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

M.A. Lachance, Editor

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Recent publications.

- 1 Golubev WI, Pfeiffer I 2014 A Study of *Schizoblastosporion starkeyi-henricii* isolates from northern and southern hemispheres of the Earth. *Microbiology* 83(5):661–665.

The species *Schizoblastosporion starkeyi-henricii* is common in peats and peaty soils. Fifteen strains of this species isolated from Europe and East Falkland Island were found homogenous in cultural, morphological and physiological characters, with the exception of one strain that was sensitive to elevated osmotic pressure.

Analyses of the D1/D2 region of the 26S rDNA and internal transcribed spacers (ITS) confirm that these strains and also Asian isolates are conspecific. Our results show that ecological factors but not geographic distances determine the global distribution of yeast fungi.

- 2 Golubev WI 2015 Intraspecific and intrageneric antagonistic activity in *Wickerhamomyces anomalus*. *Microbiology* 84 (in press).

Strains of *Wickerhamomyces anomalus* were examined for antagonistic activity. They include nomenclatural types of species which names are considered now as synonyms. More than 70% of strains exhibited antibiotic activity. Among them three groups were revealed by activity against *W. anomalus* strains

which fell into five subgroups by activity against species of their own genus and *Candida* spp. related to *Wickerhamomyces* phylogenetically. For the most part, antibiotic agents are mycocins that are different in their physico-chemical properties.

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We are grateful to Tatiana Tanaschuk (INMIV “Magarach”, Yalta, Crimea) and Kyria Boundy-Mills (Herman J. Phaff Yeast Culture Collection at University of California, Davis) for visiting their labs in June 2014 and September 2014. We were glad to accept in our lab Taiwanese colleagues Lee Ching-Fu (National Hsinchu University of Education) and Wang Pin-Han (Tonghai University) during short visit in June 2014.

The following are papers for 2014 or submitted.

- 1 Naumova ES, Sadykova AZh, Martynenko NN, Naumov GI 2014 Molecular polymorphism of β -fructosidase *SUC* genes in the *Saccharomyces* yeasts. *Molecular Biology (Moscow)*, 48 (4):573–582. © Pleiades Publishing, Ltd.
- 2 Naumov GI, Naumova ES, Glushakova AM, Kachalkin AV, Chernov IYu 2014 Finding of dairy yeasts *Kluyveromyces lactis* var. *lactis* in natural habitats. *Microbiology (Moscow)* 83 (6): 782–786. © Pleiades Publishing, Ltd.

Well-known yeasts *Kluyveromyces lactis* var. *lactis*, which are usually associated with dairy products, were discovered in nature: in woodland park soil under *Impatiens glandulifera* Royle plants. Reliable identification of the yeasts was carried out using physiological criteria (lactose and maltose utilization)

and molecular markers (nucleotide sequence of the 5.8S-ITS rDNA fragment, pulsed-field gel electrophoresis, and Southern hybridization of chromosomal DNA with the *LAC4* probe). Ecology of *Kl. lactis* var. *lactis* is discussed.

- 3 Naumov GI, Kondratieva VI, Naumova ES 2015 Hybrid sterility of the yeast *Schizosaccharomyces pombe*: genetic genus and many species in statu nascendi? *Microbiology (Moscow)*. 84 (1) (in press).

A phenomenon of ascospore death was observed in a number of *Schizosaccharomyces pombe* interstrain hybrids. Meiotic recombination of the control parental auxotrophic markers was, however, observed in a random sample of ascospores. Genetic and molecular

biological data indicated existence of at least geographical divergence of the genomes in *Sch. pombe* populations. Classification of the genus, species, and varieties of these yeasts is discussed.

- 4 Naumov GI 2015 Hyperproduction of invertase can confer inulin fermentation in selection strains of *Saccharomyces cerevisiae*. Microbiology (Moscow). (in press).

There are several recent publications on selection strains of *Saccharomyces cerevisiae* capable to ferment inulin; it is the authors' opinion that such strains possess inulinase. The review summarizes literature data, which

allow to consider that the hyperproduction of invertase, a common enzyme of *Saccharomyces cerevisiae* yeasts, takes place in selection strains.

- 5 Naumov GI 2015 The yeast *Komagataella* – genetic genus in accordance with interspecies hybridization. Microbiology (Moscow). (submitted)

Using induced complementary auxotrophic mutants and selective growth of prototrophic hybrids on minimal medium, hybridization of the type strain of *Komagataella kurtzmanii* VKPM Y-727 with the type strains of *K. pastoris* VKPM Y-3262, *K. phaffii* NRRL Y-7556, *K. populi* NRRL YB-455, *K. pseudopastoris* NRRL Y-27603 and *K. ulmi* NRRL YB-407 was demonstrated.

The data obtained suggest that the genus *Komagataella*, established earlier by phylogenetic analysis, corresponds well to the concept of genetic genus in ascomycetous fungi. According to the concept, the genetic genus is a group of hybridized species having a common mating type system. Employment of the concept of genetic genus to different yeast genera is discussed.

- 6 Naumov GI, Naumova ES, Lee Ch-Fu 2015 Towards reinstatement of the yeast genus *Zygowilliopsis* Kudriavzev (1960). Microbiology (Moscow). (submitted)

Experimental data on genetic molecular classification and identification of the yeast genus *Zygowilliopsis* are summarized. The genus is represented by at least five biological species and three varieties: *Z. californica*: var. *californica*, var. *dimennae* and var.

fukushimae. Biogeography, ecology and killer activity of *Z. californica* yeasts is considered. Heterogeneity of the taxonomic genus *Barnettozyma* Kurtzman et al. (2008) and necessity of its revision are discussed.

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We are grateful to Dr. Forbes Wardrop, Lallemand Inc., Montreal, Canada, for making it possible for us to visit Lallemand labs in Montreal in May/June 2014 for discussions and collaborative work.

The abstract of a recently published paper follows. An open access PDF is available at:
http://www.bluepenjournals.org/ijambr/pdf/2014/November/Nayyar_et_al.pdf

- 1 Ashima Nayyar, Graeme Walker, Elisabetta Canetta, Forbes Wardrop, Ashok K Adya 2014 Cell surface properties and flocculation behaviour of industrial strains of *Saccharomyces cerevisiae*.

Cellular adhesion properties of yeasts depend on the characteristics of the outer layer of the cell wall. In this study, the flocculation behaviour of four industrial strains of *Saccharomyces cerevisiae* used for production of beer, champagne, wine and fuel alcohol was evaluated; their flocculation abilities being, 42.5%, 14.8%, 13.8% and 11.6%, respectively. The brewing

yeast strain was found to be the most flocculent. Very little flocculation was observed during the lag and logarithmic phases of growth (1-15%), while during the early and late stationary phases, different strains exhibited variable flocculation patterns. Cell surface hydrophobicity (assayed using HMA and MATS) and surface charge (assayed by Alcian Blue dye retention)

played important roles in dictating flocculation behaviour in different yeast strains, as did the yeast growth phase. Percentage hydrophobicity index (HI) and % hydrophobicity of the four strains followed, respectively the same order, viz Beer (66.6, 21.5) >

Champagne (33, 10.5) > fuel alcohol (22.4, 7.4) > wine (20.5, 2.7). Our findings provide new insight into yeast cell surface properties and how these relate to behavioural characteristics of yeasts employed in industrial fermentations.

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Letter to the Editor

The *MAL4* gene in the yeast *Saccharomyces cerevisiae*

The *MAL4* gene constitutive for maltase synthesis in yeast strain 1403-7A was first described by Khan and Eaton (1971). This strain carrying the *MAL4* gene produces a very high level of maltase, 15-500 fold higher when cultured on glucose than any other family of the *MAL* genes. The presence of very high levels of cytoplasmic maltase and an active permease system allows the fermentation of sucrose in the absence of classical invertase in strain 1403-7A. This is a unique property of this strain with respect to sucrose fermentation, and we reported these results for the first time in a paper published by Khan et. al. (1973). It is important to note that two major alpha-glucosidases in yeast, namely maltase and isomaltase hydrolyse sucrose in vitro as well. Strain 1403-7A is a desirable yeast strain for industrial use as described by Badotti et.al. (2008). These authors have constructed a strain derived from 1403-7A by deleting the *AGTI* gene responsible for a high affinity permease system, otherwise similar to strain 1403-7A. This newly constructed strain, however, has several low affinity *MALXI* permease genes, namely the *MAL21*, *MAL31* and *MAL41*. The absence of the *AGTI* gene in strain 1403-7A is considered desirable as it produces a high density biomass in small batch cultures during the

fermentation process. The presence of the permease genes *MAL21* and *MAL31* in strain 1403-7A also suggests that the regulatory and maltase structural genes for these loci, namely the *MAL2* and *MAL3* should be present in the cryptic form in this strain. Cryptic genes are known to exist in microorganisms (Hall et. al. 1983).

References

- 1 Badotti F, Dario MG, Alves Jr SL, Cordioli ML, A , Miletto LC, Araujo PS de, and Stambuk BU 2008 Switching the mode of sucrose utilization by *Saccharomyces cerevisiae*. Microbial Cell Factories, 7:4
- 2 Hall BG, Yokoyama S, and Calhoun DH 1983 Role of cryptic genes in microbial evolution Mol Biol Evol. 1 (1):109-124
- 3 Khan NA and Eaton NR, 1971 Genetic Control of maltase formation in yeast. 1. Strains producing high and low basal levels of enzyme. Molec Gen Genet 112:317-322
- 4 Khan NA, Zimmermann FK and Eaton NR 1973 Genetic and biochemical evidence of sucrose fermentation by maltase in yeast. Molec. gen. Genet. 123, 43-50

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Recent publication.

- 1 Lucia Paulovičová , Ema Paulovičová, Slavomír Bystrický 2014 Immunological basis of anti-*Candida* vaccines focused on synthetically prepared cell wall mannan-derived manno-oligomers. Microbiol Immunol 58:545–551.

The increasing incidence of diseases caused by *Candida* species and complications in individuals with impaired immunity require new strategies for candidiasis treatment and prevention. The available therapies are often of limited effectiveness in immunocompromised patients, resulting in treatment failures, chronic

infections and high mortality rates. Research directed at identifying the composition of an effective vaccine is required. Mannan forms the outermost layer of the *Candida* cell wall and has an essential role in modulation of anti-*Candida* host immune responses. Therefore, *Candida* cell wall mannan and synthetically

prepared manno-oligomer-based glycoconjugates are the foci of attention in vaccine candidate development. Almost all of the existing human vaccines mediate protection through neutralizing antibodies. Th1-based and/or Th17-based cellular immune responses, rather than antibody-mediated immunity, mediate protection

against candidiasis. Findings of published studies indicate that analysis of cellular immune responses as well as antibody responses is necessary when assessing the immunomodulatory properties of manno-oligomer-based glycoconjugates that are potential anti-*Candida* vaccine candidates.

- 2 Kornélia Nemcová, Emília Breierová, Renáta Vadkertiová and Jana Molnárová 2014 The diversity of yeasts associated with grapes and musts of the Strekov winegrowing region, Slovakia. *Folia Microbiol.* published online: 25 September 2014

Many different yeast species have been isolated from grapes and musts worldwide. The diversity and frequency of yeasts depend on a number of factors such as the grape variety, the physical damage of the grapes, the weather conditions and the chemical composition of must. A total of 366 isolates were associated with the three grape cultivars: Blue Frankish, Green Veltliner and Sauvignon blanc over four consecutive years. Yeast cultures were isolated from the grapes and from the fermenting musts after the first and seventh days. The ascomycetous yeasts of the genera *Aureobasidium*, *Candida*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Saccharomyces* and *Saccharomycopsis* together with basidiomycetous yeasts of the genera *Cryptococcus*, *Dioszegia*, *Filobasidium*, *Rhodotorula* and *Sporidiobolus*

were associated with the three grape varieties. *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, *Pichia kudriavzevii* and *Sporidiobolus pararoseus* were found on the berries in significant amounts. *P. kluyveri* and *P. kudriavzevii* were more associated with the damaged grapes, whereas *Sp. pararoseus* with intact ones. *H. uvarum* and *M. pulcherrima* were present on both types of grapes almost equally. The yeast composition and quantitative representation of yeast species varied over the grape varieties and the years examined. Although the basidiomycetous species formed a significant proportion of the yeast population in some individual grape variety/year combinations, the ascomycetous species were dominant.

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The following papers have been published recently.

- 1 Kordialik-Bogacka E, Diowksza A 2014 Metal uptake capacity of modified *Saccharomyces pastorianus* biomass from different types of solution. *Environ Sci Pollution Res* 21:2223–2229.

In this paper, we investigate the effect of different biomass pretreatments on metal ion uptake by various biosorbents. Heat-treated as well as caustic-treated and ground biomass of *Saccharomyces pastorianus* was used to remove copper, lead and cadmium from various solutions. Untreated yeast was used as the control sample. The effect of yeast modification on sorption capacity depended on the different types of heavy metal ions and whether they were in single- or multi-component solutions. The highest uptake of copper and lead from a single-metal solution was obtained from heat-treated cells. Ground biomass was the most

efficient at cadmium removal. However, the sorption capacity of the modified biomass did not improve when metal ions were removed from multi-component solutions. Indeed, the results in this paper show that optimizing metal removal from single-cation solutions can lead to decreased sorption capacity in multi-component solutions. Therefore, while adjusting the procedure of biomass modification, not only the nature of the metal ion being sorbed but also the chemical composition of the metal ion solution should be taken into account.

- 2 Rajkowska K, Kunicka-Styczyńska A, Maroszyńska M, Dąbrowska M 2014 The effect of thyme and tea tree oils on morphology and metabolism of *Candida albicans*. *Acta Biochim Polonica*, 61(2):305–310.

Members of *Candida* species cause significant problems in medicine and in many industrial branches

also. In order to prevent from *Candida* sp. development, essential oils are more and more frequently applied as

natural, non-toxic, non-pollutive and biodegradable agents with a broad spectrum of antimicrobial activity. The aim of the research was to determine changes in morphology and metabolic properties of *Candida albicans* in the presence of thyme and tea tree oils. Changes of enzymatic activity of isolates were observed in the presence of both tested essential oils, and they were primarily associated with loss or decrease of activity of all enzymes detected for control. Furthermore, only for 3 out of 11 isolates additional activity of N-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase and trypsin was detected. Vivid changes in biochemical profiles were found after treatment with tea tree oil and they were related to loss of ability to

assimilate D-xylose, D-sorbitol and D-trehalose. The main differences in morphology of isolates compared to the control strain concerned formation of pseudohyphae structures. Both examined essential oils caused changes in cell and colony morphology, as well as in the metabolism of *Candida albicans*. However, the extent of differences depends on the type and concentration of an essential oil. The most important finding is the broad spectrum of changes in yeast enzymatic profiles induced by thyme and tea tree oils. It can be supposed that these changes, together with loss of ability to assimilate saccharides could significantly impact *Candida albicans* pathogenicity.

3 Kregiel D, Berłowska J 2014 Effect of quaternary ammonium silane coating on adhesive immobilization of industrial yeasts. *Chemical Papers* 68:308-315.

The aim of the research was to study how the surface coating with quaternary ammonium silanes influences the attachment of industrial yeast cells. Three brewery and distillery strains belonging to *Saccharomyces cerevisiae*, and one strain of *Debaryomyces occidentalis* with high amylolytic activity were used in this study. Native chamotte carriers were modified using two organo-silanes with different functional groups containing ethanolamine ([3-N(N,N,N-dimethyl(2-hydroxyethyl)ammonio)propyl]trimethoxysilane) and octan-1-amine (octylamine) ([3-N(N,N,N-dimethyl-octylammonio)propyl]trimethoxysilane). The yeast cell surface charge was evaluated

using an alcian blue retention assay. To determine the adhesion efficiency, light microscopy and methylene blue staining of cells were used. The viability of immobilized cells was confirmed by [2-chloro-4-(2,3 dihydro-3-methyl(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenylquinolinium iodide] (FUN@1) staining. Modification of chamotte carriers increased the biomass load significantly; however, organo-silane with octylamine showed strong anti-yeast properties. This paper describes the use of inexpensive porous chamotte covered with active organo-silanes with quaternary ammonium groups as a way to improve yeast cell adhesion efficiency.

4 Wilkowska A, Kregiel D, Guneser O, Karagul Yuceer Y 2014 Growth and by-product profiles of *Kluyveromyces marxianus* cells immobilized in foamed alginate. *Yeast* - DOI: 10.1002/yea.3044.

The aim of this research was to study how the yeast cell immobilization technique influences the growth and fermentation profiles of *Kluyveromyces marxianus* cultivated on apple/chokeberry and apple/cranberry pomaces. The encapsulation of cells was performed by droplet formation from a foamed alginate solution. The growth and metabolic profiles were evaluated for both

free and immobilized cells. Culture media with fruit waste produced good growth of free as well as immobilized yeast cells. The fermentation profiles of *K. marxianus* were different with each waste material. The most varied aroma profiles were noted for immobilized yeast cultivated on apple/chokeberry pomace.

5 Berłowska J, Kregiel D, Rajkowska K 2014 Biodiversity of brewery yeast strains and their fermentative activities. *Yeast* - DOI: 10.1002/yea.3041.

We investigated the genetic, biochemical, fermentative and physiological characteristics of brewery yeast strains and performed a hierarchical cluster analysis to evaluate their similarity. We used five different ale and lager yeast strains, originating from different European breweries and deposited at the National Collection of Yeast Cultures (UK). Ale and lager strains exhibited

different genomic properties, but their assimilation profiles and pyruvate decarboxylase activities corresponded to their species classifications. The activity of another enzyme, succinate dehydrogenase, varied between different brewing strains. Our results confirmed that ATP and glycogen content, and the activity of the key metabolic enzymes succinate

dehydrogenase and pyruvate decarboxylase, may be good general indicators of cell viability. However, the genetic properties, physiology and fermentation capacity

of different brewery yeasts are unique to individual strains.

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Y1000+ Project Funded by the Dimensions of Biodiversity Program of the National Science Foundation (DEB-1442148).

In 2015, along with the labs of Antonis Rokas (Vanderbilt) and Cletus P. Kurtzman (USDA), we are embarking on an ambitious 5-year project to sequence and analyze the genomes of the type strains of all described species of *Saccharomycotina* yeasts. This project seeks to perform global analyses of the tempo and mode of yeast genome evolution, investigate illuminating metabolic case studies in detail, and provide the community with vital resources to support genetic, taxonomic, and ecological studies. We do not intend to compete with smaller scale studies focused on specific questions; when appropriate, we will collaborate or avoid specific taxa. We seek community

support to prioritize clades and strains and, in the rare cases where they are not already available, to provide strains for genome sequencing and high-throughput phenotyping. Several key labs and strain collection curators have already agreed to collaborate. A project website will be set up soon, and links will be provided from the Hittinger Lab website:

<http://hittinger.genetics.wisc.edu>.

Both postdoctoral and graduate training opportunities are available for researchers and students interested in working on this project or other projects in the lab:

<http://hittinger.genetics.wisc.edu/People/Join/index.html>.

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<http://hittinger.genetics.wisc.edu/Research/Funding/PostDocAd2014.html>.

Recent publications.

- 1 Alexander WG, Doering DT, Hittinger CT 2014 High-efficiency genome editing and allele replacement in prototrophic and wild strains of *Saccharomyces*. *Genetics* 198: 859-66.

Current genome editing techniques available for *Saccharomyces* yeast species rely on auxotrophic markers, limiting their use in wild and industrial strains and species. Taking advantage of the ancient loss of thymidine kinase in the fungal kingdom, we have developed the herpes simplex virus thymidine kinase gene as a selectable and counterselectable marker that forms the core of novel genome engineering tools called the Haploid Engineering and Replacement Protocol (HERP) cassettes. Here we show that these cassettes

allow a researcher to rapidly generate heterogeneous populations of cells with thousands of independent chromosomal allele replacements using mixed PCR products. We further show that the high efficiency of this approach enables the simultaneous replacement of both alleles in diploid cells. Using these new techniques, many of the most powerful yeast genetic manipulation strategies are now available in wild, industrial, and other prototrophic strains from across the diverse *Saccharomyces* genus.

- 2 Almeida P, Gonçalves C, Teixeira S, Libkind D, Bontrager M, Masneuf-Pomarede I, Albertin W, Durrens P, Sherman DJ, Marullo P, Hittinger CT, Gonçalves P, Sampaio JP 2014 A Gondwanan imprint on global diversity and domestication of wine and cider yeast *Saccharomyces uvarum*. *Nat Commun* 5: 4044.

In addition to *Saccharomyces cerevisiae*, the cryotolerant yeast species *S. uvarum* is also used for wine and cider fermentation but nothing is known about its natural history. Here we use a population genomics approach to investigate its global phylogeography and domestication fingerprints using a collection of isolates obtained from fermented beverages and from natural environments on five continents. South American isolates contain more genetic diversity than that found in the Northern Hemisphere. Moreover, coalescence analyses suggest that a Patagonian sub-population gave

rise to the Holarctic population through a recent bottleneck. Holarctic strains display multiple introgressions from other *Saccharomyces* species, those from *S. eubayanus* being prevalent in European strains associated with human-driven fermentations. These introgressions are absent in the large majority of wild strains and gene ontology analyses indicate that several gene categories relevant for wine fermentation are over-represented. Such findings constitute a first indication of domestication in *S. uvarum*.

- 3 Peris D, Sylvester K, Libkind D, Gonçalves P, Sampaio JP, Alexander WG, Hittinger CT. 2014. Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Mol Ecol* 23: 2031-45.

Reticulate evolution can be a major driver of diversification into new niches, especially in disturbed habitats and at the edges of ranges. Industrial fermentation strains of yeast provide a window into these processes, but progress has been hampered by a limited understanding of the natural diversity and distribution of *Saccharomyces* species and populations. For example, lager beer is brewed with *Saccharomyces pastorianus*, an allopolyploid hybrid of *S. cerevisiae* and *S. eubayanus*, a species only recently discovered in Patagonia, Argentina. Here, we report that genetically diverse strains of *S. eubayanus* are readily isolated from Patagonia, demonstrating that the species is well established there. Analyses of multilocus sequence data strongly suggest that there are two diverse and highly differentiated Patagonian populations. The low nucleotide diversity found in the *S. eubayanus* moiety of

hybrid European brewing strains suggests that their alleles were drawn from a small subpopulation that is closely related to one of the Patagonian populations. For the first time, we also report the rare isolation of *S. eubayanus* outside Patagonia, in Wisconsin, USA. In contrast to the clear population differentiation in Patagonia, the North American strains represent a recent and possibly transient admixture of the two Patagonian populations. These complex and varied reticulation events are not adequately captured by conventional phylogenetic methods and required analyses of Bayesian concordance factors and phylogenetic networks to accurately summarize and interpret. These findings show how genetically diverse eukaryotic microbes can produce rare but economically important hybrids with low genetic diversity when they migrate from their natural ecological context.

- 4 Leducq JB, Charron G, Samani P, Dubé AK, Sylvester K, James B, Almeida P, Sampaio JP, Hittinger CT, Bell G, Landry CR 2014 Local climatic adaptation in a widespread microorganism. *Proc Biol Sci* 281: 20132472.

Exploring the ability of organisms to locally adapt is critical for determining the outcome of rapid climate changes, yet few studies have addressed this question in microorganisms. We investigated the role of a heterogeneous climate on adaptation of North American populations of the wild yeast *Saccharomyces paradoxus*. We found abundant among-strain variation for fitness components across a range of temperatures, but this variation was only partially explained by climatic variation in the distribution area. Most of fitness

variation was explained by the divergence of genetically distinct groups, distributed along a north-south cline, suggesting that these groups have adapted to distinct climatic conditions. Within-group fitness components were correlated with climatic conditions, illustrating that even ubiquitous microorganisms locally adapt and harbour standing genetic variation for climate-related traits. Our results suggest that global climatic changes could lead to adaptation to new conditions within groups, or changes in their geographical distributions.

- 5 Hittinger CT 2013 *Saccharomyces* diversity and evolution: a budding model genus. Trends Genet 29:309-17.

Saccharomyces cerevisiae is one of the best-understood and most powerful genetic model systems. Several disciplines are now converging to turn *Saccharomyces* into an exciting model genus for evolutionary genetics and genomics. Yeast taxonomists and ecologists have dramatically expanded and clarified *Saccharomyces* diversity, more than doubling the number of bona fide species since 2000. High-quality genome sequences are available (or soon will be) for all seven known species. Haploid laboratory strains are

enabling a deep integration of classic genetic approaches with modern genomic tools. Population genomic surveys and quantitative trait mapping of variation within species are underway across the genus. Finally, several case studies have illuminated general and novel genetic mechanisms of evolution. Expanding strain collections, low-cost genome sequencing, and tools for precise genetic manipulation promise to usher in a golden era for this surprisingly diverse genus as an evolutionary model.

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The following are the abstracts of papers published recently.

- 1 Harner, NK, PK Bajwa, MB Habash, JT Trevors, GD Austin & H Lee 2014 Mutants of the pentose-fermenting yeast *Pachysolen tannophilus* tolerant to hardwood spent sulfite liquor and acetic acid. Antonie van Leeuwenhoek 105:29-43.

A strain development program was initiated to improve the tolerance of the pentose-fermenting yeast *Pachysolen tannophilus* to inhibitors in lignocellulosic hydrolysates. Several rounds of UV mutagenesis followed by screening were used to select for mutants of *P. tannophilus* NRRL Y2460 with improved tolerance to hardwood spent sulfite liquor (HW SSL) and acetic acid in separate selection lines. The wild type (WT) strain grew in 50 % (v/v) HW SSL while third round HW SSL mutants (designated UHW301, UHW302 and UHW303) grew in 60 % (v/v) HW SSL, with two of these isolates (UHW302 and UHW303) being viable and growing, respectively, in 70 % (v/v) HW SSL. In defined liquid

media containing acetic acid, the WT strain grew in 0.70 % (w/v) acetic acid, while third round acetic acid mutants (designated UAA301, UAA302 and UAA303) grew in 0.80 % (w/v) acetic acid, with one isolate (UAA302) growing in 0.90 % (w/v) acetic acid. Cross-tolerance of HW SSL-tolerant mutants to acetic acid and vice versa was observed with UHW303 able to grow in 0.90 % (w/v) acetic acid and UAA302 growing in 60 % (v/v) HW SSL. The UV-induced mutants retained the ability to ferment glucose and xylose to ethanol in defined media. These mutants of *P. tannophilus* are of considerable interest for bioconversion of the sugars in lignocellulosic hydrolysates to ethanol.

- 2 Harner NK, X Wen, PK Bajwa, GD Austin, CY Ho, MB Habash, JT Trevors & H Lee 2014 Genetic improvement of native xylose-fermenting yeasts for ethanol production. J Indust Microbiol Biotechnol <http://dx.doi.org/10.1007/s10295-014-1535-z>

Lignocellulosic substrates are the largest source of fermentable sugars for bioconversion to fuel ethanol and other valuable compounds. To improve the economics of biomass conversion, it is essential that all sugars in potential hydrolysates be converted efficiently into the desired product(s). While hexoses are fermented into ethanol and some high-value chemicals, the bioconversion of pentoses in hydrolysates remains inefficient. This remains one of the key challenges in lignocellulosic biomass conversion. Native pentose-fermenting yeasts can ferment both glucose and xylose

in lignocellulosic biomass to ethanol. However, they perform poorly in the presence of hydrolysate inhibitors, exhibit low ethanol tolerance and glucose repression, and ferment pentoses less efficiently than the main hexoses glucose and mannose. This paper reviews classical and molecular strain improvement strategies applied to native pentose-fermenting yeasts for improved ethanol production from xylose and lignocellulosic substrates. We focus on *Pachysolen tannophilus*, *Scheffersomyces (Candida) shehatae*, *Scheffersomyces (Pichia) stipitis* and *Spathaspora*

passalidarum which are good ethanol producers among the native xylose-fermenting yeasts. Strains obtained thus far are not robust enough for efficient ethanol

production from lignocellulosic hydrolysates and can benefit from further improvements.

XIX National Laboratory of Industrial Microbiology, Department of Biology, Faculty of Sciences, Alzahra University, and Microorganism Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran. Communicated by S. Nasr <shaghayegh2963@yahoo.com>.

Recent publications.

- 1 Shaghayegh Nasr, Mohammad Reza Soudi, Seyed Abolhassan Shahzadeh Fazeli, Hai DT Nguyen, Matthias Lutz & Marcin Piątek 2014 Expanding evolutionary diversity in the Ustilaginomycotina: *Fereydouniaceae* fam. nov. and *Fereydounia* gen. nov., the first urocystidalean yeast lineage. *Mycol Progress* 13:1217-1226,

The systematic position of two strains of a yeastlike fungus isolated from plant remnants on the Kharg Island in the Persian Gulf of Iran is evaluated using morphological, physiological and phylogenetic analyses. In culture, this fungus produced cylindrical cells that reproduced by polar budding on short stalks. Production of ballistoconidia and blastospores was observed. The carbon source assimilation spectrum was broad, but fermentation ability was absent. Phylogenetic analyses of the nuclear SSU, LSU (D1/D2 domain), and ITS rDNA revealed that this fungus represents a new

lineage in the Urocystidales of the subphylum Ustilaginomycotina. Based on the comparison of phenotypic characters, physiology, and DNA sequences, a new genus and species *Fereydounia khargensis* (IBRC-M 30116T=CBS 13305T) is described for this fungus and accommodated in the novel family *Fereydouniaceae*. This is the first report of anamorphic saprobic fungus residing in the Urocystidales, stressing the remarkable evolutionary diversity in the subphylum Ustilaginomycotina.

- 2 Shaghayegh Nasr, Mohammad Reza Soudi, Seyyedeh Maryam Zamanzadeh Nasrabadi, Mahdi Moshtaghi Nikou, Ali Hatef Salmanian & Hai DT Nguyen 2014 *Basidioascus persicus* sp. nov., a yeast-like species of the order Geminibasidiales isolated from soil. *Int J Syst Evol Microbiol* 64:3046–3052.

A novel species of basidiomycetes was isolated from kitchen garden soil in Shahryar city, Tehran province, Iran. Molecular and conventional methods were employed to identify and classify this single isolate. Morphologically, the isolate was considered yeast-like with hyaline and oval cells reproducing by monopolar budding, forming ballistoconidia, hyphae, arthroconidia and didymospores. Basidia and basidiospores resembling those produced by *Basidioascus* species were observed. Sequencing and Bayesian phylogenetic analysis of rRNA genes and the internal transcribed spacer region revealed its sister relationship to described

species of the genus *Basidioascus*. Assimilation and fermentation tests, cell-wall carbohydrate analysis and enzyme activity tests were performed to provide insight into the metabolism of the isolate. Based on morphology, physiology and phylogeny of rRNA gene sequences, the isolate was shown to represent a novel species of the genus *Basidioascus*, described as *Basidioascus persicus* sp. nov. (holotype IBRC P1010180T5ex-type IBRC M30078T5isotype CBS 12808T). The MycoBank number of the novel species is MB 804703. An emended description of the genus *Basidioascus* is also provided.

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

Following the suggestion of a reviewer of JGAM to present a phylogenetic tree and to include in this tree our cell wall sugar data, we have completely rewritten our recent manuscript and gave it a new title. Our last scientific publication gives a comprehensive overview of our work on cell wall sugars with the new molecular taxonomy.

- 1 Prillinger, H., Lopandic, K. Yeast types of the Basidiomycota using cell wall sugars and ribosomal DNA sequences.

The cell wall carbohydrate composition was correlated with the D1/D2 based phylogeny to estimate its significance in evolution of the Basidiomycota. The majority of investigated isolates showed three main cell wall sugar types: the *Microbotryum*-, the *Ustilago*- and the *Tremella*-type. The *Microbotryum*-type (mannose-glucose-galactose-fucose) corresponded with the subphylum Pucciniomycotina, the *Ustilago*-type (glucose-mannose-galactose) with the Ustilaginomycotina and the *Tremella*-type (glucose-mannose-xylose) with the Agaricomycotina. However, in a number of isolates additional carbohydrates were also identified. A sporadic appearance of rhamnose and xylose within the Microbotryomycetes of the Pucciniomycotina, or glucose-mannose pattern within the Agaricomycetes, and galactose or fucose within the

Tremellomycetes of the Agaricomycotina indicated that the cell wall carbohydrate composition characterise rather classes or subclasses than subphyla. The appearance of rhamnose, that is also present in the cell walls of the Taphrinales of the Ascomycota, may indicate a basal position of the Pucciniomycotina in the evolution of the Basidiomycota. This result is in conflict with the D1/D2 based phylogeny, suggesting that the Ustilaginomycotina occupy a basal position in the neighbor-joining tree. The occurrence of the glucose-mannose pattern of the *Saccharomyces*-type of the ascomycetous yeasts and in the highest evolved basidiomycetous yeast isolates (Agaricaceae) from two *Cyphomyrmex* species suggests that the Saccharomycotina are basal in the phylogenetic tree of the Ascomycota.

XI Centre International de Ressources Microbiennes (CIRM-Levures), INRA/AgroParisTech, 78850 Thiverval-Grignon, France - <http://www6.inra.fr/cirm/Levures>. Communicated by Serge Casaregola <serge@grignon.inra.fr>.

The following papers were recently published.

- 1 Jacques N, Louis-Mondesir C, Coton M, Coton E, Casaregola S 2014 Two novel *Saccharomycopsis* species isolated from black olive brines and a tropical plant. Description of *Saccharomycopsis olivae* f. a., sp. nov. and *Saccharomycopsis guyanensis* f. a., sp. nov. Reassignment of *Candida amapae* to *Saccharomycopsis amapae* f. a., comb. nov., *Candida lassenensis* to *Saccharomycopsis lassenensis* f. a., comb. nov. and *Arthroascus babjevae* to *Saccharomycopsis babjevae* f. a., comb. nov. Int J Syst Evol Microbiol. 64:2169-2175. doi: 10.1099/ijs.0.060418-0

Three yeast strains related to members of the genus *Saccharomycopsis* were isolated. One strain (CLIB 1310) was isolated from olive brines of fermented black olives in France and two strains (CLIB 1454 and CLIB 1455) were isolated from a plant in French Guiana. Sequence analyses based on the D1/D2 domains of the nuclear large subunit rRNA gene, small-subunit rRNA gene and partial EF-1 α gene revealed that the strains represented two novel taxa exhibiting extensive sequence divergence from the previously described species of the genus *Saccharomycopsis*. Two novel species are described to accommodate these

newly isolated strains: *Saccharomycopsis olivae* sp. nov. (type strain CLIB 1310(T) = CBS 12701(T)) and *Saccharomycopsis guyanensis* sp. nov. (type strain CLIB 1455(T) = CBS 12914(T) and strain CLIB 1454). Both strains CLIB 1454 and CLIB 1455(T) displayed identical sequences but differed in their ability to metabolize sorbitol and in their morphology on agar medium. *Candida amapae*, *Candida lassenensis* and *Arthroascus babjevae* belonging to the *Saccharomycopsis* clade, are reassigned to *Saccharomycopsis* as novel combinations.

- 2 Stackebrandt E, Smith D, Casaregola S, Varese GC, Verkleij G, Lima N, Bridge P 2014 Deposit of microbial strains in public service collections as part of the publication process to underpin good practice in science. Springerplus 3:208. doi: 10.1186/2193-1801-3-208

Despite recommendations to release microbial resources to the community post-publication, the reality is far from satisfying. A workshop discussed the need for a coordinated and effective deposition policy for 'key' microbial strains and proposes a set of criteria to facilitate their deposition into public service collections.

The majority of authors either contacted directly or during submission of manuscripts to several international, mainly European bacteriology journals agreed to this set of 'key strain' criteria and to the voluntarily deposition of resources into public resource centres.

- 3 Kunze G, Gaillardin C, Czernicka M, Durrens P, Martin T, Böer E, Gabaldón T, Cruz JA, Talla E, Marck C, Goffeau A, Barbe V, Baret P, Baronian K, Beier S, Bleykasten C, Bode R, Casaregola S, Despons L, Fairhead C, Giersberg M, Gierski PP, Hähnel U, Hartmann A, Jankowska D, Jubin C, Jung P, Lafontaine I, Leh-Louis V, Lemaire M, Marcet-Houben M, Mascher M, Morel G, Richard GF, Riechen J, Sacerdot C, Sarkar A, Savel G, Schacherer J, Sherman DJ, Stein N, Straub ML, Thierry A, Trautwein-Schult A, Vacherie B, Westhof E, Worch S, Dujon B, Souciet JL, Wincker P, Scholz U, Neuvéglise C. 2014. The complete genome of *Blastobotrys (Arxula) adenivorans* LS3 - a yeast of biotechnological interest. *Biotechnol Biofuels*. 7:66. doi: 10.1186/1754-6834-7-66

The industrially important yeast *Blastobotrys (Arxula) adenivorans* is an asexual hemiascomycete phylogenetically very distant from *Saccharomyces cerevisiae*. Its unusual metabolic flexibility allows it to use a wide range of carbon and nitrogen sources, while being thermotolerant, xerotolerant and osmotolerant. The sequencing of strain LS3 revealed that the nuclear genome of *A. adenivorans* is 11.8 Mb long and consists of four chromosomes with regional centromeres. Its closest sequenced relative is *Yarrowia lipolytica*, although mean conservation of orthologs is low. With 914 introns within 6116 genes, *A. adenivorans* is one of the most intron-rich hemiascomycetes sequenced to date. Several large species-specific families appear to result from multiple rounds of segmental duplications of tandem gene arrays, a novel mechanism not yet described in yeasts. An analysis of

the genome and its transcriptome revealed enzymes with biotechnological potential, such as two extracellular tannases (Atan1p and Atan2p) of the tannic-acid catabolic route, and a new pathway for the assimilation of n-butanol via butyric aldehyde and butyric acid. The high-quality genome of this species that diverged early in Saccharomycotina will allow further fundamental studies on comparative genomics, evolution and phylogenetics. Protein components of different pathways for carbon and nitrogen source utilization were identified, which so far has remained unexplored in yeast, offering clues for further biotechnological developments. In the course of identifying alternative microorganisms for biotechnological interest, *A. adenivorans* has already proved its strengthened competitiveness as a promising cell factory for many more applications.

XII 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 Aro-Kärkkäinen N, Toivari M, Maaheimo H, Ylilauri M, Pentikäinen OT, Andberg M, Oja M, Penttilä M, Wiebe MG, Ruohonen L, Koivula A 2014 L-Arabinose/D-galactose 1-dehydrogenase of *Rhizobium leguminosarum* bv. *trifolii* characterised and applied for bioconversion of L-arabinose to L-arabonate with *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* (Sept 19, 2014).

Four potential dehydrogenases identified through literature and bioinformatic searches were tested for l-arabonate production from l-arabinose in the yeast *Saccharomyces cerevisiae*. The most efficient enzyme, annotated as a d-galactose 1-dehydrogenase from the pea root nodule bacterium *Rhizobium leguminosarum* bv. *trifolii*, was purified from *S. cerevisiae* as a homodimeric protein and characterised. We named the enzyme as a l-arabinose/d-galactose 1-dehydrogenase (EC 1.1.1.-), R1 AraDH. It belongs to the Gfo/Idh/MocA protein family, prefers NADP⁺ but uses also NAD⁺ as a cofactor, and showed highest catalytic efficiency (k_{cat}/K_m) towards l-arabinose, d-galactose and d-fucose. Based on nuclear magnetic resonance (NMR) and modelling studies, the enzyme prefers the α -pyranose

form of l-arabinose, and the stable oxidation product detected is l-arabino-1,4-lactone which can, however, open slowly at neutral pH to a linear l-arabonate form. The pH optimum for the enzyme was pH 9, but use of a yeast-in-vivo-like buffer at pH 6.8 indicated that good catalytic efficiency could still be expected in vivo. Expression of the R1 AraDH dehydrogenase in *S. cerevisiae*, together with the galactose permease Gal2 for l-arabinose uptake, resulted in production of 18 g of l-arabonate per litre, at a rate of 248 mg of l-arabonate per litre per hour, with 86 % of the provided l-arabinose converted to l-arabonate. Expression of a lactonase-encoding gene from *Caulobacter crescentus* was not necessary for l-arabonate production in yeast.

- 2 Magnus Carlquist, Brian Gibson, Yonca Karagul Yuceer, Adamantini Paraskevopoulou, Mari Sandell, Angel I. Angelov, Velitchka Gotcheva, Angel D. Angelov, Marlene Etschmann, Gustavo de Billerbeck, Gunnar Lidén - Process engineering for bioflavour production with metabolically active yeast – a minireview. Yeast DOI: 10.1002/yea.3058

Flavours are biologically potent molecules of large commercial interest in the food, cosmetics, detergent and pharmaceutical industry. The production of flavours can take place by either extraction of plant materials, chemical synthesis, through biological conversion of precursor molecules or through *de novo* synthesis. The latter alternatives are gaining importance through the rapidly growing fields of systems biology

and metabolic engineering giving efficient production hosts. One potential production host for bioflavour production is yeast. In this mini-review, we give an overview of bioflavour production in yeast. Two specific examples – production of 2-phenylethanol and vanillin - are used to illustrate process challenges and strategies used.

- 3 Brian Gibson and Gianni Liti - *Saccharomyces pastorianus*: genomic insights inspiring innovation for industry. Yeast DOI: 10.1002/yea.3033

A combination of biological and non-biological factors has led to the interspecific hybrid yeast species *Saccharomyces pastorianus* becoming one of the world's most important industrial organisms. This yeast is used in the production of lager-style beers, the fermentation of which requires very low temperatures compared to other industrial fermentation processes. This group of organisms has benefited from both the whole-genome duplication in its ancestral lineage and the subsequent hybridization event between *S. cerevisiae* and *S. eubayanus*, resulting in strong fermentative ability. The hybrid has key traits, such as

cold tolerance and good maltose- and maltotriose utilizing ability, inherited either from the parental species or originating from genetic interactions between the parent genomes. Instability in the nascent allopolyploid hybrid genome may have contributed to rapid evolution of the yeast to tolerate conditions prevalent in the brewing environment. The recent discovery of *S. eubayanus* has provided new insights into the evolutionary history of *S. pastorianus* and may offer new opportunities for generating novel industrially-beneficial lager yeast strains.

- 4 Brian Gibson, Kristoffer Krogerus, Jukka Ekberg, Adrien Monroux, Laura Mattinen, Jari Rautio & Virve Vidgren - Variation in α -acetolactate production within the hybridlager yeast group *Saccharomyces pastorianus* and affirmation of the central role of the ILV6 gene. Yeast - DOI: 10.1002/yea.3026

A screen of 14 *S. pastorianus* lager-brewing strains showed as much as a nine-fold difference in wort total diacetyl concentration at equivalent stages of fermentation of 15°Plato brewer's wort. Two strains (A153 and W34), with relatively low and high diacetyl production, respectively, but which did not otherwise differ in fermentation performance, growth or flavour production, were selected for further investigation. Transcriptional analysis of key genes involved in valine biosynthesis showed differences between the two strains that were consistent with the differences in wort diacetyl concentration. In particular, the ILV6 gene, encoding a regulatory subunit of acetohydroxy acid synthase, showed early transcription (only 6 h after inoculation) and up to five-fold greater expression in W34 compared to A153. This earlier transcription was

observed for both orthologues of ILV6 in the *S. pastorianus* hybrid (*S. cerevisiae* × *S. eubayanus*), although the *S. cerevisiae* form of ILV6 in W34 also showed a consistently higher transcript level throughout fermentation relative to the same gene in A153. Overexpression of either form of ILV6 (by placing it under the control of the PGK1 transporter) resulted in an identical two-fold increase in wort total diacetyl concentration relative to a control. The results confirm the role of the Ilv6 subunit in controlling α -acetolactate/diacetyl concentration and indicate no functional divergence between the two forms of Ilv6. The greater contribution of the *S. cerevisiae* ILV6 to acetolactate production in natural brewing yeast hybrids appears rather to be due to higher levels of transcription relative to the *S. eubayanus* form.

- 5 Koivuranta KT, Ilmén M, Wiebe MG, Ruohonen L, Suominen P, Penttilä M 2014 L-lactic acid production from D-xylose with *Candida sonorensis* expressing a heterologous lactate dehydrogenase encoding gene. *Microb Cell Fact* 13:107.

Bioplastics, like polylactic acid (PLA), are renewable alternatives for petroleum-based plastics. Lactic acid, the monomer of PLA, has traditionally been produced biotechnologically with bacteria. With genetic engineering, yeast have the potential to replace bacteria in biotechnological lactic acid production, with the benefits of being acid tolerant and having simple nutritional requirements. Lactate dehydrogenase genes have been introduced to various yeast to demonstrate this potential. Importantly, an industrial lactic acid producing process utilising yeast has already been implemented. Utilisation of D-xylose in addition to D-glucose in production of biochemicals such as lactic acid by microbial fermentation would be beneficial, as it would allow lignocellulosic raw materials to be utilised in the production processes. The yeast *Candida sonorensis*, which naturally metabolises D-xylose, was genetically modified to produce L-lactic acid from D-xylose by integrating the gene encoding L-lactic acid dehydrogenase (ldhL) from *Lactobacillus helveticus* into its genome. In microaerobic, CaCO₃-buffered conditions a *C. sonorensis* ldhL transformant having two copies of the ldhL gene produced 31 g l⁻¹ lactic acid from 50 g l⁻¹ D-xylose free of ethanol. Anaerobic

production of lactic acid from D-xylose was assessed after introducing an alternative pathway of D-xylose metabolism, i.e. by adding a xylose isomerase encoded by XYLA from *Piromyces* sp. alone or together with the xylulokinase encoding gene XKS1 from *Saccharomyces cerevisiae*. Strains were further modified by deletion of the endogenous xylose reductase encoding gene, alone or together with the xylitol dehydrogenase encoding gene. Strains of *C. sonorensis* expressing xylose isomerase produced L-lactic acid from D-xylose in anaerobic conditions. The highest anaerobic L-lactic acid production (8.5 g l⁻¹) was observed in strains in which both the xylose reductase and xylitol dehydrogenase encoding genes had been deleted and the xylulokinase encoding gene from *S. cerevisiae* was overexpressed. Integration of two copies of the ldhL gene in *C. sonorensis* was sufficient to obtain good L-lactic acid production from D-xylose. Under anaerobic conditions, the ldhL strain with exogenous xylose isomerase and xylulokinase genes expressed and the endogenous xylose reductase and xylitol dehydrogenase genes deleted had the highest L-lactic acid production.

- 6 John Londesborough, Peter Richard, Mari Valkonen and Kaarina Viljanen 2014 Effect of C-Terminal Protein Tags on Pentitol and L-Arabinose Transport by *Ambrosiozyma monospora* Lat1 and Lat2 Transporters in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 80:2737-2745.

Functional expression in heterologous hosts is often less successful for integral membrane proteins than for soluble proteins. Here, two *Ambrosiozyma monospora* transporters were successfully expressed in *Saccharomyces cerevisiae* as tagged proteins. Growth of *A. monospora* on L-arabinose instead of glucose caused transport activities of L-arabinose, L-arabitol, and ribitol, measured using L-[1-³H]arabinose, L-[¹⁴C]arabitol, and [¹⁴C]ribitol of demonstrated purity. *A. monospora* LAT1 and LAT2 genes were cloned earlier by using their ability to improve the growth of genetically engineered *Saccharomyces cerevisiae* on L-arabinose. However, the L-arabinose and pentitol transport activities of *S. cerevisiae* carrying LAT1 or LAT2 are only slightly greater than those of control strains. *S. cerevisiae* carrying the LAT1 or LAT2 gene fused in frame to the genes for green fluorescent protein

(GFP) or red fluorescent protein (mCherry) or adenylate kinase (AK) exhibited large (>3-fold for LAT1; >20-fold for LAT2) increases in transport activities. Lat1-mCherry transported L-arabinose with high affinity ($K_m \approx 0.03$ mM) and L-arabitol and ribitol with very low affinity ($K_m \geq 75$ mM). The Lat2-GFP, Lat2-mCherry, and Lat2-AK fusion proteins could not transport L-arabinose but were high-affinity pentitol transporters (K_m s ≈ 0.2 mM). The L-arabinose and pentitol transport activities of *A. monospora* could not be completely explained by any combination of the observed properties of tagged Lat1 and Lat2, suggesting either that tagging and expression in a foreign membrane alters the transport kinetics of Lat1 and/or Lat2 or that *A. monospora* contains at least one more L-arabinose transporter.

- 7 Mojzita D, Oja M, Rintala E, Wiebe M, Penttilä M, Ruohonen L 2014 Transcriptome of *Saccharomyces cerevisiae* during production of D-xylonate. BMC Genomics 15:763.

Production of D-xylonate by the yeast *S. cerevisiae* provides an example of bioprocess development for sustainable production of value-added chemicals from cheap raw materials or side streams. Production of D-xylonate may lead to considerable intracellular accumulation of D-xylonate and to loss of viability during the production process. In order to understand the physiological responses associated with D-xylonate production, we performed transcriptome analyses during D-xylonate production by a robust recombinant strain of *S. cerevisiae* which produces up to 50 g/L D-xylonate. Comparison of the transcriptomes of the D-xylonate producing and the control strain showed considerably higher expression of the genes controlled by the cell wall integrity (CWI) pathway and of some genes previously identified as up-regulated in response to other organic acids in the D-xylonate producing strain. Increased phosphorylation of Slt2 kinase in the D-xylonate producing strain also indicated that D-xylonate production caused stress to the cell wall. Surprisingly, genes encoding proteins involved in translation, ribosome structure and RNA metabolism, processes which are commonly down-regulated under conditions

causing cellular stress, were up-regulated during D-xylonate production, compared to the control. The overall transcriptional responses were, therefore, very dissimilar to those previously reported as being associated with stress, including stress induced by organic acid treatment or production. Quantitative PCR analyses of selected genes supported the observations made in the transcriptomic analysis. In addition, consumption of ethanol was slower and the level of trehalose was lower in the D-xylonate producing strain, compared to the control. The production of organic acids has a major impact on the physiology of yeast cells, but the transcriptional responses to presence or production of different acids differs considerably, being much more diverse than responses to other stresses. D-Xylonate production apparently imposed considerable stress on the cell wall. Transcriptional data also indicated that activation of the PKA pathway occurred during D-xylonate production, leaving cells unable to adapt normally to stationary phase. This, together with intracellular acidification, probably contributes to cell death.

- 8 V Nedović, B Gibson, TF Mantzouridou, B Bugarski, V Djordjević, A Kalušević, A Paraskevopoulou, M Sandell, D. Šmogrovičová & M Yilmaztekin - Aroma formation by immobilized yeast cells in fermentation processes. Yeast DOI: 10.1002/yea.3042

Immobilized cell technology has shown a significant promotional effect on the fermentation of alcoholic beverages such as beer, wine and cider. However, genetic, morphological and physiological alterations occurring in immobilized yeast cells impact on aroma formation during fermentation processes. The focus of this review is exploitation of existing knowledge on the biochemistry and the biological role of flavour production in yeast for the biotechnological production of aroma compounds of industrial importance, by means of immobilized yeast. Various

types of carrier materials and immobilization methods proposed for application in beer, wine, fruit wine, cider and mead production are presented. Engineering aspects with special emphasis on immobilized cell bioreactor design, operation and scale-up potential are also discussed. Ultimately, examples of products with improved quality properties within the alcoholic beverages are addressed, together with identification and description of the future perspectives and scope for cell immobilization in fermentation processes.

- 9 Nygård Y, Maaheimo H, Mojzita D, Toivari M, Wiebe M, Resnekov O, Gustavo Pesce C, Ruohonen L & Penttilä M 2014 Single cell and in vivo analyses elucidate the effect of xylC lactonase during production of D-xylonate in *Saccharomyces cerevisiae*. Metab Eng 25:238-47.

D-xylonate is a potential platform chemical which can be produced by engineered *Saccharomyces cerevisiae* strains. In order to address production constraints in more detail, we analysed the role of lactone ring opening in single cells and populations. Both D-xylonolactone and D-xylonate were produced

when the *Caulobacter crescentus* xylB (D-xylose dehydrogenase) was expressed in *S. cerevisiae*, with or without co-expression of xylC (D-xylonolactonase), as seen by (1)H NMR. XylC facilitated rapid opening of the lactone and more D-xylonate was initially produced than in its absence. Using in vivo (1)H NMR analysis of

cell extracts, culture media and intact cells we observed that the lactone and linear forms of D-xylonic acid were produced, accumulated intracellularly, and partially exported within 15-60min of D-xylose provision. During single-cell analysis of cells expressing the pH sensitive fluorescent probe pHluorin, pHluorin fluorescence was gradually lost from the cells during D-xylonate production, as expected for cells with decreasing intracellular pH. However, in the presence

of D-xylose, only 9% of cells expressing xylB lost pHluorin fluorescence within 4.5h, whereas 99% of cells co-expressing xylB and xylC lost fluorescence, a large proportion of which also lost vitality, during this interval. Loss of vitality in the presence of D-xylose was correlated to the extracellular pH, but fluorescence was lost from xylB and xylC expressing cells regardless of the extracellular condition.

- 10 Nygård Y, Mojzita D, Toivari M, Penttilä M, Wiebe MG, Ruohonen L 2014 The diverse role of Pdr12 in resistance to weak organic acids. *Yeast* 31:219-32.

Resistance to weak organic acids is important relative to both weak organic acid preservatives and the development of inhibitor tolerant yeast as industrial production organisms. The ABC transporter Pdr12 is important for resistance to sorbic and propionic acid, but its role in tolerance to other weak organic acids with industrial relevance is not well established. In this study, yeast strains with altered expression of PDR12 and/or CMK1, a protein kinase associated with post-transcriptional negative regulation of Pdr12, were exposed to seven weak organic acids: acetic, formic, glycolic, lactic, propionic, sorbic and levulinic acid. These are widely used as preservatives, present in

lignocellulosic hydrolysates or attractive as chemical precursors. Overexpression of PDR12 increased tolerance to acids with longer chain length, such as sorbic, propionic and levulinic acid, whereas deletion of PDR12 increased tolerance to the shorter acetic and formic acid. The viability of all strains decreased dramatically in acetic or propionic acid, but the Δ pdr12 strains recovered more rapidly than other strains in acetic acid. Furthermore, our results indicated that Cmk1 plays a role in weak organic acid tolerance, beyond its role in regulation of Pdr12, since deletion of both Cmk1 and Pdr12 resulted in different responses to exposure.

- 11 Signori L, Passolunghi S, Ruohonen L, Porro D, Branduardi P 2014 Effect of oxygenation and temperature on glucose-xylose fermentation in *Kluyveromyces marxianus* CBS712 strain. *Microb Cell Factories* 13:51.

The yeast *Kluyveromyces marxianus* features specific traits that render it attractive for industrial applications. These include production of ethanol which, together with thermotolerance and the ability to grow with a high specific growth rate on a wide range of substrates, could make it an alternative to *Saccharomyces cerevisiae* as an ethanol producer. However, its ability to co-ferment C5 and C6 sugars under oxygen-limited conditions is far from being fully characterized. In the present study, *K. marxianus* CBS712 strain was cultivated in defined medium with glucose and xylose as carbon source. Ethanol fermentation and sugar consumption of CBS712 were investigated under different oxygen supplies (1.75%, 11.00% and 20.95% of O₂) and different temperatures (30°C and 41°C). By decreasing oxygen supply, independently from the temperature, both biomass production as well as sugar utilization rate were progressively reduced. In all the tested conditions xylose consumption followed glucose exhaustion. Therefore, xylose metabolism was mainly affected by

oxygen depletion. Loss in cell viability cannot explain the decrease in sugar consumption rates, as demonstrated by single cell analyses, while cofactor imbalance is commonly considered as the main cause of impairment of the xylose reductase (KmXR) - xylitol dehydrogenase (KmXDH) pathway. Remarkably, when these enzyme activities were assayed in vitro, a significant decrease was observed together with oxygen depletion, not ascribed to reduced transcription of the corresponding genes. In the present study both oxygen supply and temperature were shown to be key parameters affecting the fermentation capability of sugars in the *K. marxianus* CBS712 strain. In particular, a direct correlation was observed between the decreased efficiency to consume xylose with the reduced specific activity of the two main enzymes (KmXR and KmXDH) involved in its catabolism. These data suggest that, in addition to the impairment of the oxidoreductive pathway being determined by the cofactor imbalance, post-transcriptional and/or post-translational regulation of the pathway enzymes

contributes to the efficiency of xylose catabolism in micro-aerobic conditions. Overall, the presented work provides novel information on the fermentation

capability of the CBS712 strain that is currently considered as the reference strain of the genus *K. marxianus*.

XIII Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany - www.dsmz.de. Communicated by Andrey Yurkov <andrey.yurkov@dsmz.de>.

Recent papers.

- 1 Prior R, Görges K, Yurkov A, Begerow D 2014 New isolation method for endophytes based on enzyme digestion. *Mycological Progress* 13: 849-856.

Investigation of the diversity and ecology of endophytic fungi has gained popularity in recent decades. Thereby, culture-independent methods were very instrumental as they revealed greater species richness and challenged the efficiency of isolation methods. However, culture-based methods remain the only possibility to provide strains for future studies. To improve the efficiency of endophyte isolation, we compared seven different artificial media that included a variety of plant extracts. Furthermore, we developed a method based on enzymatic digestion of plant tissue

to improve the isolation efficiency in terms of species richness. The effect of additional plant extract in media that contained yeast and malt extract was of minor significance, whereas isolations using enzymatic digestion of plant leaf tissue combined with a 1:10 dilution of nutrients revealed a much higher diversity of species. Using this protocol, we isolated three times as many species from the same leaves than with previously described methods. This is reflected by the large number of singletons obtained in culture-independent approaches to study the diversity of endophytic fungi.

- 2 Selbmann L, Turchetti B, Yurkov A, Cecchini C, Zucconi L, Isola D, Buzzini P, Onofri S 2014 Description of *Taphrina antarctica* f.a. sp. nov., a new anamorphic ascomycetous yeast species associated with Antarctic endolithic microbial communities and transfer of four *Lalaria* species in the genus *Taphrina*. *Extremophiles: life under extreme conditions* 18: 707-721.

In the framework of a large-scale rock sampling in Continental Antarctica, a number of yeasts have been isolated. Two strains that are unable to grow above 20°C and that have low ITS sequence similarities with available data in the public domain were found. The D1/D2 LSU molecular phylogeny placed them in an isolated position in the genus *Taphrina*, supporting their affiliation to a not yet described species. Because the new species is able to grow in its anamorphic state only, the species *Taphrina antarctica* f.a. (forma asexualis) sp. nov. has been proposed to accommodate both strains (type strain DBVPG 5268T, DSM 27485T and CBS 13532T). *Lalaria* and *Taphrina* species are dimorphic ascomycetes, where the anamorphic yeast

represents the saprotrophic state and the teleomorph is the parasitic counterpart on plants. This is the first record for this genus in Antarctica; since plants are absent on the continent, we hypothesize that the fungus may have focused on the saprotrophic part of its life cycle to overcome the absence of its natural host and adapt environmental constraints. Following the new International Code of Nomenclature for Algae, Fungi and Plants (Melbourne Code 2011) the reorganization of *Taphrina*–*Lalaria* species in the teleomorphic genus *Taphrina* is proposed. We emend the diagnosis of the genus *Taphrina* to accommodate asexual saprobic states of these fungi. *Taphrina antarctica* was registered in MycoBank under MB 808028.

- 3 Yurkov A, Kachalkin A, Daniel H-M, Groenewald M, Libkind D, de Garcia V, Zalar P, Gouliamova D E, Boekhout T, Begerow D 2014 Two yeast species *Cystobasidium psychroaquaticum* f.a. sp. nov. and *Cystobasidium rietchieii* f.a. sp. nov. isolated from natural environments, and the transfer of members of *Rhodotorula minuta* clade to the genus *Cystobasidium*. *Antonie van Leeuwenhoek* (in press), doi: 10.1007/s10482-014-0315-0

Many species of dimorphic basidiomycetes are known only in their asexual phase and typically those pigmented in different hues of red have been classified in the large polyphyletic genus *Rhodotorula*. These yeasts are ubiquitous and include a few species of some clinical relevance. The phylogenetic distribution of *Rhodotorula* spans three classes: *Microbotryomycetes*, *Cystobasidiomycetes* and *Exobasidiomycetes*. Here, the presented multi-gene analyses resolved phylogenetic relationships between the second largest group of *Rhodotorula* and the mycoparasite *Cystobasidium fimetarium* (Cystobasidiales, Cystobasidiomycetes, Pucciniomycotina). Based on the results, we propose the transfer of nine species belonging to the *Rhodotorula minuta* clade into the genus *Cystobasidium*. As a result, the clinically relevant species *R. minuta* will be renamed *Cystobasidium minutum*. This proposal follows ongoing reassessments of the anamorphic genus *Rhodotorula* reducing the polyphyly of this genus. The delimitation of the *R. minuta* clade from *Rhodotorula* species

comprised in Sporidiobolales including the type species *Rhodotorula glutinis* is an important step to overcome obsolete generic placements of asexual basidiomycetous yeasts. Our proposal will also help to distinguish most common red yeasts from clinical samples such as members of Sporidiobolales and Cystobasidiales. The diagnosis of the genus *Cystobasidium* is amended by including additional characteristics known for the related group of species. The taxonomic change enables us to classify two novel species with the phylogenetically related members of the *R. minuta* clade in *Cystobasidium*. The recently from natural environments isolated species are described here as *Cystobasidium psychroaquaticum* f.a. sp. nov. (K-833^T = KBP 3881^T = VKPM Y-3653^T = CBS 11769^T = MUCL 52875^T = DSM 27713^T) and *Cystobasidium rietchiei* f.a. sp. nov. (K-780^T = KBP 4220^T = VKPM Y-3658^T = CBS 12324^T = MUCL 53589^T = DSM 27155^T). The new species were registered in MycoBank under MB 809336 and MB 809337, respectively.

- 4 Solis MJL, Yurkov A, dela Cruz TE, Unterseher M 2015 Leaf-inhabiting endophytic yeasts are abundant but unevenly distributed in three *Ficus* species from botanical garden greenhouses in Germany. *Mycological Progress* (in press) - doi: 10.1007/s11557-014-1019-6

Yeasts of both Asco- and Basidiomycota occur in various ecological zones of any geographic regions and climatic conditions, but environmental yeast research was often conducted in either extreme habitats or the phyllosphere. Here, we report on the occurrence of foliar endophytic yeasts of three tropical *Ficus* species from two German greenhouses in Greifswald and Berlin. Living leaves were collected and subjected to dilution-to-extinction cultivation. Fungal colonies were used for morphological analyses, microsatellite-primed fingerprinting, sequencing and phylogeny of the internal

transcribed spacer (ITS) DNA. Fifteen percent (~200 colonies) of all fungal isolates belonged to the genera *Cryptococcus* (Filobasidiales) and *Rhodotorula* (Sporidiobolales and Cystobasidiales) that split into 23 species / operational taxonomic units. No other yeast-forming taxa were isolated. Both side- and host specific variations in species composition and abundance were observed, however, statistics did not support significant associations. Further evidence exist that gardening practices, such as moving potted plants, could influence fungal endophytic communities.

Book chapter.

- 5 Begerow D, Schäfer, AM, Kellner R, Yurkov A, Kemler M, Oberwinkler F, Bauer R 2014 Ustilaginomycotina. In: McLaughlin DJ, Spatafora JW (eds) *The Mycota. Systematics and Evolution* 7A:295-329 - doi: 0.1007/978-3-642-55318-9_11

Ustilaginomycotina represents one of three subphyla of the Basidiomycota, comprising 10 orders, 26 families, and 121 genera, with more than 1,700 species in total. Whereas most of the species are plant parasites with a biphasic life cycle and largely strong host preference, some species are recognized solely from their anamorphic phase. In addition, Ustilaginomycotina

contains some lineages that parasitize hosts outside the plant kingdom, for example, the mammal-pathogenic genus *Malassezia*. In this chapter we summarize the most recent systematic studies of Ustilaginomycotina by highlighting the unique characters of monophyletic lineages. Furthermore, evolutionary trends are discussed.

- 6 Carvalho C, Yurkov A, Fonseca A, Sampaio JP 2014 PYCC - a repository of Mediterranean yeast diversity, 33rd Annual Meeting of the European Culture Collections' Organisation, Valencia, Spain, June 2014. URL: <http://congresos.adeituv.es/ecco/>

PYCC is a research biological collection committed to the preservation, distribution and study of yeasts. It was founded in 1952 by Prof. Nicolau van Uden and currently holds approximately 3000 strains, representing ca. 800 species and 140 genera. About 1300 strains are unique to PYCC and for those isolates with information on sampling site and substrate, molecular sequence data has been obtained and is available through PYCC website. One of PYCC's goals is to become an internationally recognised repository of yeast diversity from the Mediterranean. In fact, of the PYCC holdings for which ecological data is available, 42% correspond to yeast strains found in Mediterranean

ecosystems or regional fermented foods and beverages. Those strains include starter cultures for selected foods and beverages, such as the varieties of bread and wine that make part of the Mediterranean diet, recently recognised by UNESCO as an Intangible Cultural Heritage of Humanity. PYCC also maintains a considerable collection of natural isolates mainly from plant substrates or soils collected from different Mediterranean ecosystems for which relevant ecological and molecular information (e.g. DNA-barcodes) is available online. Molecular information on PYCC strains is available and searchable through MycoBank (www.mycobank.org) services.

- 7 Röhl O, Carvalho C, Sampaio JP, Yurkov AM 2014 Investigation of Mediterranean soil reveals insights into species community. Proc. of the International Mycological Congress, Bangkok, Thailand, August 2014, p. 756. URL: <http://www.imc10.com>

In the frame of the project aiming at yeast biodiversity in soils of three major forest types in Portugal, we investigated details about species composition, diversity and distribution patterns. Soil samples were collected in Arrábida National Park, a mountain area of undisturbed Mediterranean maquis but with two markedly different micro-climatic zones. This leads to the formation of distinct vegetation types, forests to shrubs. In order to assess the influence of environmental factors on small spatial scale, we collected soils on northern (forest formation) and southern (forest and shrub formations) slopes. Solid media inoculated with soil suspensions was used to isolate yeasts. Strains were grouped with PCR-fingerprinting and identified by rDNA sequencing. Our

study revealed strong differences between the three sampling sites, both in yeast numbers and community compositions. Species frequently occurred in one forest were not detected in other sampling sites. Basidiomycetous species like *Cryptococcus aerius*, *Cr. terreus*, *Curvibasidium pallidocorallinum* and *Cystofilobasidium capitatum* for example are dominant agents in some but not all sampling sites. The study yielded several novel yeast species, including members of the genus *Cystofilobasidium* that was prominent in two of three forest soils of Serra da Arrábida. This work was partly supported by Fundação para a Ciência e a Tecnologia (Portugal), projects PTDC/BIA-BIC/4585/2012 and PEst-OE/BIA/UI0457/2011.

- 8 Yurkov AM, Röhl O, Guerreiro M, Begerow D, Sampaio JP, Fonseca A 2014 Partitioning yeast diversity on biome, biotope, plot and species scales. Proc. of the International Mycological Congress, Bangkok, Thailand, August 2014, p. 337. URL: <http://www.imc10.com>

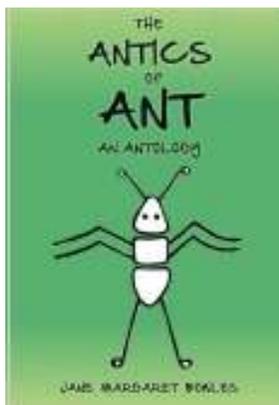
Complete species recovery and robust species identification are both crucial for accurate biodiversity assessment of yeasts in the environment. We set out to analyse the relationship between species richness values in soils and sampling at several hierarchical levels: (i) different plots within a forest sampled in the same season, (ii) forests of the same type studied in the same season, and (iii) forests of the same type studied in different seasons. By using species richness estimations, we determined the adequate sampling effort in a

habitat. Our results revealed that yeast communities in soils are: (1) generally species-poor in a single plot; (2) highly dissimilar between plots or across spa4al and environmental transects; (3) globally diverse with up to 25% more species discovered with every new forest or season sampled; (4) understudied and may contain up to 20% hitherto undescribed species. Furthermore, we assessed species boundaries in several clades of Tremellomycetes and tested for the presence of cryptic species from the same environments using multi-locus

sequence analysis (MLSA) approaches. Our results showed that ITS-LSU rRNA sequences are often unable to distinguish cryptic species and demonstrated the usefulness of network-based methods over traditional phylogenetic trees for adequate species

delimitation. This work was partly supported by Fundação para a Ciência e a Tecnologia (Portugal), projects PTDC/BIA- MIC/113051/2009, PTDC/BIA- BIC/4585/2012, Pest-OE/BIA/UI0457/2011.

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Communicated by MA Lachance <lachance@uwo.ca>.



I've put together a book entitled *The Antics of Ant*, which is a collection of simple drawings by my late wife, Jane Margaret Bowles, where she explores the word ANT. It has nothing to do with yeasts, but readers should find it entertaining no matter what. The book is suitable for all ages and can be purchased at <https://www.createspace.com/4861181> and other reputable online bookstores.

Recently accepted papers.

- 1 de Vega C, Guzmán B, Steenhuisen SL, Johnson SD, Herrera CM, Lachance MA 2014 *Metschnikowia drakensbergensis* sp. nov. and *Metschnikowia caudata* sp. nov., two endemic yeasts associated with *Protea* flowers in South Africa. *Int J Syst Evol Microbiol* 64(Pt 11):3724-3732.

In a taxonomic study of yeasts recovered from nectar of flowers and associated insects in South Africa, 11 strains were found to represent two novel species. Morphological and physiological characteristics and sequence analyses of the large-subunit rRNA gene D1/D2 region, as well as the actin, RNA polymerase II and elongation factor 2 genes, showed that the two novel species belonged to the genus *Metschnikowia*. *Metschnikowia drakensbergensis* sp. nov. (type strain EBD-CdVSA09-2^T = CBS 13649^T = NRRL Y-63721^T; MycoBank no. MB809688; allotype EBD-CdVSA10-2^A = CBS13650^A = NRRL Y-63720^A) was recovered from nectar of *Protea roupelliae* and the beetle *Heterochelus* sp. This species belongs to the large-spored *Metschnikowia* clade and is closely related to *Metschnikowia proteae*, with which mating reactions and single-spored asci were observed. *Metschnikowia*

caudata sp. nov. (type strain EBD-CdVSA08-1(T) = CBS 13651(T) = NRRL Y-63722(T); MycoBank no. MB809689; allotype EBD-CdVSA57-2(A) = CBS 13729(A) = NRRL Y-63723(A)) was isolated from nectar of *Protea dracomontana*, *P. roupelliae* and *P. subvestita* and a honeybee, and is a sister species to *Candida hainanensis* and *Metschnikowia lobpuriensis*. Analyses of the four sequences demonstrated the existence of three separate phylotypes. Intraspecies matings led to the production of mature asci of unprecedented morphology, with a long, flexuous tail. A single ascospore was produced in all compatible crosses, regardless of sequence phylotype. The two species appear to be endemic to South Africa. The ecology and habitat specificity of these novel species are discussed in terms of host plant and insect host species.

- 2 Gomes FCO, Safar SVB, Marques AR, Medeiros AO, Santos ARO, Carvalho C, Lachance MA, Sampaio JP & Rosa CA 2014 The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of *Vriesea minarum*, an endangered bromeliad species in Brazil, and the description of *Occultifur brasiliensis* f.a., sp. nov. *Antonie van Leeuwenhoek* (Accepted December 2014).

The diversity of yeast species collected from the bromeliad tanks of *Vriesea minarum*, an endangered

bromeliad species, and their ability to produce extracellular enzymes were studied. Water samples were

collected from 30 tanks of bromeliads living in a rupestrian field site located at Serrada Piedade, Minas Gerais state, Brazil, during both the dry and rainy seasons. Thirty-six species were isolated, representing 22 basidiomycetous and 14 ascomycetous species. *Occultifur* sp., *Cryptococcus podzolicus* and *Cryptococcus* sp. 1 were the prevalent basidiomycetous species. The yeast-like fungus from the order Myriangiales, *Candida silvae* and *Aureobasidium pullulans* were the most frequent ascomycetous species. The diversity of the yeast communities obtained between seasons was not significantly different, but the yeast composition per bromeliad was different between seasons. These results suggest that there is significant spatial heterogeneity in the composition of populations of the yeast communities within bromeliad tanks,

independent of the season. Among the 352 yeast isolates tested, 282 showed at least one enzymatic activity. Protease activity was the most widely expressed extracellular enzymatic activity, followed by xylanase, amylase, pectinase and cellulase activities. These enzymes may increase the carbon and nitrogen availability for the microbial food web in the bromeliad tank of *V. minarum*. Sequence analyses revealed the existence of 10 new species, indicating that bromeliad tanks are important sources of new yeasts. The novel species *Occultifur brasiliensis*, f.a., sp. nov., is proposed to accommodate the most frequently isolated yeast associated with *V. minarum*. The type strain of *O. brasiliensis* f.a., sp. nov. is UFMG-CM-Y375^T (= CBS 12687^T). The Mycobank number is MB 809816.

Presentations at meetings.

- 3 Lachance MA 2014 The impact of DNA sequencing on yeast species descriptions. 14th congress of the International Union of Microbiological Societies, Montréal, Québec).
- 4 Lachance MA 2014 The large-spored *Metschnikowia* species as a model for speciation. 10th International Mycological congress, Bangkok, Thailand.
- 5 Lachance MA 2014 Species concepts and species descriptions: from art to science. 10th International Mycological congress, Bangkok, Thailand.

International Commission on Yeasts

Minutes of Meeting held on Saturday, October 11, 2014, Perla Hotel, Nova Gorica

Attending Commissioners

Sakkie Pretorius, Diethard Mattanovich, Johan Thevelein, Angelica Ganga, Hana Sychrova, Sylvie Dequin, Anna Maraz, Pietro Buzzini, Akihiko Kondo, Hiroshi Takagi, Patricia Lappe Oliveras, Teun Boekhout, Jack Pronk, Bernard Prior, Florian Bauer, Hyun Ah Kang, Amparo Querol, Jens Nielsen, Hüseyin Erten, Andrei Sibirny, Charles Abbas, Patrizia Romano, Lisa Granchi, James C. du Preez, Anne Gschaedler.

New Commissioners

John Morrissey (Ireland), Patick Fickers (Belgium), Liliana Godoy Olivares (Chile), Ian Roberts (UK), and Steve Oliver (UK).

Invited Guest

Duccio Cavalieri (Italy) representing 27th ICYGMB International Conference on Yeast Genetics And Molecular Biology that will be held in Trento, Italy.

Agenda

- Chair Introductory Remarks
- Tribute to Jure Piskur
- Expanding Commission's country representation
- Endorsement and reaching out to other yeast meetings
- 2013 ISSY Slovakia Meeting
- Attendance at IUMS 2014 Montreal

- Continued affiliation with IUMS; Teun Boekhout new Division Chair; Singapore IUMS 2017
- Introduction of new attending ICY commissioners in attendance

Future Meeting Updates

- ISSY 32 Italy (Sept. 13-17, 2015, Perugia)
- ICY 14 Japan (Sept. 11-15, 2016 Awaji Yumebutai International Conference Center, Awaji Island, Japan)
- ISSY 33 Ireland (June or July 2017, Cork)
- ISSY 34 Argentina (Bariloche, date TBD, 2018)
- ISSY 35 Proposal from Turkey (date/venue TBD, 2019)
- ICY 15 Proposal from Austria (date/venue TBD, 2020)

Chair's Notes and Feedback

Vote taken to approve new Commissioners attending and absent.

Commissioners attending were advised of a proposal from current Commissioners representing Portugal to nominate three new members for Portugal to replace the three current members. The attending members expressed some concern about this proposal and the desire to maintain some continuity and broad representation. Chair will advise current Portugal representatives of this concern prior to proceeding.

Overlap of ISSY 32 and ICYGMB 27 Italy meetings was discussed. Pietro Buzzini and Duccio Cavalieri will work together to coordinate efforts and co-hosting of special topic sessions as well as

transportation from Northern Italy to Perugia. The Chair endorses the concept of joined efforts. ICY commissioners involved in organizing other yeast meetings are encouraged to notify the Chair in advance of other yeast meetings so as to minimize conflict in dates and venues.

Commissioners endorsed the idea of Ireland as a host of the 2017 ISSY. The meeting will be held in Cork June/July 2017 with Argentina hosting the 2018 meeting instead. The Chair encourages Ireland, Argentina, Turkey, and Austria to provide an update on plans and proposals at the ISSY 32.

Several ICY commissioners attending stressed the need to continue with specialized narrow topic meetings (ISSY) as it differentiates us from other meetings and enables a greater focus on areas not covered elsewhere. The Chair shares this view and is also concerned about redundancy of topics. ISSY organizers are encouraged to maintain the old practice of picking a narrow theme topic.

The organizers are also encouraged to think of alternate months to Sept. as Universities in the US and other countries are in session and it will be difficult for many that have teaching responsibility to attend. In the past many of our meetings were in the later part of August.

Some commissioners expressed concern about the lengthy detailed presentations for currently approved future meetings. In future we will limit all presentations to five minutes and link up to website where detailed information can be accessed. New proposals will be given 10 minutes so as to allow for greater discussion prior to a formal vote.

Recent meeting

ISSY 31 - Nova Gorica and Vipava, Slovenia, October 9-12, 2014

ISSY 31 took place in Nova Gorica and Vipava, Slovenia, between October 9 and 12, 2014. The venues were the Perla Hotel in Nova Gorica, where also the majority of participants were accommodated, and the Lanthieri Palace in Vipava. The main topics of the symposium were yeast fermentations, from basic concepts to applications, and from aspects of biodiversity and evolution to genetic regulation. ISSY 31 was also dedicated to the memory of late Jure Piškur who originally conceived the symposium. The organizing committee strived to organize the conference

such that it would be the place to celebrate Jure's life and work. There were seven plenary sessions and 16 parallel sessions. The parallel sessions included three dedicated to EU projects that had their annual meetings just after the conference: Cornucopia, YeastCell, and YeSVitE. Within the sessions, there were 159 speakers presenting, of whom 24 had been invited. The organizing committee followed a decision that no poster sessions would be organized at ISSY 31, and thus all the participants who had submitted an eligible abstract were given an opportunity to present their work in the form of

a short lecture; the majority were 10 minutes long, including the time for discussion. Three prizes for the Best Young Speakers were awarded to Elena Kuzmin (Toronto, Canada), Peter Gajdoš (Bratislava, Slovakia) and Lina Lindahl (Göteborg, Sweden). The total number of attendees was 240, from 37 different countries: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Italy, Japan, Lithuania, Mexico, New Zealand, Poland, Portugal, Russia, Slovakia, Slovenia, South Africa, South Korea, Spain, Sweden, The Netherlands, Turkey, UK, Ukraine, Uruguay and USA. Twenty seven ICY

commissioners registered for the ISSY31 and participated in the ICY Commissioners' meeting on October 11. The organizing committee received very positive feedback on the organization of the ISSY31. Participants expressed their content with the scientific program as well as with the general organization. Prior to the symposium, some concerns had been expressed regarding the packed schedule, but the received feedback on this issue was also largely positive. The local community (wine makers, tourist society) in Vipava contributed to the social program on October 11, which was warmly appreciated by the attendees.

Uroš Petrovič

ISSY31 Organizing Committee Executive Chairman

Forthcoming Meetings

42nd Annual Conference of the Commission on Yeast of the Czechoslovak Society for Microbiology

The 42nd Annual Conference on Yeasts is still being planned for 19 - 22 May 2015 at Smolenice Castle, Slovakia. On-line registration will opened shortly on <http://yeastconference.sk/> in December, 2014.

32nd Specialized Symposium on Yeasts (ISSY 32) Perugia, September 13-17 2015



On behalf of the Organizing Committee, I cordially invite you to the 32nd Specialized Symposium on Yeasts, which will be held in Perugia, Italy, in September 13-17, 2015.

General topic: Yeast Biodiversity and Biotechnology in the Twenty-First Century. The program will include opening and keynote lectures, selected oral presentations, and poster sessions covering all aspects of yeast ecology, physiology, taxonomy, food and non-food biotechnology, genetic and genomic. Special sessions will be dedicated to non-conventional yeasts and yeasts culture Collections. For further details on the program see: www.issy32.com

This Symposium back to Perugia after 27 years (the last was ISSY7, held in 1988). In this context, ISSY32 is dedicated to Prof. Alessandro Martini, Chair of ISSY7, yeast biologist and great Master of science and life.

ISSY32 is sponsored by the International Commission on Yeasts (ICY), by the Italian Society for Agricultural, Food and Environmental Microbiology (SIMTREA), by the Italian Society for General Microbiology and Microbial Biotechnology (SIMGBM).

Venue: ISSY32 will be held at Hotel Giò–Perugia

Congress Centre, Perugia, Italy:

<http://www.hotelgio.it/en/#.VGIGh2cQNpB>.

Hotel Giò – Perugia Congress Centre (inside the Hotel Giò Wine e Jazz Area with its 206 rooms) includes a number of meeting rooms and an Auditorium for up to 700 places. 2 Halls (Jazz Hall and Wine Hall) with areas expressly developed for informal meeting, living bar for coffee breaks and comfortable working and reading corners.

About the place: Perugia is a cosmopolitan city and home of two Universities. It hosts a worldwide famous jazz Festival during summertime and its University for Foreigners is a well-known place in which learn Italian. It is a walled city on a hilltop with amazing views over the valley and has several historical monuments and a lovely central square. Its history goes back to the ninth century BC. Besides, the beauty of the

surrounding towns (Assisi, Todi) and nature (Trasimeno lake, Marmore falls) and the rich cultural heritage of the region will provide an excellent opportunity for informal encounters.

Perugia connections: the city Airport of Perugia (<http://www.airport.umbria.it/en>) represents the main access gateway from some European cities. Alternatively, the two airports of Rome, Fiumicino

(<http://www.adr.it/web/aeroporti-di-roma-en/pax-fco-fiumicino>

and Ciampino

(<http://www.adr.it/web/aeroporti-di-roma-en/pax-cia-ciampino>), which are about two hours from Perugia by public transport, are the closest possibilities.

I am looking forward to seeing you on middle September 2015 in Perugia, Italy.

Pietro Buzzini

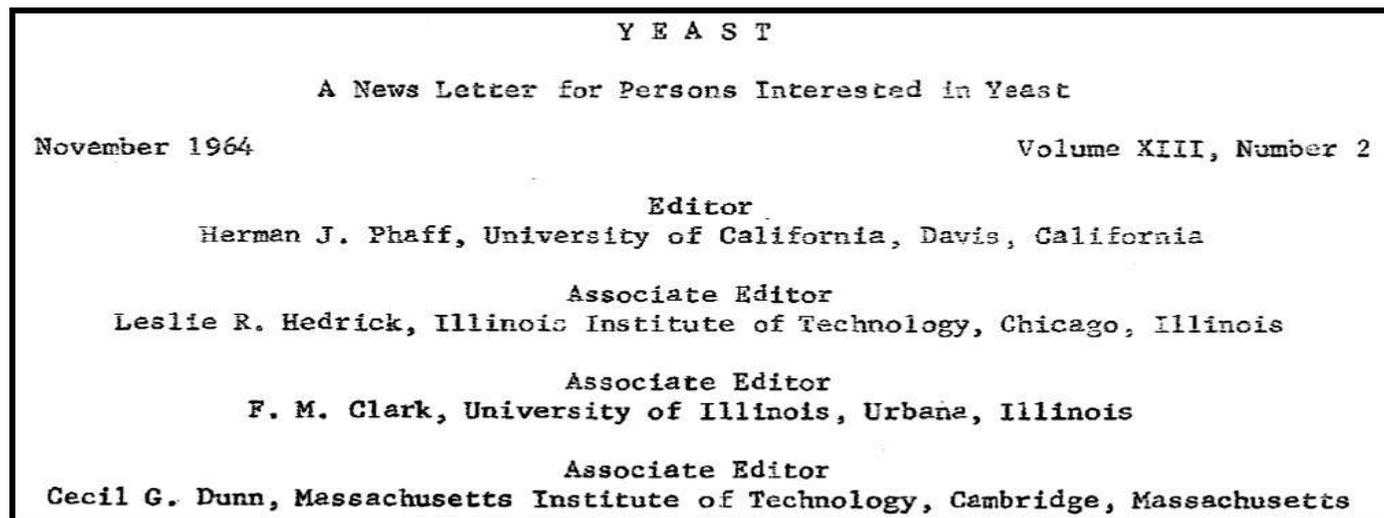
Chair of the Organizing Committee of ISSY32.

Department of Agricultural, Food and Environmental Science,

Industrial Yeasts Collection DBVPG (www.dbvpg.unipg.it)

University of Perugia, Perugia (Italy)

50 Years Ago



Miss W. Ch. Slooff of CBS reported that type strains of 9 new yeast species were deposited in the collection.

Dr. N. J W. Kreger-van Rij sent a copy of her doctoral dissertation to the Editor, who provided an abbreviated form of the Summary in the News Letter. The thesis contained revisions of the classification of genera *Endomycopsis*, *Pichia* and *Debaryomyces*, which would be included in the next edition of “The Yeasts”. The number of carbon sources used in assimilation tests was increased from 5 in the 1952 edition of “The Yeasts” to 31 in this study. Carbon and nitrogen assimilation, spore morphology, and other characteristics were measured for 51 strains of

Endomycopsis, 220 strains of *Pichia*, and 67 strains of *Debaryomyces*. A summary was given of the several genera and species proposed by others that were rejected, and many species that were reclassified. A diagnosis of each genus and keys to the species in each genus are in the dissertation.

The abstract of the Ph.D. dissertation of **Dr. Donald G. Ahearn**, University of Miami, was published in the News Letter. He compared properties of marine and terrestrial carotenogenic yeasts. The systematics of yeasts in the subfamily Rhodotoruloideae were revised based on an expanded number of physiological tests.

Dr. Henri Saëz of the Parc Zoologique de Paris reported publication of an observation that blood ingested by female ticks contains numerous blastospores, and *Candida tropicalis* was cultured. The new species *Geotrichum vanriji* was described.

Professor V. I. Kudriavzev of the USSR Academy of Sciences, reported that the Dept. of Type Cultures of Microorganisms was set up in 1958 to organize the USSR All-Union Collection of Type Cultures of filamentous fungi, yeasts, bacteria, and actinomyces. Professor Kudriavzev sent Phaff a catalog of type cultures. "All cultures, in any quantity, are supplied free of charge." The Department requested that all scientists, in USSR and abroad, send cultures of newly described species.

Dr. C. C. Lindgren, Southern Illinois University, shared a list of six publications since the last Yeast News Letter, covering genetics in *Saccharomyces*.

An abstract of a presentation by **Dr. Thomas Brock** of Indiana University discussed the activity of enzymes against linkages in the cell wall of *Hansenula wingei*, related to conjugation. This was presented at the Annual Meeting of the American Society for Cell Biology, and published in the Journal of Cell Biology.

The absorption of amino acids from wort by various species of yeast was the subject of an abstract presented by **John S Pierce** of the Arthur Guinness Son and Company, London, published in J. Inst. Brew.

Drs. M. J. Lewis and H. J. Phaff of the University of California Davis described two publications, "Glycine assimilation and the Stickland mechanism in brewer's yeast" accepted in the Journal of the Institute of Brewing, and "Release of nitrogenous substances by brewers' yeast" in the Journal of Bacteriology. **Dr. M.W. Miller** left on a 7-month sabbatical leave at the Division of Food Preservation, C.S.I.R.O., Australia. **Dr. H. J. Phaff** spent three days at the University of Miami to discuss taxonomy of *Rhodotorula* and *Cryptococcus* with **Dr. F. J. Roth, Dr. Ahearn**, and **Mr. Fell**.

Dr. Yoshio Tani of Kyoto University, Japan, described a recent publication titled, "Physiological and Biochemical Studies of *Saccharomyces sake*" in the Journal of Fermentation Technology. Growth of this yeast species in the presence of lactic acid reduced NAD, CoA, cytochrome C, and several B-vitamins.

Professor Øjvind Winge, former director of the Physiological Department at Carlsberg Laboratory, passed away in April 1964.

Dr. Beryl Brady, who recently moved to the Departamento Microbiologia at the University of Lisbon, Portugal, requested reports on cultures of psychrophilic yeasts.

Dr. K. Kodama of Iitaga, Japan visited the laboratory of **Professor J. Boidin**, University of Lyon to discuss electron microscopy and the importance of ascospore morphology in yeast taxonomy.

Dr. N. van Uden of the University of Lisbon is writing a review on marine yeasts for the first volume of "Advances in Marine Microbiology", and requested reprints and dissertations on pertinent topics.

Dr. J. F. T. Spencer reported the isolation of yeasts from honey from bumble-bee nests in Alberta and Saskatchewan, Canada.

Dr. V. P. Cirillo joined the faculty of the State University of New York at the Stony Brook Campus, and continues studies of membrane transport in yeast.

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