of an asexual yeast that is assigned phylogenetically to a sexual genus.

It seems that the idea of assigning newly described asexual yeast species to the corresponding teleomorphic genus, provided that the affiliation is firmly established, is supported by many yeast taxonomists. A hypothetical example follows. If a new species of the profoundly polyphyletic genus *Candida* is being described and it has been proven to be well nested within the *Pichia* clade (preferably on the basis of multigene phylogenetic analysis) it is desirable to describe it as a new *Pichia* species instead of further increasing the polyphyly of the genus *Candida*, even if no ascosporulation have been observed. The acceptance of the proposal to designate asexual a species assigned to a teleomorphic genus as a *forma asexualis* (*f.a.)* would provide this information connected to the name of the species. As I am of the opinion that whether or not the sexual stage has ever been observed in the given species is very important, I support the proposal and I agree with André's justification as well.

Unfortunately, the proposal of *forma asexualis* has not received broad support from other taxonomists. However, in my opinion, whether or not *forma asexualis* is used, every reasonable effort should be made to reveal the sexual cycle of new (and extant) anamorphic yeast species. Sexual reproduction plays a fundamental role in the biology of living beings. Therefore, wherever possible, the characteristics of the sexual reproduction must be included in the description of a new yeast species. In the case of heterothallic species, the investigation of sporulation is also a useful tool in deciding whether or not closely related strains are conspecific. Mating compatibility is a foundational criterion for species circumscription, even if there are cases where conspecific strains fail to sporulate and others where closely related but distinct species may form spores. Usually only the presence or absence of sporulation and spores is reported along with the morphology of sporulation. Generally the viability of the spores and the fertility of the subsequent generation are not studied, and so not all criteria of conspecificity according to the biological species concept are tested. Nonetheless, the presence or absence of spore formation represents important information about a species. Although nowadays the phylogenetic species concept often overshadows the biological species concept, the formation of a sexual state can provide irreplaceable information, even in the absence of sequence data. An interesting example to illustrate this is the following (taken from Kurtzman (2004) Antonie van Leeuwenhoek 85: 297-304). Strains of a new heterothallic yeast species

(*Trichomonascus petasosporus*) were recovered from insect frass, which if mixed with the proper mating partner developed a sexual stage nearly identical with that of *Trichomonascus mycophagus*, an uncultured fungus known only from dried herbarium specimens. As an attempt to isolate DNA from the type material of *T. mycophagus* was unsuccessful, the author had to rely on the morphology of the sexual state. Based on the striking similarity of the unique manner of ascus formation of the two fungi, the new species was assigned to the genus *Trichomonascus*, instead of being placed in the related (then still existing) *Stephanoascus*. This important observation also resolved the uncertainties of the phylogenetic placement of *Trichomonascus*, which was earlier considered to be endomycete-like. Without taking into account ascosporulation in *T. petasosporus* we would not know that *T. mycophagus* and *T. petasosporus* are closely related, and it would be a pity.

Recent Meeting

40th Annual Conference on Yeasts of the Yeast Commission of the Czechoslovak Society for Microbiology (3rd Yeast Research in Visegrad Countries), Smolenice, Slovakia, May 8-11, 2012

The 40th Annual Conference on Yeasts was organized by the Yeast Commission of the Czechoslovak Society for Microbiology, the Institute of Chemistry and Institute of Animal Biochemistry and Genetics, SAS, and the Department of Biotechnology, Slovak University of Technology, in collaboration with Institute of Ferm. Technol. Microbiol., Technical University of Lodz, Poland, Hungarian Society for Microbiology, Hungary and Institute of Microbiology, ASC, Czech Republic. It took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, on May 8-11, 2012 and was supported by International Visegrad Fund.

Prof. Peter Raspor from the University of Ljubljana, Slovenia presented the opening memorial lecture in honour of Dr. A. Kocková-Kratochvílová"Biodiversity, bio-technology, biosafety of industrial yeasts *Saccharomyces cerevisiae*: Can we handle all issues?". The conference was attended by over 90 scientists, mainly from Czech Republic and Slovakia, but there were also several invited speakers from Austria, France, Hungary, Italy, Poland, Netherlands and Thailand.

The conference program consisted of three sessions dedicated to Biochemistry and Cell Biology, Genetics and Molecular Biology, Biotechnology and Medical Mycology. The 25 oral presentations were complemented by 11 short 5 min presentations (Poster Highlights) and 30 posters. The interesting scientific program was interrupted by some relaxed moments, such as a wine and mead presentation and tasting by the Slovak producing companies.

Further information about the activities of the Yeast Commission of the Czechoslovak Society for Microbiology can be found on the website http://www.chem.sk/yeast. The abstracts of lectures and posters are on this website. The titles of lectures of the 40th Annual Yeast Conference are listed below:

Invited Lecture to the memory of Dr. A. Kocková-Kratochvílová - Peter Raspor (Slovenia) Biodiversity, bio-technology, biosafety of industrial yeasts *Saccharomyces cerevisiae*: Can we handle all issues?

1 Kopecká M. (Czech Republic) On the nature, ultrastructure and assembly of the yeast cell wall.
2 Vecchiarelli A. (Italy): *Candida albicans* secreted aspartic proteases induce inflammatory.
3 Hagen F, Ceresini P.C, Polacheck I, Meis J F, May, R C, Klaassen C.H.W, Boekhout T. (The Netherlands): Out of the Amazonian Rainforest-ancient dispersal of the human fungal pathogen *Cryptococcus gattii.*
4 Kašperová A, Czerneková L, Weigl E, Čeřovský V, Turánek J, Raška M (Czech Republic): Testing of selected bee venom antimicrobial peptides on growth and morphogenesis of *Candida albicans.*
5 Paulovičová L, Paulovičová E, Pericolini E, Gabrielli E, Vecchiarelli A. (Slovakia/Italy): Immunomodulatory efficiency of *Candida glabrata* cell wall mannan.
6 Svobodová L, Prášilová L, Šefraná V, Bardoň J, Raclavský V, Hamal P. (Czech Republic): Differentiation of clinical isolates of *Candida utilis, C. pelliculosa* and *C. fabianii* using RAPD, McRAPD and MALDI-TOF MS.
7 Tongta A. (Tailand): Production and Purification of recombinant human growth hormone from *Pichia pastoris* in a GMP pilot scale process.
8 Dulermo T, Beopoulos A, Haddouche R, Poirier Y, Nicaud J.-M. (France): Utilization of linseed oil by various acyltransferase mutants of *Yarrowia lipolytica.*

9 Gajdoš P, Nicaud J.-M, Čertík M. (Slovakia/France)Utilization of linseed oil by various acyltransferase mutants of *Yarrowia lipolytica*.
10 Holič R, Yazawa H, Kumagai H, Uemura H. (Slovakia/Japan): Engineered high content of ricinoleic acid in fission yeast *Schizosaccharomyces pombe.*
11 Kregiel D, Czyzowska A, Ambroziak W. (Poland): Apple pomace as a valuable source for yeast biotechnology.
12 Biely P, Vršanská M. (Slovakia): *Aureobasidium pullulans* – a yeast-like microorganism with diverse biotechnological potential.
13 Brlejová M, Čertík M, Breierová E. (Slovakia): Antioxidant properties of cell wall glycoproteins from *Rhodotorula glutinis*.
14 Bizaj E, Cordente A. G, Bellon J. R, Raspor P, Curtin C. D, Pretorius I. S. (Slovenia/Australia): Novel development strategy for industrial yeasts: Case wine yeasts.
15 Ďurčanská K, Furdíková K, Ševcech J, Malík F. (Slovakia): How to improve wine? Aplication of autochthonous yeasts.
16 Klis F.M, Heilmann, C.J, Sorgo A.G. (The Netherlands) A guided tour through the wall proteome of the clinical fungus *Candida albicans.*
17 Mazáň M, Zemková Z, Blanco N, Arroyo J, Farkaš V. (Slovakia/Spain): Multiple functions of Chr proteins involved in he formation of yeast cell walls.
18 Hrušková-Heidingsfeldová, O, Dostál J, Vinterová Z, Brynda J, Šanda M, Řezáčová P, Pichová I. (Czech Republic): Secreted aspartic proteinase Sapp1p from *Candida parapsilosis*: interaction with inhibitors and with the cell surface.
19 Poloncová K, Šimová Z, Tahotná D, Holič R, Griač, P. (Slovakia) - Pdr16p and sterol metabolism of the yeast *Saccharomyces cerevisiae.*
20 Hodurova Z, Ferreira L, Balazfyova Z, Dominguez A, Gbelska Y. (Slovakia/ Spain): Does multidrug resistance-regulating transcription factor *Kl*Pdr1p affect cytosolic proteome of *Kluyveromyces lactis*?
21 Tarnowski LJ, M. Milewski M, Kurlandzka A. (Poland): Comparative analysis of cellular distribution of human cohesins SA1 and SA2 expressed in yeast and HeLa cells.
22 Gacser, A, Pfeiffer I. (Hungary): Behind the Host-Microbe Interactions: Functional and structural analysis of virulence factors of *Candida parapsilosis*.
23 Haslinger D, Chiocchetti A. , Karl T. , Bösch M. , Kellermann J, Waltes R, Poustka F. , Bauer J.W, Freitag C.M. , Hintner H, Lottspeich F. , Wiemann S, Klauck SM, Breitenbach-Koller H. (Austria/ Germany): Proteomic approach identifies putative pathomechanisms in autism.

Communicated by Emilia Breierová

Forthcoming Meeting

ISSY30 - High Tatras-Stará Lesná, Slovakia

ISSY30 is planned for 18-22 September 2013 at the Congress Centre Academia, Stará Lesná, Slovakia. Readers are encouraged to look for updates on the forthcoming ISSY30 website: www.issy2013.org

Brief News Items

Address Change: Andrey Yurkov

Andrey Yurkov starts his new postdoctoral project entitled "Yeast communities in Mediterranean ecosystems: exploration of genetic diversity, ecological adaptations and biotechnological potential" at the CREM - Center for Microbial Resources, Universidade Nova de Lisboa, Caparica, Portugal. A part of this project will be dedicated to the Portuguese Yeast Culture Collection. The project aims to understand better the microbial diversity harbored by Mediterranean ecosystems through the analysis of molecular data for yeast strains deposited in the PYCC collection. Hence, this project will contribute to the ongoing activity of PYCC, which includes revision of yeast strains available at PYCC, barcoding of yeast species from Mediterranean ecosystems and the reassessment of species boundaries of the most representative species using multi-locus sequence typing. The data gathered during this project will be stored in PYCC database: http://pycc.bio-aware.com

Future correspondence could be addressed to:

Dr. Andrey Yurkov
Portuguese Yeast Culture Collection,
Centro de Recursos Microbiologicos (CREM),
Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa,
Campus de Caparica,
2829-516 Caparica, Portugal

E-mail: <a.yurkov@campus.fct.unl.pt>
My previous e-mail addresses will also remain active.

New laboratory: Chris Todd Hittinger

Chris Todd Hittinger has opened a new yeast research lab in the Laboratory of Genetics at the University of Wisconsin-Madison (http://hittinger.genetics.wisc.edu/).

The Hittinger Lab studies the diversity of yeast carbon metabolism as a model for how complex gene networks evolve. Training opportunities in molecular genetics and functional and evolutionary genomics are available for highly qualified students and postdocs.

Internal appointments within Lesaffre (Marcq-en-Baroeul, 17 January 2012)

Following the arrival of Christophe de Saint Louvent as CEO of Lesaffre at the beginning of July 2011, a new organisation was set up and resulted in internal appointments.

Emmanuel Lorette, age 59, becomes Europe Bread-making President. A graduate of ESC Lille and CPA-HEC, he began his career at La Redoute in the Purchase Department. After having been head of one of the CEPI department (training and consulting in companies), he joined Lesaffre in 1995 as a purchase co-ordinator. He then became general manager for Morocco and then Poland, before becoming the President for Central Europe in 2008.

Vincent Saingier, age 42, graduated from the INSA Toulouse and CPA-HEC, becomes Latin America Bread making President. He began his career with Lesaffre in 1992 as a technical manager in Spain. He later occupied different production and general manager functions for Hungary, Italy, China and France before becoming Overseas Bread making President in 2009.

Jean-Philippe Poulin, age 43, becomes Overseas Bread making President in 2009. With an advanced degree in financial management, he spent twelve years in the Soufflet group of which eight years as general manager of the flour milling activity. He joined Lesaffre in 2009 to take charge of the ingredients business unit.

Hervé Bolze, age 53, becomes Bread making Marketing and Commercial Co-ordination Vice President. A graduate of the EPSCI (ESSEC group) with a MBA IBEAR (University of South California-USA) and a Master degree from IPADE (Mexico), he joined Lesaffre in 1985 as an export sales manager. In 1990, he settled down in Mexico to take charge of the sales and marketing department of Latin America, then that of the American continent, and finally of the Central American region after its creation.

Paolo Rossi, age 54, becomes Nutrition and Health President. A graduate of the University of Milan, he joined Lesaffre in 2001 as Chief Executive Officer for Italy before taking over the management of the Western European region after its creation in 2004. He had previously worked in different agro-food companies in Italy and in an English multi-national chemical company.

Founded in1853, Lesaffre, an independent French family-run group, has now become a leader in the domain of bread-making yeast and yeast extracts. Lesaffre has unique technical skills that it has thoroughly mastered, and enjoys top-level expertise in the production, fermentation and transformation of yeast in all its forms. Present on all five continents with more than 45 production sites and large number of distribution subsidiaries, Lesaffre adapts to the precise needs of its clients. Its products are distributed in more than 160 countries. The group employs 7000 people out of which 1600 are in France. It made a turnover of 1.3 billion euros in 2010.

For more information: www.lesaffre.com
Contact: Anne-Sophie Joly
Tel: 33 3 28 52 00 56 asophie.joly@rp-carrees.com

50 Years Ago

Y E A S T
A News Letter for Persons Interested in Yeast
May 1962, Volume XI, Number 1

Mrs. N. **Kreger-van Rij** of CBS announced that type strains of seven new yeast species were received at the CBS.

Dr. **van Uden** will attend the International Congress of Microbiology and the A.I.B.S. meetings during August in Montreal, Canada and Corvallis Oregon respectively; he is looking forward to seeing again the old friends in the States.

Dr. **Margaret di Menna** reported on a survey of soil yeasts in New Zealand. "[D]ominant species have been, with monotonous regularity, *Cryptococcus albidus, Cryptococcus terreus* and *Candida curvata."*

"The following article will appear soon in the Bulletin de la Société Mycologique de France "Les levures à spores réniformes" by **J. Boidin, F. Abadie, J.L.Jacob and M.C. Pignal**. [...] The authors propose to assign yeasts with [kidney or cresent-shaped ascospores] to the genus Guilliermondella Nads. et Krassiln."

Dr. **D. M. Reynolds** of the University of California Davis described studies of the spore discharge mechanism in *Sporobolomyces*, using a high-speed motion picture camera with a framing rate of 1000 pictures per second.

Dr. **H. Phaff** of the University of California Davis announced that **Dr. Michael Lewis**, from the University of Birmingham, England, joined his group to work on nitrogen excretion by brewer's yeast. "Dre. J.F.T. Spencer, Prairie Regional Laboratory, Saskatoon, Sask., Canada, will spend a sabbatical year at Davis to work in the area of yeast ecology and taxonomy."

Dr. **S. Windisch** of the Institut für Gärungsgewerbe, Berlin, described studies of genetics of *Saccharomyces* yeasts, including ploidy in bottom-fermenting beer yeasts. They devised a method to study genetics of homothallic strains, by crossing with LiCl-resistant heterothallic haploids.

Dr. **M. Solotorovsky** of Rutgers studied the fate of *Candida albicans* in experimental infections in mice using fluorescent abtibodies.

Dr. **G. C. Ainsworth** of the Commonwealth Mycological Institute, Surrey, England commented on Article 37 of the 1961 edition of the International Code of Botanical Nomenclature, which requires that a nomenclatural type must be designated, and requested that taxonomists submit bibliographical citations to be recorded in the Index of Fungi.

A listing of the names and institutional affiliation of 301 subscribers to the newsletter was published. 193 were in the USA and Canada.