Yeast

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

Editorials

Takashi Nakase (1939-2018)

Dr. Takashi Nakase, former Director of the Japan Collection of Microorganisms, died recently. A major contributor to yeast systematics, Dr. Nakase was a pioneer of yeast molecular chemotaxonomy and published extensively in the field. I am particularly grateful for his generous hospitality, in 1991, when he hosted me for a memorable visit of JCM and arranged meetings with several Japanese yeast systematists and ecologists in Tokyo, Nagoya, Nara, and Osaka. Dr. Masako Takashima has kindly contributed a summary of the highlights of Dr. Nakase's scientific career.

Inna Pavlovna Babjeva (1927-2018)

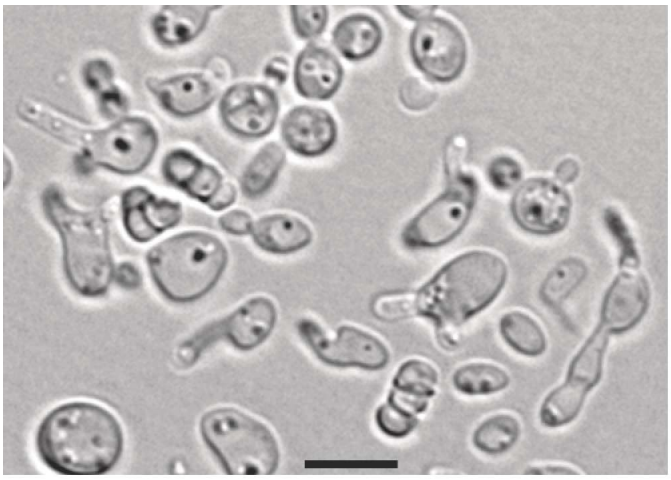
We are saddened by the loss of Dr. Inna Babjeva, a long-time contributor to the study of yeast ecology and diversity, and a beloved teacher, mentor, and colleague. Dr. Babjeva's pioneering studies of soil yeasts are particularly noteworthy. Our thanks to Dr. Andrey Yurkov for preparing an obituary.

ISSY 34 - Bariloche, Argentina

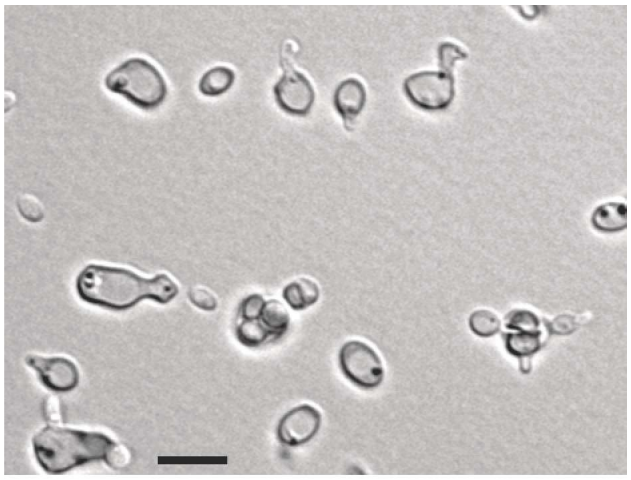
In the tradition begun in 1971, the 34th International Specialized Symposium on Yeasts continued the process of raising the bar by offering a wonderful array of scientific presentations and social events. Well attended by nearly three hundred participants, the symposium was preceded by a special taxonomy workshop in honour of the late Clete Kurtzman and followed by a brewing workshop, both of which attracted a large proportion of ISSY delegates. The plenary lectures were complemented by innovative e-posters, where presenters delivered short, condensed presentations to small audiences gathered around large computer screens. Bariloche is set on the edge of a spectacular Andean lake and the symposium took place the first week of the Patagonian spring season. On opening day, attendees were treated to a chairlift ride to the top of a local mountain in the midst of a raging snowstorm, following which they were entertained by traditional performing artists in the spectacular setting of Llao Llao, in the Andean foothills. A first-hand introduction to beers brewed with *Saccharomyces eubayanus*, discovered in Patagonia, included samples provided by international (Heineken), regional, and local (brew-pubs) brewers. The banquet featured Japanese Banzais (ten-thousand years of life!), a mind-blowing magician who dazzled all guests, and a first-rate rock band, featuring surprise guest performers from the yeast researcher community. Congratulations to Diego Libkind and his team for a job exquisitely done!

M.A. Lachance, Editor

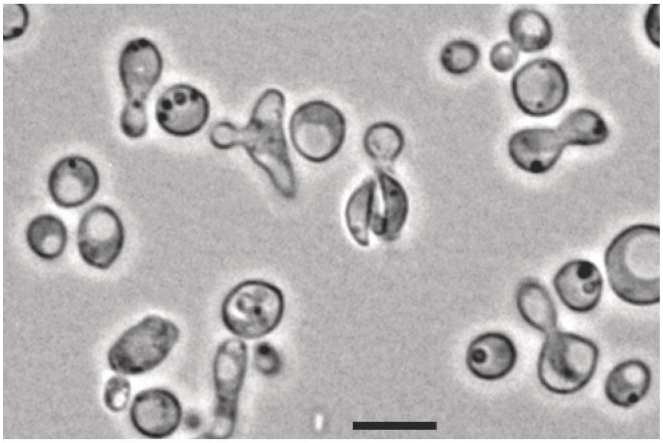
In defense of yeast sexual life cycles: five new *formae sexuales*
Photomicrographs by Maudy Smith
Bar = 5 µm



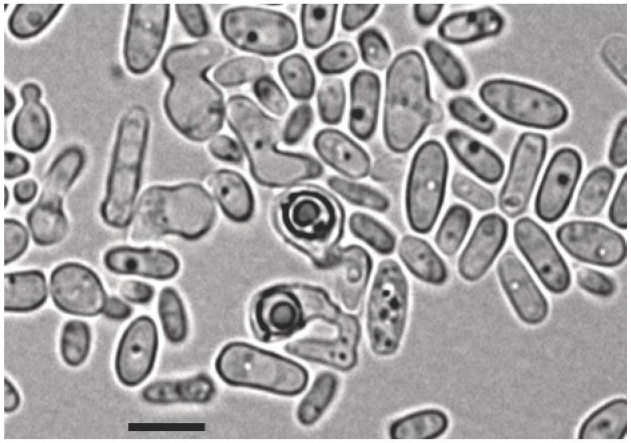
Ogataea chumphonensis



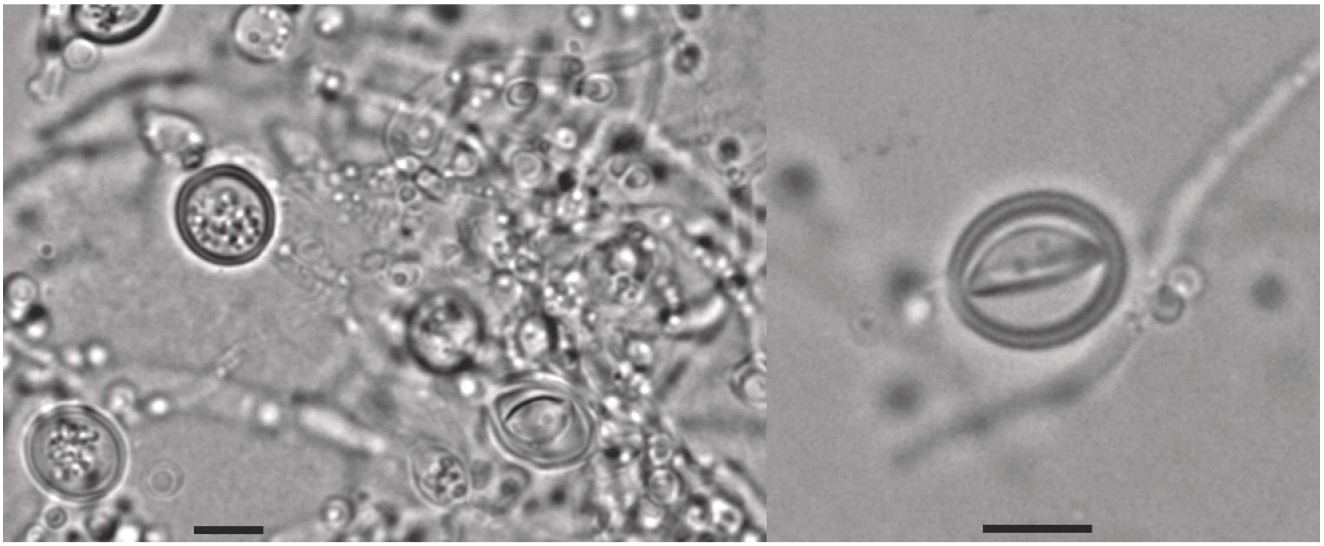
Ogataea mattraensis



Cyberlindnera nakhonratchasimensis



Martiniozyma asiatica



Sugiyamaella paludigena

I Yeast Collection, Westerdijk Fungal Biodiversity Institute, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands, Communicated by M. Groenewald <m.groenewald@westerdijkinstitute.nl> and M. Th. Smith <m.smith@westerdijkinstitute.nl>.

The teleomorph states were observed in the following type strains of species introduced initially as anamorphic taxa. See images in the preceding page.

Ogataea chumphonensis Limtong, Koowadjanakul, Jindam. & Yongmanitchai ex M. Groenew. & M.T. Sm. sp. nov.

Asci are formed by the type strains CBS 12096 after mother-bud conjugation or between independent cells at 25 C after 2 weeks on GPYA, releasing 1-4 hat-shaped ascospores (Upper left photo).

Candida chumphonensis was assigned to the *Ogataea* clade on basis of molecular parameters (Koowadjanakul et al.,2011). *C. chumphonensis* Limtong, Koowadjanakul, Jindam. & Yongmanitchai (2011). Antonie van Leeuwenhoek 100: 214 was invalidly published due to Art. 40.7 of the code of nomenclature and therefore the following name is proposed:

Ogataea chumphonensis Limtong, Koowadjanakul, Jindam. & Yongmanitchai ex M. Groenew. & M.T. Sm. sp.. nov.

Holotype: CBS 12096 preserved in metabolically inactive condition.

Culture ex-type CBS 12096

MycoBank No.: MB828451

Ogataea mattraensis Limtong, Koowadjanakul, Jindam. & Yongmanitchai ex M. Groenew. & M.T. Sm. sp. nov.

Asci are formed by the type strains CBS 12097 after mother-bud conjugation or between independent cells at 25 C after 2 weeks on GPYA, releasing 1-4 hat-shaped ascospores (Upper right photo).

Candida mattranensis was assigned to the *Ogataea* clade on basis of molecular parameters (Koowadjanakul et al.,2011). *C. mattranensis* Limtong, Koowadjanakul, Jindam. & Yongmanitchai (2011). Antonie van Leeuwenhoek 100: 214 was invalidly published due to Art. 40.7 of the code of nomenclature and therefore the following name is proposed:

Ogataea mattraensis Limtong, Koowadjanakul, Jindam. & Yongmanitchai ex M. Groenew. & M.T. Sm. sp. nov.

Holotype: CBS 12097 preserved in metabolically inactive condition.

Culture ex-type CBS 12097.

MycoBank No.: MB828452

Cyberlindnera nakhonratchasimensis (Jindam. & Nakase) M. Groenew. & M.T. Sm., sp. nov.

Asci are formed by the type strains CBS 11706 directly, after mother-bud conjugation or between independent cells at 25 C after 2 weeks on GPYA, releasing 1-4 crescent shaped ascospores (Middle left photo).

Candida nakhonratchasimensis was assigned to the *Cyberlindnera* clade on basis of molecular parameters (Daniel et al., 2014). *C. nakhonratchasimensis* Jindam. & Nakase (2004). J Gen Appl Microbiol 50: 266 was invalidly published due to Art. 40.7 of the code of nomenclature and the following name is proposed:

Cyberlindnera nakhonratchasimensis Jindam. & Nakase ex M. Groenew. & M.T. Sm. sp. nov.

Holotype: CBS 11706 preserved in metabolically inactive condition.

Culture ex-type CBS 11706.

MycoBank No.: MB828453

Martiniozyma asiatica (Limtong, Kaewwich., Am-In, Nakase, C.F. Lee, Yongman. & Srisuk) Kurtzman

Applying DNA sequence analysis, Kurtzman (2015) erected the genus *Martiniozym*a to harbor two *Candida* species, of which one was reclassified as *Martiniozyma asiatica* (Limtong et al.) Kurtzman with CBS 10863 as the Type strain of this species. So far the sexual state had not been obtained. In 2018, two compatible strains were received from M.A. Lachance and registered as CBS 15371 and CBS 15372. After 1-2 weeks of incubation at 25 C on V8 ascosporulation medium, conjugation and ascus formation were obtained between CBS 15371 and CBS 15372. One round warty ascospore per ascus was formed (Middle right photo). To determine the mating type of the type strain of the species, CBS 10863 was pair-wise mixed with the tester strains CBS 15371 and CBS 15372.

Conjugation and ascus formation was observed between CBS 10863 and CBS 15372, although the formation of the teleomorph was not as efficient as between CBS 15371 and CBS 15372. Arbitrarily, mating type alpha is assigned to CBS 10863 and mating type a to CBS 15372.

Sugiyamaella paludigena *(Golubev & Blagod.) H. Urbina & M. Blackw*

Applying DNA sequence analysis, Urbina et al. (2013) reclassified *Candida paludigena* Golubev & Blagod.as *Sugiyamaella paludigena* (Golubev & Blagod.) H. Urbina & M. Blackw with CBS 8005 as the Type strain of this species. So far the sexual state had not been obtained. CBS 8005 was found to be compatible with CBS 212.83 and CBS 213.83 forming asci from conjugating hyphae. After 4 weeks of incubation on DMA and PDA at 25 C as well as 15 C mature, persistent asci with an apical cell were observed containing 1 ellipsoidal ascospore (Bottom right photo).

References

Daniel, H-M., Lachance, M.-A. & Kurtzman, C.P. (2014) On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. Antonie van Leeuwenhoek 106: 67-84

Jindamorakot, S., Am-in, S., Thuy, T.T., Duy, N.D., Kawasaki, H., Potacharoe, W., Limtong, S., Tanticharoen, M. & Nakase, T. (2004) *Candida easanensis* sp.nov., *Candida pattaniensis* sp.nov. and *Candida nakhonratchasimensis* sp.nov., three new species of yeasts isolated from frass in Thailand. J.Gen.Appl.Microbiol. 50: 261-269

Koowadjanakul, N., Jindamorakot, S., Yongmanitchai, W. & Limtong, S. (2011) *Ogataea phyllophila* sp.nov., *Candida chumphonensis* sp. nov. and *Candida mattranensis* sp.nov., three methylotrophic yeast species from phylloplane in Thailand. Antonie van Leeuwenhoek 100: 207-217

Kurtzman, C.P. (2015) Description of *Martiniozyma* gen.nov. and transfer of seven *Candida* species to *Sartunispora* as new combinations. Antonie van leeuwenhoek 108: 803-809

Limtong, S., Kaewwichian, R., Am-In, S., Nakase, T., Lee, C.F., Yongmanitchai, W. & Srisuk, N. (2010) *Candida asiatica* sp. nov., an anamorphic ascomycetous yeast species isolated from natural samples from Thailand. Antonie van Leeuwenhoek 98: 475-481

Urbina, H., Frank, R. & Blackwell, M. (2013) *Scheffersomyces cryptocercus*: a new xylose-fermenting yeast associated with the gut of wood roaches and new combinations in the *Sugiyamaella* yeast clade. Mycologia 105: 650-660.

II Yeast Culture Collection, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, Posbus 339, Bloemfontein 9300, Republic of South Africa. Communicated by Aurelia Van Wyk <VanWykAG@ufs.ac.za>.

Here is a summary of current research being conducted at the collection.

Isolation and identification of *Lipomyces* species and other cycloheximide resistant yeasts from different habitats.

The genus *Lipomyces* belongs to the family *Lipomycetaceae*. The species of these soil inhabitants are also called oleaginous yeasts, because they produce large quantities of intracellular lipids (i.e. > 65% w/w, on dry cell mass). There are 22 accepted species in the *Lipomyces* genus. One of the key characteristics of *Lipomyces* is their resistance to 5 $g.L^{-1}$ cycloheximide (CYH). Cycloheximide is a heterocyclic glutirimide antibiotic, inhibiting protein synthesis in eukaryotes. They are also able to grow on heterocyclic nitrogen compounds such thymine and imidazole as sole energy source. The first aim of this project was to identify 46 unidentified *Lipomyces* strains isolated from soils of South Africa as well as 32 unidentified *Lipomyces* strains isolated from soils of other countries, deposited in the UNESCO-MIRCEN Yeast Culture Collection. The second aim was to isolate and identify *Lipomyces species* and

other cycloheximde resistant yeasts from indigenous flowers by using standard procedures used to isolate *Lipomyces* species. Identification was done by sequencing the D1/D2 domain of the LSU rRNA of all yeasts strains including those isolated from flowers. Forty five of the unidentified *Lipomyces* strains isolated from soil of South Africa, as well as 31 *Lipomyces* strains islolated from soils of other countries, were identified as known *Lipomyces* species. Twenty-five *Lipomyces tetrasporus* species were identified from South African soil and fourteen from soils of other countries. *Lipomyces tetrasporus* is distributed worldwide and has only been isolated from soil, making it the most abundant species found in this study. The gene sequence analysis of one of the *Lipomyces* strains, isolated from South African soil, revealed a 96 % similarity to a *Lipomyces* sp. Also, the gene sequence analysis of the *Lipomyces* strain isolated from the soil of Japan, showed a 97% similarity to a *Lipomyces* species. Gene sequences of 96% and 97% similarity to *Lipomyces*, are likely an indication of new species. Therefore they should be characterized further and one the tests includes sequence analysis of the ITS regions. For the flower yeasts, no *Lipomyces* species were isolated; however, other yeasts strains were isolated and identified. They belong mostly to the basidiomycetous yeasts, *Rhodotorula* and *Papiliotrema*. From the flower, Cotyledon orbiculata, a strain was isolated that showed a 95% similarity to a *Papiliotrema* sp. Also, from the flower *Pelargonium fragrens*, a strain was isolated that revealed a 97 % similarity to a *Filobasidium* sp. These are likely to be new species belonging to *Papiliotrema* and *Filobasidium*, respectively. These strains should also be characterised further by gene sequence analysis of the ITS regions. An indication of a novel species isolated, is when the nucleotide difference (0 - 0.05 %) between two strains of a species are not more than zero – three. All of the *Rhodotorula* strains from the flowers, were resistant to 5 $g.L^{-1}$ cycloheximide. The only other yeast strain, was a possible new *Papiliotrema* isolate, isolated from the flower *Cotyledon orbiculata*. From this study, two possible novel *Lipomyces* species, one from South African soil and the other from Japan, were identified through the gene sequence analysis of the D1/D2 domain of the LSU rRNA. Futhermore, two possible novel species were isolated from indigenous flowers from South Africa, identified as a *Papiliotrema* sp. and a *Filobasidium* sp.

III Culture Collection of Yeast (CCY), Institute of Chemistry, Slovak Academy of Sciences Dúbravská cesta 9, 845 38, Bratislava, Slovakia. Communicated by Emília Breierová <Emilia.Breierova@savba.sk>.

Recent publications.

1 Stratilová B, Klaudiny J, Řehulka P, Stratilová E, Mészárosová C, Garajová S, Pavlatovská B, Řehulková H, Kozmon S, Šesták S, Firáková Z, Vadkertiová R. 2018. Characterization of a long-chain α-galactosidase from *Papiliotrema flavescens.* World J Microbiol Biotechnol 34:1-14.

α-Galactosidases are assigned to the class of hydrolases and the subclass of glycoside hydrolases (GHs). They belong to six GH families and include the only characterized α-galactosidases from yeasts (GH 27, *Saccharomyces cerevisiae*). The present study focuses on an investigation of the lactose-inducible α-galactosidase produced by *Papiliotrema flavescens*. The enzyme was present on the surface of cells and in the cytosol. Its temperature optimum was about 60 °C and the pH optimum was 4.8; the pH stability ranged from 3.2 to 6.6. This α-galactosidase also exhibited transglycosylation activity. The cytosol α-galactosidase with a molecular weight about 110 kDa, was purified using a combination of liquid chromatography techniques. Three intramolecular peptides were determined by the partial structural analysis of the sequences of the protein isolated, using MALDI-TOF/TOF mass spectrometry. The data obtained recognized the first yeast α-galactosidase, which belongs to the GH 36 family. The bioinformatics analysis and homology modeling of a 210 amino acids long C-terminal sequence (derived from cDNA) confirmed the correctness of these findings. The study was also supplemented by the screening of capsular cryptococcal yeasts, which produce the surface lactose-inducible α- and β-galactosidases. The production of the lactose-inducible α-galactosidases was not found to be a general feature within the yeast strains examined and, therefore, the existing hypothesis on the general function of this enzyme in cryptococcal capsule rearrangement cannot be confirmed.

2 Breierová E, Čertík M, Márová I. and Vadkertiová R. 2018. The Effect of Zn(II) ions and reactive oxygen on the uptake of zinc and production of carotenoids by selected red yeasts. Chem Biodiversity 15:e1800069

Three strains of red yeast *Rhodosporidium kratochvilovae, Rhodotorula glutinis* and *Sporidiobolus salmonicolor* were studied for their responses to the presence metal stress, oxidative stress and a combination of these stress factors. For all yeast strains, the production of β-carotene increased in stress conditions. The combination of H_2O_2 and Zn(II) significantly activated the pathways for the production of torularhodin in the strain *R. glutinis* (from 250 to 470 μg^{-1} DCW) as well as β-carotene (from 360 to 1100 μg^{-1} DCW) and torulene (from 100 to 360 μg^{-1} DCW) in Sp. salmonicolor. Strains of *R. glutinis* and *Rh. kratochvilovae* bound the majority of Zn(II) ions to the fibrillar part of the cell walls, whereas the strain Sp. salmonicolor bound them to both extracellular polymers and the fibrillar part of the cell walls. A decrease in the ability of yeasts to tolerate higher concentrations of Zn(II) in the presence of free radicals (hydrogen peroxide) was also found.

The 7th meeting on Chemistry & Life 2018 was held 12-14 September in Brno, Czech Republic. The following papers were presented.

3 Breierová E, Šoltésová D, Stratilová B. and Sasinková V. Analysis of yeast exoproducts related to antifungal activity of *Metschnikowia pulcherrima.*

4 Dudášová H, Vadkertiová R. Diversity of yeasts colonizing aboveground plant organs of fruit trees and the soil related to these trees.

5 Horváthová A, Čurillová N, Vadkertiová M, Vyšehradská L, Márová I, Dudášová H, Stratilová B, Maldi TOF analysis for verification of collection yeasts and classification of new isolates.

The 5th European Section meeting of the International Academy of Cardiovascular Sciences (IACS-ES) was held at 23-26 May 2018 at Smolenice Castel, Slovakia. (ISBN 978-80-224-1649-8). The following paper was presented.

6 Frimmel K, Sotníková R, Navarová J, Knezl V, Križák J, Breierová E, Okruhlicová Ľ. Treatment of moderate inflammation with carotenoids and their effect on connexins expression on left ventricle of the heart.

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

The following chapter has been published in December 2017.

1 Kręgiel D, Pawlikowska E, Antolak H. 2017. Non-conventional yeasts in fermentation processes. W: Old Yeasts - New Questions. Lucas Cândida, Pais Célia (Eds.) Chapter 10. Pg 87-116. INTECH. ISBN 978-953-51-5515-7. DOI: 10.5772/intechopen.70404.

Traditionally the term 'yeast' means *Saccharomyces cerevisiae* and its close relatives. This yeast is used in traditional fermentation processes, mainly for ethanol formation, baking, winemaking and beer production. The classical carbon substrates for typical yeast processes are glucose or sucrose, however, the successful expansion of industrial biotechnology drives research toward the utilization of alternative carbon sources. New technologies require very specific challenges and differ from those found in conventional fermentation processes. Most microbial habitats, especially in modern biotechnological processes, do not provide culture media rich in mono- and disaccharides. They include fermentation environments with various compositions of carbon and energy sources as well as the presence of various cytotoxic compounds which inhibit the growth of industrial yeasts. About 1500 various yeast species have been identified nowadays. Microbiologists and biotechnologists have named all non-S. cerevisiae yeasts as 'non-conventional' yeasts. Their features present a potential that can be used for non-

conventional processes. Non-*Saccharomyces* strains provide alternative metabolic routes for substrate utilization and product formation. The diversity of these yeasts includes many species possessing useful, and sometimes uncommon, metabolic features potentially interesting for biotechnology. The selected strains of non-conventional yeasts could be used as pure or mixed cultures for improving industrial fermentations.

The following abstracts have been presented.

2 Pawlikowska E, Berlowska J, Kregiel D. 2018. Yeast *Metschnikowia pulcherrima* with potential as a biocontrol agent. XXX Jubilee Conference Processing and Energy in Agriculture PTEP 2018 Brzece, Serbia. Conference materials, page 33.

The reduction of chemical pesticides is the global desired trend in modern biotechnology. Many people have been exposed to artifical substances and, although the human health threats are not known fully, animal studies have found evidence of damage to neurological, immune and reproductive systems. In addition, chemical pesticides and insecticides are flowing off yards and gardens, contaminating some streams and rivers at concentrations that can kill small creatures vital to the survival of fish and other aquatic life. The safer alternatives in comparison to chemicals are natural, biological agents. Such interesting alternative may be the use of yeast *Metschnikowia pulcherrima*. The inhibitory effect may be connected with both the production of pulcherrimic acid and enzymatic activities. The aim of the study was to characterize collection strains belonging to *M. pulcherrima*, obtained from two reputable European collections – The National Collection of Yeast Cultures (NCYC, United Kingdom) and The Culture Collection of Yeasts (CCY, Slovakia). Enzymatic assays were carried out using the commercial API ZYM tests (bioMérieux). The potential antimicrobial activity of yeast strains was studied by observations of pulcherrimin production – the complex of pulcherrimin acid and iron ions Fe (III). For tested strains of *M. pulcherrima* the significant differences in enzyme profiles were obtained. All strains showed the activity of α-glucosidase and leucine arylamidase. However, the widest spectrum of enzymatic activity was observed for strains: NCYC2321, CCY145 and CCY149. These yeasts produced esterase, acid phosphatase and phosphohydrolase. Moreover, the strains are able to secrete pucherrimic acid that complexed Fe(III) ions and formed red pulcherimin. The pulcherrimic acid-ferric ion complex creates the environment unsuitable for growth of microbes that require more iron for growth. For example, the iron-limited environment is lethal to germinating conidia of phytopatogenic molds. Therefore, this biocontrol activity enables pulcherrimin-producing strains to naturally antagonize competing microorganisms. In conclusion, the isolated *M. pulcherrima* strains are promising candidates as a biological agent. These studies suggest that proper greenhouse/storage experiments should be conducted to confirm desirable attributes of yeasts as potential biocontrol agents. **Acknowledgments**: This work was supported by the National Centre for Research and Development under Grant BIOSTRATEG2/296369/5/NCBR/2016.

3 Pawlikowska E, Breierova E, James SA, Kregiel D. 2018. In search of natural biocontrol agents *Metschnikowia* species. 45TH Annual Conference on Yeast, Smolenice, Slovakia. Conference materials, page 53.

Different yeast species have been used as effective biocontrol agents against a variety of plant pathogens. Biocontrol yeasts inhibit the growth of targeted pathogens by different ways and mechanisms. Competition for nutrients and space, secretion of specific lytic enzymes, secretion of inhibitory metabolites as well as stress tolerance are the main modes of action. When searching for new biocontrol agents, researchers often focus on epiphytic yeasts isolated from natural environments. Such yeasts are especially useful as their occurrence in a specific habitat is often as the result of successful competition against other microorganisms. In this study, we have isolated *Metschnikowia* spp. strains from flowers and fruits in Poland. The plant material was collected from April to September 2017 in the Lodz Region, at two small orchards where traditional organic management is employed. Yeast screening was carried out using YPD agar with Fe(III) ions supplemented with streptomycin or chloramphenicol, and adjusted to pH 4,6. Species identity was determined by sequence analysis of the D1/D2 domains of the large subunit (LSU) rRNA gene, with the isolates identified as *Metschnikowia andauensis* and *M. sinensis*. Yeast

isolates were further characterized on their ability to produce pulcherrimic acid as well as to secrete α- and β-glucosidase and leucine arylamidase. Overall, their assimilation profiles were similar to those displayed by collection strains of *M. pulcherrima*. However, the isolates showed a wider range of growth temperatures (8 - 30°C), pH level (3 - 9) as well as tolerance to increased concentrations of ethanol (8%), glucose (30%) and peroxides (50 mM). In conclusion, our results demonstrate the potential of epiphytic yeasts belonging to the genus *Metschnikowia* as biocontrol agents. **Acknowledgments**: The authors thank the Collection of Industrial Microorganisms LOCK105 Poland, the Culture Collection of Yeasts Slovakia and the National Collection of Yeast Cultures UK for the essential and financial support.

V Laboratory of Genetics, Genome Center of Wisconsin, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin, Madison, WI 53706, USA. Communicated by Chris Todd Hittinger <cthittinger@wisc.edu>.

The Hittinger Lab is now on Twitter! Follow us @HittingerLab.

The Y1000+ Project (http://y1000plus.org) has released the largest number of budding yeast genomes to date! Read about it in *Cell* in the manuscript by Shen/Opulente/Kominek/Zhou et al.

1 Shen XX, Opulente DA, Kominek J, Zhou X, Steenwyk JL, Buh KV, Haase MAB, Wisecaver JH, Wang M, Doering DT, Boudouris JT, Schneider RM, Langdon QK, Ohkuma M, Endoh R, Takashima M, Manabe RI, Čadež N, Libkind D, Rosa CA, DeVirgilio J, Hulfachor AB, Groenewald M, Kurtzman CP, Hittinger CT, Rokas A. 2018. Tempo and mode of genome evolution in the budding yeast subphylum. *Cell* epub https://doi.org/10.1016/j.cell.2018.10.023.

Budding yeasts (subphylum Saccharomycotina) are found in every biome and are as genetically diverse as plants or animals. To understand budding yeast evolution, we analyzed the genomes of 332 yeast species, including 220 newly sequenced ones, which represent nearly one-third of all known budding yeast diversity. Here, we establish a robust genus-level phylogeny comprising 12 major clades, infer the timescale of diversification from the Devonian period to the present, quantify horizontal gene transfer (HGT), and reconstruct the evolution of 45 metabolic traits and the metabolic toolkit of the budding yeast common ancestor (BYCA). We infer that BYCA was metabolically complex and chronicle the tempo and mode of genomic and phenotypic evolution across the subphylum, which is characterized by very low HGT levels and widespread losses of traits and the genes that control them. More generally, our results argue that reductive evolution is a major mode of evolutionary diversification.

2 Krause DJ, Kominek J, Opulente DA, Shen XX, Zhou X, Langdon QK, DeVirgilio J, Hulfachor AB, Kurtzman CP, Rokas A, Hittinger CT. 2018. Functional and evolutionary characterization of a secondary metabolite gene cluster in budding yeasts. Proc Natl Acad Sci USA 115: 11030-11035.

Secondary metabolites are key in how organisms from all domains of life interact with their environment and each other. The iron-binding molecule pulcherrimin was described a century ago, but the genes responsible for its production in budding yeasts have remained uncharacterized. Here, we used phylogenomic footprinting on 90 genomes across the budding yeast subphylum Saccharomycotina to identify the gene cluster associated with pulcherrimin production. Using targeted gene replacements in *Kluyveromyces lactis*, we characterized the four genes that make up the cluster, which likely encode two pulcherriminic acid biosynthesis enzymes, a pulcherrimin transporter, and a transcription factor involved in both biosynthesis and transport. The requirement of a functional putative transporter to utilize extracellular pulcherrimin-complexed iron demonstrates that pulcherriminic acid is a siderophore, a chelator that binds iron outside the cell for subsequent uptake. Surprisingly, we identified homologs of the putative transporter and transcription factor genes in multiple yeast genera that lacked the biosynthesis genes and could not make pulcherrimin, including the model yeast *Saccharomyces cerevisiae*. We deleted these previously uncharacterized genes and showed they are also required for pulcherrimin utilization in *S. cerevisiae*, raising the possibility that other genes of unknown function are linked to

secondary metabolism. Phylogenetic analyses of this gene cluster suggest that pulcherrimin biosynthesis and utilization were ancestral to budding yeasts, but the biosynthesis genes and, subsequently, the utilization genes, were lost in many lineages, mirroring other microbial public goods systems that lead to the rise of cheater organisms.

3 Langdon QK, Peris D, Kyle B, Hittinger CT. 2018. sppIDer: a species identification tool to investigate hybrid genomes with high-throughput sequencing. Mol Biol Evol 35: 2835-2849.

The genomics era has expanded our knowledge about the diversity of the living world, yet harnessing high-throughput sequencing data to investigate alternative evolutionary trajectories, such as hybridization, is still challenging. Here we present sppIDer, a pipeline for the characterization of interspecies hybrids and pure species, that illuminates the complete composition of genomes. sppIDer maps short-read sequencing data to a combination genome built from reference genomes of several species of interest and assesses the genomic contribution and relative ploidy of each parental species, producing a series of colorful graphical outputs ready for publication. As a proof-of-concept, we use the genus *Saccharomyces* to detect and visualize both interspecies hybrids and pure strains, even with missing parental reference genomes. Through simulation, we show that sppIDer is robust to variable reference genome qualities and performs well with low-coverage data. We further demonstrate the power of this approach in plants, animals, and other fungi. sppIDer is robust to many different inputs and provides visually intuitive insight into genome composition that enables the rapid identification of species and their interspecies hybrids. sppIDer exists as a Docker image, which is a reusable, reproducible, transparent, and simple-to-run package that automates the pipeline and installation of the required dependencies (https://github.com/GLBRC/sppIDer; last accessed September 6, 2018).

4 Eizaguirre JI, Peris D, Rodríguez ME, Lopes CA, De Los Ríos P, Hittinger CT, Libkind D. 2018. Phylogeography of the wild Lager-brewing ancestor (*Saccharomyces eubayanus*) in Patagonia. Environ Microbiol 20: 3732-3743.

Saccharomyces eubayanus is the close relative of the Lager-brewing yeast and was firstly found in North Patagonia associated with *Nothofagus* trees. In recent years additional strains were found in North America, Asia and New Zealand, and genomic analyses showed the existence of two main populations of this yeast, both of them present in Patagonia. Here, we performed the most comprehensive study of *S. eubayanus* in Patagonia natural environments (400 samples) and confirmed that this region has the highest isolation success rate for this species described worldwide (more than 10-fold). The genetic characterization of 200 isolates (*COX2*, *DCR1*, *intFR*) revealed five geographically structured subpopulations. We hypothesized that marine ingressions and glaciations, which shaped the Patagonian landscape, contributed on population differentiation. The first large screening of fermentation performance of 60 wild *S. eubayanus* strains indicated which subpopulations would be more suitable for beer production.

5 Higgins DA, Young MK, Tremaine M, Sardi M, Fletcher JM, Agnew M, Liu L, Dickinson Q, Peris D, Wrobel RL, Hittinger CT, Gasch AP, Singer SW, Simmons BA, Landick R, Thelen MP, Sato TK. 2018. Natural variation in the multidrug efflux pump *SGE1* underlies ionic liquid tolerance in yeast. Genetics 210: 219-234.

Imidazolium ionic liquids (IILs) have a range of biotechnological applications, including as pretreatment solvents that extract cellulose from plant biomass for microbial fermentation into sustainable bioenergy. However, residual levels of IILs, such as 1-ethyl-3-methylimidazolium chloride ([C2C1im]Cl), are toxic to biofuel-producing microbes, including the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* strains isolated from diverse ecological niches differ in genomic sequence and in phenotypes potentially beneficial for industrial applications, including tolerance to inhibitory compounds present in hydrolyzed plant feedstocks. We evaluated >100 genome-sequenced *S. cerevisiae* strains for tolerance to [C2C1im]Cl and identified one strain with exceptional tolerance. By screening a library of genomic DNA fragments from the [C2C1im]Cl-tolerant strain for improved IIL tolerance, we identified *SGE1*, which encodes a plasma membrane multidrug efflux pump, and a previously

uncharacterized gene that we named ionic liquid tolerance 1 (*ILT1*), which encodes a predicted membrane protein. Analyses of SGE1 sequences from our panel of *S. cerevisiae* strains together with growth phenotypes implicated two single nucleotide polymorphisms (SNPs) that associated with IIL tolerance and sensitivity. We confirmed these phenotypic effects by transferring the *SGE1* SNPs into a [C2C1im]Cl-sensitive yeast strain using CRISPR/Cas9 genome editing. Further studies indicated that these SNPs affect Sge1 protein stability and cell surface localization, influencing the amount of toxic IILs that cells can pump out of the cytoplasm. Our results highlight the general potential for discovering useful biotechnological functions from untapped natural sequence variation and provide functional insight into emergent *SGE1* alleles with reduced capacities to protect against IIL toxicity.

6 Kuang MC, Kominek J, Alexander WG, Cheng J-F, Wrobel RL, Hittinger CT. 2018. Repeated cis-regulatory tuning of a metabolic bottleneck gene during evolution. Mol Biol Evol 35: 1968-1981.

Repeated evolutionary events imply underlying genetic constraints that can make evolutionary mechanisms predictable. Morphological traits are thought to evolve frequently through cis-regulatory changes because these mechanisms bypass constraints in pleiotropic genes that are reused during development. In contrast, the constraints acting on metabolic traits during evolution are less well studied. Here we show how a metabolic bottleneck gene has repeatedly adopted similar cis-regulatory solutions during evolution, likely due to its pleiotropic role integrating flux from multiple metabolic pathways. Specifically, the genes encoding phosphoglucomutase activity (*PGM1*/*PGM2*), which connect *GAL*actose catabolism to glycolysis, have gained and lost direct regulation by the transcription factor Gal4 several times during yeast evolution. Through targeted mutations of predicted Gal4-binding sites in yeast genomes, we show this galactose-mediated regulation of *PGM1*/*2* supports vigorous growth on galactose in multiple yeast species, including *Saccharomyces uvarum* and *Lachancea kluyveri*. Furthermore, the addition of galactose-inducible *PGM1* alone is sufficient to improve the growth on galactose of multiple species that lack this regulation, including *Saccharomyces cerevisiae*. The strong association between regulation of *PGM1*/*2* by Gal4 even enables remarkably accurate predictions of galactose growth phenotypes between closely related species. This repeated mode of evolution suggests that this specific cis-regulatory connection is a common way that diverse yeasts can govern flux through the pathway, likely due to the constraints imposed by this pleiotropic bottleneck gene. Since metabolic pathways are highly interconnected, we argue that cis-regulatory evolution might be widespread at pleiotropic genes that control metabolic bottlenecks and intersections.

7 Baker EP, Hittinger CT. Evolution of a novel chimeric maltotriose transporter in *Saccharomyces eubayanus* from parent proteins unable to perform this function. bioRxiv https://doi.org/10.1101/431171.

At the molecular level, the evolution of new traits can be broadly divided between changes in gene expression and changes in protein structure. For proteins, the evolution of novel functions is generally thought to proceed through sequential point mutations or recombination of whole functional units. In *Saccharomyces*, the uptake of the sugar maltotriose into the cell is the primary limiting factor in its utilization, but maltotriose transporters are relatively rare, except in brewing strains. No known wild strains of *Saccharomyces eubayanus*, the cold-tolerant parent of hybrid lager-brewing yeasts (*Saccharomyces cerevisiae x S. eubayanus*), are able to consume maltotriose, which limits their ability to fully ferment malt extract. In one strain of *S. eubayanus*, we found a gene closely related to a known maltotriose transporter and were able to confer maltotriose consumption by overexpressing this gene or by passaging the strain on maltose. Even so, most wild strains of *S. eubayanus* lack native maltotriose transporters. To determine how this rare trait could evolve in naive genetic backgrounds, we performed an adaptive evolution experiment for maltotriose consumption, which yielded a single strain of *S. eubayanus* able to grow on maltotriose. We mapped the causative locus to a gene encoding a novel chimeric transporter that was formed by an ectopic recombination event between two genes encoding transporters that are unable to import maltotriose. In contrast to classic models of the evolution of novel protein functions, the recombination breakpoints occurred within functional domains. Thus, the ability of the new protein to carry maltotriose was likely acquired through epistatic interactions between independently evolved

substitutions. By acquiring multiple mutations at once, the transporter rapidly gained a novel function, while bypassing potentially deleterious intermediate steps. This study provides an illuminating example of how recombination between paralogs can establish novel interactions among substitutions to create adaptive functions.

8 Kominek J, Doering DT, Opulente DA, Shen XX, Zhou X, DeVirgilio J, Hulfachor AB, Kurtzman CP, Rokas A, Hittinger CT. Eukaryotic acquisition of a bacterial operon. bioRxiv https://doi.org/10.1101/399394.

Operons are a hallmark of bacterial genomes, where they allow concerted expression of multiple functionally related genes as single polycistronic transcripts. They are rare in eukaryotes, where each gene usually drives expression of its own independent messenger RNAs. Here we report the horizontal operon transfer of a catecholate-class siderophore biosynthesis pathway from Enterobacteriaceae into a group of closely related yeast taxa. We further show that the co-linearly arranged secondary metabolism genes are actively expressed, exhibit mainly eukaryotic transcriptional features, and enable the sequestration and uptake of iron. After transfer to the eukaryotic host, several genetic changes occurred, including the acquisition of polyadenylation sites, structural rearrangements, integration of eukaryotic genes, and secondary loss in some lineages. We conclude that the operon genes were likely captured in the shared insect gut habitat, modified for eukaryotic gene expression, and maintained by selection to adapt to the highly-competitive, iron-limited environment.

9 Li XC, Peris D, Hittinger CT, Sia EA, Fay JC. Mitochondria-encoded genes contribute to the evolution of heat and cold tolerance among *Saccharomyces* species. bioRxiv https://doi.org/10.1101/390500.

Over time, species evolve substantial phenotype differences. Yet, genetic analysis of these traits is limited by reproductive barriers to those phenotypes that distinguish closely related species. Here, we conduct a genome-wide non-complementation screen to identify genes that contribute to a major difference in thermal growth profile between two *Saccharomyces* species. *S. cerevisiae* is capable of growing at temperatures exceeding 40°C, whereas *S. uvarum* cannot grow above 33°C but outperforms *S. cerevisiae* at 4°C. The screen revealed only a single nuclear-encoded gene with a modest contribution to heat tolerance, but a large effect of the species' mitochondrial DNA (mitotype). Furthermore, we found that, while the *S. cerevisiae* mitotype confers heat tolerance, the *S. uvarum* mitotype confers cold tolerance. Recombinant mitotypes indicate multiple genes contribute to thermal divergence. Mitochondrial allele replacements showed that divergence in the coding sequence of *COX1* has a moderate effect on both heat and cold tolerance, but it does not explain the entire difference between the two mitochondrial genomes. Our results highlight a polygenic architecture for interspecific phenotypic divergence and point to the mitochondrial genome as an evolutionary hotspot for not only reproductive incompatibilities, but also thermal divergence in yeast.

10 Baker EP, Peris D, Moriarty RV, Li XC, Fay JC, Hittinger CT. Mitochondrial DNA and temperature tolerance in lager yeasts. bioRxiv https://doi.org/10.1101/391946.

A growing body of research suggests that the mitochondrial genome (mtDNA) is important for temperature adaptation. In the yeast genus *Saccharomyces*, species have diverged in temperature tolerance, driving their use in high or low temperature fermentations. Here we experimentally test the role of mtDNA in temperature tolerance in synthetic and industrial hybrids (*Saccharomyces cerevisiae x Saccharomyces eubayanus*, or *Saccharomyces pastorianus*), which cold-brew lager beer. We find that the relative temperature tolerances of hybrids correspond to the parent donating mtDNA, allowing us to modulate lager strain temperature preferences. The strong influence of mitotype on the temperature tolerance of otherwise identical hybrid strains provides support for the mitochondrial climactic adaptation hypothesis in yeasts and demonstrates how mitotype has influenced the world's most commonly fermented beverage.

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The following papers were published during 2017-18.

1 Sha SP, Suryavanshi MS, Jani K, Sharma A, Shouche Y, Tamang JP. (2018). Diversity of yeasts and molds by culture-dependent and culture-independent methods for mycobiome surveillance of traditionally prepared dried starters for the production of Indian alcoholic beverages. Frontiers Microbiol 9:2237. doi: 10.3389/fmicb.2018.02237

Marcha, thiat, dawdim, hamei, humao, khekhrii, chowan and *phut* are traditionally prepared dried starters used for production of various ethnic alcoholic beverages in North East states of India. The surveillance of mycobiome associated with these starters have been revealed by culture-dependent methods using phenotypic and molecular tools. We identified *Wickerhamomyces anomalus*, *Pichia anomala, Saccharomycopsis fibuligera, Pichia terricola, Pichia kudriavzevii,* and *Candida glabrata* by ITS-PCR. The diversity of yeasts and molds in all 40 samples was also investigated by culture-independent method using PCR-DGGE analysis. The average distributions of yeasts showed *Saccharomyces cerevisiae* (16.5%), *Saccharomycopsis fibuli-gera* (15.3%), *Wickerhamomyces anomalus* (11.3%), *Sm. malanga* (11.7%), *Kluyveromyces marxianus* (5.3%), *Meyerozyma* sp. (2.7%), *Candida glabrata* (2.7%), and many strains below 2%. About 12 strains of molds were also identified based on PCR-DGGE analysis which inlcuded *Aspergillus penicillioides* (5.0%), *Rhizopusoryzae* (3.3%), and sub-phylum: *Mucoro-mycotina* (2.1%). Different techniques used in this paper revealed the diversity and differences of mycobiome species in starter cultures of India which may be referred as base-line data for further research.

2 Shangpliang HNK, Rai R, Keisam S, Jeyaram K, Tamang JP. (2018). Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. Scientific Rep 8: 1532 DOI.10.1038/s41598-018-19524-6.

Naturally fermented milk (NFM) products are popular ethnic fermented foods in Arunachal Pradesh and Sikkim states of India. The present study is the first to have documented the bacterial community in 54 samples of NFM products viz. *chhurpi*, *churkam, dahi* and *gheu*/*mar* by high-throughput Illumina amplicon sequencing. Metagenomic investigation showed that *Firmicutes* (*Streptococcaceae*, *Lactobacillaceae*) and *Proteobacteria* (*Aceto-bacteraceae*) were the two predominant members of the bacterial communities in these products. *Lactococcus lactis* and *Lactobacillus helveticus* were the predominant lactic acid bacteria while *Acetobacter* spp. and *Gluconobacter* spp. were the predominant acetic acid bacteria present in these products.

3 Anupma A, Pradhan P, Sha SP, Tamang JP. (2018). Traditional skill of ethnic people of the Eastern Himalayas and North East India in preserving microbiota as dry amylolytic starters. Indian J Trad Knowledge 17(1): 184-190.

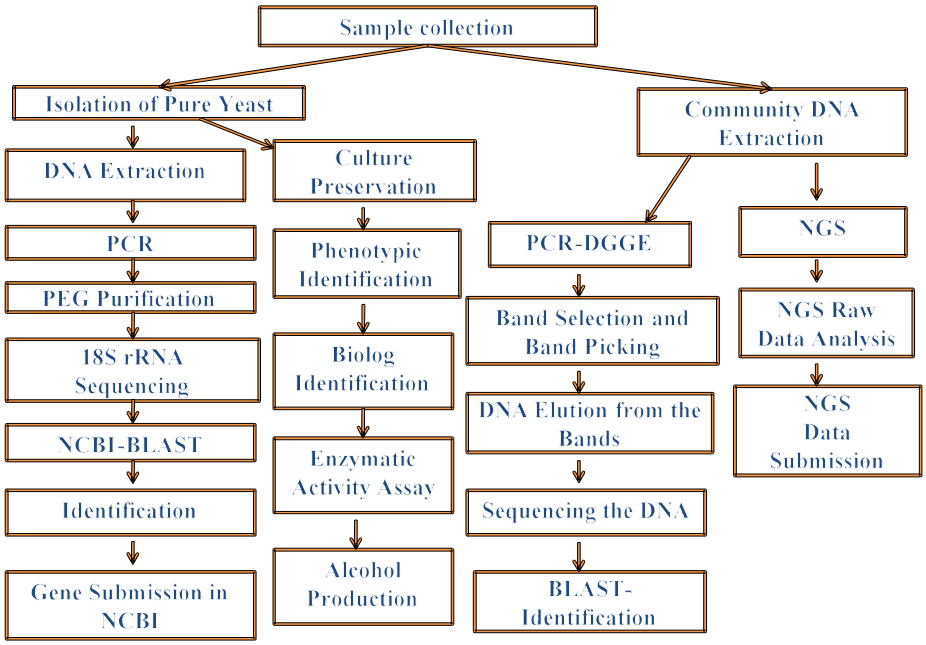
Preparation of ethnic fermented beverages using dry amylolytic starters is an integral part of socio-cultural practice of different ethnic groups of people residing in the Eastern Himalayan region of Nepal, Bhutan and India including North-East India. Alcoholic beverages are produced by traditional fermentation using specific amylolytic starters, which are prepared in different ways by diverse ethnic groups. This study is aimed at documenting the traditional skill of various ethnic groups of people of North East India and the Eastern Himalayas in preserving microbiota as dry amylolytic starters generally used for preparation of alcoholic beverages.

The following PhD Thesis was awarded by Sikkim University, Gangtok, India in October 2018.

Candidate: Dr. Shankar Prasad Sha. **Title**: Studies on yeasts diversity in some amylolytic starters of north east india using culture-dependent and culture-independent techniques. **Supervisor**: Professor Dr. Jyoti Prakash Tamang.

The major objectives of this Thesis were to document indigenous knowledge of people of North East India on production of traditionally prepared non-food amylolytic starters in the form of dry, solid, oval-flat cake-like starters viz. *marcha* of Sikkim, *humao* of Assam, *hamei* of Manipur, *thiat* of Meghalaya, *phut* of Arunachal Pradesh, *khekhrii* of Nagaland, *chowan* of Tripura and *dawdim* of Mizoram; and to investigate the yeast communities by culture-dependent and culture-independent methods; and also to estimate the α-amylase and glucoamylase activities and alcohol productivity of the identified yeast strains. Starter-making technology reflects the traditional method of 'sub-culturing' of desirable inocula from previous batch to new culture using rice as base substrates by back-sloping, and are produced at home for commercial use in few villages where starter-makers sell the products for livelihood. This is the first report on complete profiles of mycobiome communities with vast diversity as well as their enzymatic and alcohol-producing abilities associated with traditionally prepared amylolytic starters of North East India. This Thesis has also documented the traditional practicing of "ethno-microbiology" by diverse groups of ethnic people of North East India which involves the process of conservation and crude sub-culturing of functional microbiome using back-sloping method. This is the worth documentation and recognition of the age-old wisdom and native skill of the ethnic people of North East India for alcohol production using amylolytic starters cultures.

Schematic representation of complete PhD work



VII Department of Soil Biology, Faculty of Soil Science, Lomonosov Moscow State University, 119991 Leninskie gory, 1/12 Moscow, Russia and All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS, 142290, pr. Nauki 5, Pushchino, Russia. Communicated by A.V. Kachalkin <kachalkin_a@mail.ru>.

Recent publications.

1 Abdullabekova DA, Magomedova ES, Magomedov GG, Aliverdieva DA, Kachalkin AV. 2017. Yeast communities of chestnut soils under vineyards in Dagestan. Eurasian Soil Sci 50:1463-1467.

The study of yeast communities in chestnut soils (Kastanozems) under vineyards in the Republic of Dagestan made it possible to isolate 20 yeast species. Most of the yeasts under vineyards belonged to ascomycetes, among which species of the *Saccharomycetaceae* family (in particular, *Saccharomyces cerevisiae*) comprised a significant part. The obtained results indicate that the soils under vineyards keep the pool of microbial diversity and ensure preservation of many species typical for grapes. The method of enrichment culture on grape juice medium proved to be more efficient than other methods of analysis with respect to the number of isolated species and the rate of their detection. However, implementation of different techniques to study yeasts' diversity can give somewhat different results; a set of methods should be used for an integrated analysis.

2 Tepeeva AN, Glushakova AM, Kachalkin AV. 2018. Yeast communities of the Moscow city soils. Microbiology 87:407-415.

Yeast abundance and diversity were studied in the soils (topsoil) of Moscow city: urban soils under lawn vegetation and close to the areas of household waste disposal, as well as in zonal soddy-podzolic soils (retisols) in parks (Losiny Ostrov and Izmailovo). The numbers of soil yeasts were similar in all studied urban biocenoses (on average 3.5 Ч 10^3 CFU/g). From all studied soils, 54 yeast species were isolated. The highest yeast diversity was found in the soils adjacent to the areas of household waste storage. Soils from different urban sites were found to have different ratios of ascomycetous and basidiomycetous yeasts: basidiomycetes predominated in urban soils under lawn vegetation, while in the areas close to the waste disposal sites their share was considerably lower. The differences between the studied urban soils were also found in the structure of soil yeast complexes. In urban soils with high anthropogenic impact, the isolation frequency of clinically important yeast species (*Candida parapsilosis, C. tropicalis, Diutina catenulata,* and *Pichia kudriavzevii*) was as high as 35% of all studied samples, while its share in the community was 17%. The factors responsible for development of specific features of yeast communities in various urban soils are discussed in the paper.

3 Tepeeva AN, Glushakova AM, Kachalkin AV. 2018. The influence of heating mains on yeast communities in urban soils // Eurasian Soil Science 51:460-466.

The number and species diversity of yeasts in urban soils (urbanozems) affected by heating mains and in epiphytic yeast complexes of grasses growing above them were studied. The number of yeasts in the soil reached 10^3–10^4 CFU/g; on the plants, 10^7 CFU/g. Significant (by an order of magnitude) increase in the total number of soil yeasts in the zone of heating mains in comparison with the surrounding soil was found in winter period. Overall, 25 species of yeasts were isolated in our study. Yeast community of studied urbanozems was dominated by the *Candida sake*, an eurybiont of the temperate zone and other natural ecotopes with relatively low temperatures, but its share was minimal in the zone of heating mains. In general, the structure of soil and epiphytic yeast complexes in the zones of heating mains differed from that in the surrounding area by higher species diversity and a lower share of pigmented species among the epiphytic yeasts. The study demonstrated that the number and species structure of soil yeast communities in urban soils change significantly under the influence of the temperature factor and acquire a mosaic distribution pattern.

4 Kachalkin A. 2018. Yeast collection of the Soil biology department in Lomonosov Moscow State University (KBP MSU). XXXVII Annual Meeting of the European Culture Collections Organisation. Moscow.

The Yeast collection of the Soil Biology Department in Lomonosov Moscow State University (KBP MSU, WDCM CCINFO 1173) was founded by Dr. Inna Pavlovna Bab'eva in 1958. At first the Collection started with strains isolated by I.P. Bab'eva, I.S. Reshetova, and co-workers from different soils from the former USSR. Collection was enriched also with strains from different isolation sources such as phylloplane, tree exudates, fungi, insects and others related with the biogeocenotic approach to the study of yeast ecology. The Collection plays an important role in the students' education process, yeast strains are used in teaching bachelors, masters and PhD-students at the Soil Biology Department. The Collection holds presently more than 1800 yeast strains from natural environments, that makes it the third-largest yeast collection in Russia, after VKM and VKPM. Today, the KBP MSU contains 355 species of yeast fungi, together with undescribed species, what belonging to 134 genera. The Collection is expanding with scientifically interesting strains in the realization of ecological and biogeographical projects. The geographical diversity of the yeasts maintained in the Collection is reflected by 30 different countries of the strains' origin. Nevertheless, the main part (57%) of the yeast cultures deposited in the KBP MSU now have the Russian origin. The Collection has necessary facilities for sequence-based species identification. The yeasts are stored in glycerol at -80 °C. The KBP MSU is not commercial and shares strains with researches and other collections on the basis of research agreements. The KBP MSU is funded by research grants from the Russian Foundation for Basic Research (RFBR) and the project of Russian Science Foundation (RSF) called "Noah's Ark" for consolidation available university collections into a single depository. The online version of the KBP MSU catalogue is available at https://depo.msu.ru.

VIII Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Communicated by Marizeth Groenewald <m.groenewald@westerdijkinstitute.nl> and Teun Boekhout <t.boekhout@westerdijkinstitute.nl>.

The following are our publications so far for 2017-2018.

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2 Arastehfar A, Fang W, Badali H, Vaezi A, Jiang W, Liao W, Pan W, Hagen F & Boekhout, T. (2018) Low-cost tetraplex PCR for the global spreading multi-drug resistant fungus, *Candida auris* and its phylogenetic relatives. Front Microbiol 9: 1-8. doi: 10.3389/fmicb.2018.01119.

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4 Arastehfar A, Fang W, Pan W, Liao W, Yan L & Boekhout T. (2018) Identification of Nine Cryptic Species of *Candida albicans, C. glabrata*, and *C. parapsilosis* complexes using One-step multiplex PCR. BMC Infect Dis (in press). https://doi.org/10.1186/s12879-018-3381-5.

5 Aslani A, Janbabaei G, Abbastabar M, Meis JF, Babaeian M, Khodavaisy S, Boekhout T & Badali H. (2018) Identification of uncommon oral yeasts from cancer patients by MALDI-TOF mass spectrometry. BMC Infect Dis 18: 24.

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8 Fotedar R, Kolecka A, Boekhout T, Fell JW, Ananad A, Al Malaki A, Zeyara A, Al-Marri M. (2018) *Naganishia qatarensis* sp. nov, a novel basidiomycetous yeast species from a hypersaline marine environment in Qatar. Int J Syst Evol Microbiol.

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11 Groenewald M, Lorenzo Lombard L, de Vries M, Lopez AG, Smith M, Crous PW. (2018) Diversity of yeast species from Dutch garden soil and the description of six novel Ascomycetes FEMS yeast research (DOI: 10.1093/femsyr/foy076).

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

The following are abstracts of recently published papers and attended conferences from the lab members.

1 Miloshev G, Staneva D, Uzunova K, Vasileva B, Draganova-Filipova M, Zagorchev P, Georgieva M. 2018. Linker histones and chromatin remodeling complexes maintain genome stability and control cellular ageing. Mechanisms of Ageing and Development, https://doi.org/10.1016/j.mad.2018.07.002

Linker histones are major players in chromatin organization and *per se* are essential players in genome homeostasis. As the fifth class of histone proteins the linker histones not only interact with DNA and core histones but also with other chromatin proteins. These interactions prove to be essential for the higher levels of chromatin organization like chromatin loops, transcription factories and chromosome territories. Our recent results have proved that *Saccharomyces cerevisiae* linker histone – Hho1p, physically interacts with the actin-related protein 4 (Arp4) and that the abrogation of this interaction

through the deletion of the gene for the linker histone in *arp4* mutant cells leads to global changes in chromatin compaction. Here, we show that the healthy interaction between the yeast linker histone and Arp4p is critical for maintaining genome stability and for controlling cellular sensitivity to different types of stress. The abolished interaction between the linker histone and Arp4p leads the mutant yeast cells to premature ageing phenotypes. Cells die young and are more sensitive to stress. These results unambiguously prove the role of linker histones and chromatin remodelling in ageing by their cooperation in pertaining higher-order chromatin compaction and thus maintaining genome stability. *Acknowledgments:* This work was supported by the Bulgarian National Research Fund [grant number: DN 11/15] and NATO Science for Peace and Security programme [grant number: NATO SPS MYP G5266].

2 Staneva DN, Georgieva MK, Miloshev GA. 2018. *Kluyveromyces lactis* harbors an intact and functional linker histone gene encoding an important chromatin component. 22nd International Chromosome Conference (ICC 2018), 2nd-5th September 2018, Prague, Czech Republic.

The histone H1/H5 family members, also named linker histones, are essential constituents of eukaryotic chromatin with both structural and regulatory functions. By interactions with the linker DNA and with the other histones, H1 proteins mediate the formation and moreover the maintenance of the higher-order chromatin structures by which epigenetically modulate gene expression. Although H1 histones are widespread and evolutionary relatively well conserved chromatin proteins, it has long been considered that certain yeast species like *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces lactis* do not possess linker histones. The whole genome sequencing of these lower eukaryotes revealed some puzzling findings. The presence of a single histone H1-encoding gene in S. *cerevisiae* (*YPL127C*) was detected, the lack of a clear homolog of H1/H5 gene family in *S. pombe* was confirmed, while a pseudogene was reported in *K. lactis*. Here, we present experimental evidence for the existence of a functional linker histone gene in *K. lactis*, designated as *KlLH1*. Our data obtained after sequencing of the *KlLH1* together with other additional results confirm beyond doubt the proper transcription of the gene as well as *KlLH1* mRNA splicing and translation. Results about the involvement of KlH1p in bulk chromatin organization, studied by the method of Yeast Chromatin Comet assay (YChCA) demonstrate the important roles which this family of histones plays in the cellular fate. *Acknowledgments:* This work was partially supported by the National Research Fund [Grants DN 11/15; DMU 02/8] and NATO SPS Program [Grant No. MYP G5266].

3 Miloshev G, Staneva D, Georgieva M. 2018. The linker histones in higher order chromatin structures and their involvement in cellular norm and pathology. COST Action CA15214 Work Group Meeting "Epigenetic Significance of Higher-Order Chromatin Structures and Their Dynamics in Norm and Pathology", 21st – 22nd June, 2018, Sofia, Bulgaria.

Chromatin plays role of a media and appliance for the epigenetic mechanisms by which cells adapt to changes in the environment. The accuracy of chromatin organization is crucial for the response of cell to the environmental stimuli including different kind of mechanical stress. The extreme complexity especially at higher levels of organization chromatin represents a serious issue in the study of mechanobiological properties of its activity. It has always been suggested that the fifth class histones – the linker histones (H1 family) plays a fine but significant role in the regulation of gene activity. This function of linker histones remains obscure and controversial but it has be proven beyond doubt that H1 family are deeply involved in the building and maintenance of higher-order chromatin structures. Moreover, linker histones are engaged in guarding of the genome integrity both in norm and under stress conditions. However, as to date little is known about the intimate mechanisms of higher-order chromatin structures and linker histones input in the normal and pathological processes. We have followed the dynamics of higher-order chromatin structures at a single-cell level and were able to show that linker histone is responsible for the formation and maintenance of the second level of chromatin compaction, the so called "30 nm fiber". Furthermore, by the developed by us method for studying of the higher-order levels of chromatin organization – the Chromatin Comet Assay (ChCA) we have proven that linker histones are involved in the next level of chromatin organization - loop formation. The

comparison of the normally organized chromatin loops with that of cells devoid of linker histones poses some thought-provoking questions about the epigenetic significance of linker histones and higher-order chromatin structures both in norm and pathology.

Acknowledgments: This work is partially supported by the Bulgarian National Research Fund [grant number: DN 11/15] and NATO Science for Peace and Security programme [grant number: NATO SPS MYP G5266].

4 M Draganova-Filipova, M Georgieva, D Staneva, B Vasileva, P Zagorchev, G Miloshev. 2018. UVA/B stress differently influences cells during their ageing. Cell Symposia Aging and Metabolism. 23rd-25th September 2018, Sitges, Spain.

Cellular senescence normally is progressing slowly in the living cells but can be accelerated by various internal or external factors leading to different pathological conditions. UVA/B irradiation is an important stress factor which affects not only proliferation capacity of the cell but also change its epigenetic information at the level of chromatin organization. The aim of our study was to determine and compare cell vitality and chromatin changes in yeast and high eukaryotic cell cultures in normal under UVA/B stress conditions. We determined cell viability and chromatin organization in *Saccharomyces cerevisiae* chromatin mutants and two cell lines (fibroblast McCoy-Plovdiv and fibrosarcoma HT1080) before and after 460, 4600 and 16435mJ UVA/B irradiation. The results show that cell vitality changes under the stress but also confirm the key role of chromatin organization in senescence as well as cellular capacity to respond and cope with stress.

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Recent publications.

1 Higgins DA, Young MK, Tremaine M, Sardi M, Fletcher JM, Agnew M, Liu L, Dickinson Q, Peris D, Wrobel RL, Hittinger CT, Gasch AP, Singer SW, Simmons BA, Landick R, Thelen MP, Sato TK. 2018. Natural variation in the multidrug efflux pump *SGE1* underlies ionic liquid tolerance in yeast. Genetics 210: 219-234

Imidazolium ionic liquids (IILs) have a range of biotechnological applications, including as pretreatment solvents that extract cellulose from plant biomass for microbial fermentation into sustainable bioenergy. However, residual levels of IILs, such as 1-ethyl-3-methyl imidazolium chloride ([C2C1im]Cl), are toxic to biofuel-producing microbes, including the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* strains isolated from diverse ecological niches differ in genomic sequence and in phenotypes potentially beneficial for industrial applications, including tolerance to inhibitory compounds present in hydrolyzed plant feedstocks. We evaluated over one-hundred genome-sequenced *S. cerevisiae* strains for tolerance to [C2C1im]Cl and identified one strain with exceptional tolerance. By screening a library of genomic DNA fragments from the [C2C1im]Cl-tolerant strain for improved IIL tolerance, we identified *SGE1*, which encodes a plasma membrane multidrug efflux pump, and a previously uncharacterized gene that we named *ILT1*, which encodes a predicted membrane protein. Analyses of *SGE1* sequences from our panel of *S. cerevisiae* strains together with growth phenotypes implicated two single nucleotide polymorphisms (SNPs) that associated with IIL tolerance and sensitivity. We confirmed these phenotypic effects by transferring the *SGE1* SNPs into a [C2C1im]Cl-sensitive yeast strain using CRISPR/Cas9 genome editing. Further studies indicated that these SNPs affect Sge1 protein stability and cell surface localization, impacting the amount of toxic IILs that cells can pump out of the cytoplasm. Our results highlight the general potential for discovering useful biotechnological functions from untapped natural sequence variation and provide functional insight into emergent *SGE1* alleles with reduced capacities to protect against IIL toxicity.

2 Langdon QK, Peris D, Kyle B, Hittinger CT. 2018. sppIDer: a species identification tool to investigate hybrid genomes with high-throughput sequencing. Mol Biol Evol *In press*

The genomics era has expanded our knowledge about the diversity of the living world, yet harnessing high-throughput sequencing data to investigate alternative evolutionary trajectories, such as hybridization, is still challenging. Here we present sppIDer, a pipeline for the characterization of interspecies hybrids and pure species, that illuminates the complete composition of genomes. sppIDer maps short-read sequencing data to a combination genome built from reference genomes of several species of interest and assesses the genomic contribution and relative ploidy of each parental species, producing a series of colorful graphical outputs ready for publication. As a proof-of-concept, we use the genus *Saccharomyces* to detect and visualize both inter-species hybrids and pure strains, even with missing parental reference genomes. Through simulation, we show that sppIDer is robust to variable reference genome qualities and performs well with low-coverage data. We further demonstrate the power of this approach in plants, animals, and other fungi. sppIDer is robust to many different inputs and provides visually intuitive insight into genome composition that enables the rapid identification of species and their interspecies hybrids. sppIDer exists as a Docker image, which is a reusable, reproducible, transparent, and simple-to-run package that automates the pipeline and installation of the required dependencies (https://github.com/GLBRC/sppIDer).

3 Eizaguirre JI, Peris D, Rodríguez ME, Lopes CA, De Los Ríos P, Hittinger CT, Libkind D. 2018. Phylogeography of the wild Lager-brewing ancestor (*Saccharomyces eubayanus*) in Patagonia. Environmental Microbiology *In press*

Saccharomyces eubayanus is the close relative of the Lager brewing yeast and was firstly found in North Patagonia associated with *Nothofagus* trees. In recent years additional strains were found in North America, Asia and New Zealand, and genomic analyses showed the existence of two main populations of this yeast, both of them present in Patagonia. Here, we performed the most comprehensive study of *S. eubayanus* in Patagonia natural environments (400 samples) and confirmed that this region has the highest isolation success rate for this species described worldwide (more than 10 fold). The genetic characterization of 200 isolates (*COX2*, *DCR1*, *intFR*) revealed five geographically structured subpopulations. We hypothesized that marine ingressions and glaciations, which shaped the Patagonian landscape, contributed on population differentiation. The first large screening of fermentation performance of 60 wild *S. eubayanus* strains indicated which subpopulations would be more suitable for beer production.

4 Li XC, Peris D, Hittinger CT, Sia EA, Fay JC. - Biorxiv *Preprint* - Mitochondria-encoded genes contribute to the evolution of heat and cold tolerance among *Saccharomyces* species.

Over time, species evolve substantial phenotype differences. Yet, genetic analysis of these traits is limited by reproductive barriers to those phenotypes that distinguish closely related species. Here, we conduct a genome-wide non-complementation screen to identify genes that contribute to a major difference in thermal growth profile between two *Saccharomyces* species. *S. cerevisiae* is capable of growing at temperatures exceeding 40°C, whereas *S. uvarum* cannot grow above 33°C but outperforms *S. cerevisiae* at 4°C. The screen revealed only a single nuclear-encoded gene with a modest contribution to heat tolerance, but a large effect of the species' mitochondrial DNA (mitotype). Furthermore, we found that, while the *S. cerevisiae* mitotype confers heat tolerance, the *S. uvarum* mitotype confers cold tolerance. Recombinant mitotypes indicate multiple genes contribute to thermal divergence. Mitochondrial allele replacements showed that divergence in the coding sequence of *COX1* has a moderate effect on both heat and cold tolerance, but it does not explain the entire difference between the two mitochondrial genomes. Our results highlight a polygenic architecture for interspecific phenotypic divergence and point to the mitochondrial genome as an evolutionary hotspot for not only reproductive incompatibilities, but also thermal divergence in yeast.

Baker ECP, Peris D, Moriarty RV, Li XC, Fay JC, Hittinger CT. - Biorxiv *Preprint* - Mitochondrial DNA and temperature tolerance in lager yeasts.

A growing body of research suggests that the mitochondrial genome (mtDNA) is important for temperature adaptation. In the yeast genus *Saccharomyces*, species have diverged in temperature tolerance, driving their use in high or low temperature fermentations. Here we experimentally test the role of mtDNA in temperature tolerance in synthetic and industrial hybrids (*Saccharomyces cerevisiae* x *Saccharomyces eubayanus*, or *Saccharomyces pastorianus*), which cold-brew lager beer. We find that the relative temperature tolerances of hybrids correspond to the parent donating mtDNA, allowing us to modulate lager strain temperature preferences. The strong influence of mitotype on the temperature tolerance of otherwise identical hybrid strains provides support for the mitochondrial climactic adaptation hypothesis in yeasts and demonstrates how mitotype has influenced the world's most commonly fermented beverage.

New reviews.

5 Peris D, Pérez-Torrado R, Hittinger CT, Barrio E, Querol. 2018. A On the origins and industrial applications of *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii* hybrids. Yeast 5: 51-69.

Companies based on alcoholic fermentation products, such as wine, beer, and biofuels, use yeasts to make their products. Each industrial process utilizes different media conditions, which differ in sugar content, the presence of inhibitors, and fermentation temperatures. *Saccharomyces cerevisiae* has traditionally been the main yeast responsible for most fermentation processes. However, the market is changing due to the consumer demands or external factors, such as climate change. Some processes, such as biofuel production or winemaking, require new yeasts to solve specific challenges, especially those associated with sustainability, novel flavors, and altered alcohol contents. One of the proposed solutions is the application of yeast hybrids. The lager beer market has been dominated by *S. cerevisiae* x *Saccharomyces eubayanus* hybrids. However, several less thoroughly studied hybrids have been isolated from other diverse industrial processes. Here we focus on *S. cerevisiae* x *Saccharomyces kudriavzevii* hybrids, which have been isolated from diverse industrial conditions that include wine, ale beer, cider, and dietary supplements. Emerging data suggest an extended and complex story of adaptation of these hybrids to traditional industrial conditions. *S. cerevisiae* x *S. kudriavzevii* hybrids are also being explored for new industrial applications, such as biofuels. This review describes the past, present, and future of *S. cerevisiae* x *S. kudriavzevii* hybrids.

6 Peris D, Pérez-Torrado R. 2018. La biodiversidad de levaduras como fuente de innovación en la industria cervecera. TecniFood Magazine 118: 76-78 (In Spanish).

XI Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary. Communicated by Matthias Sipiczki <gecela@post.sk>.

List of papers published in 2018.

1 Sipiczki M, Selim SA. 2018. Antagonistic yeasts from a salt-lake region in Egypt: identification of a taxonomically distinct group of phylloplane strains related to *Sporisorium*. Antonie van Leeuwenhoek - doi: 10.1007/s10482-018-1184-8.

2 Acs-Szabo L, Papp LA, Antunovics Z, Sipiczki M, Miklos I. 2018. Assembly of *Schizosaccharomyces cryophilus* chromosomes and their comparative genomic analyses revealed principles of genome evolution of the haploid fission yeasts. Sci Rep 8:14629. doi: 10.1038/s41598-018-32525-9.

3 Sipiczki M, Horvath E, Pfliegler WP. 2018. Birth-and-death evolution and reticulation of ITS segments of *Metschnikowia andauensis* and *Metschnikowia fructicola* rDNA repeats. Front Microbiol 9:1193. doi: 10.3389/fmicb.2018.01193. eCollection 2018.

4 Mohd Tap R, Kamarudin NA, Ginsapu SJ, Ahmed Bakri AR, Ahmad N, Amran F, Sipiczki M. 2018. Draft genome sequence of *Candida pseudohaemulonii* isolated from the blood of a neutropenic patient. Genome Announc 6: pii: e00166-18. doi: 10.1128/genomeA.00166-18.

5 Csoma H, Acs-Szabo L, Papp LA, Sipiczki M. 2018. Application of different markers and data-analysis tools to the examination of biodiversity can lead to different results: a case study with *Starmerella bacillaris* (synonym *Candida zemplinina*) strains. FEMS Yeast Res 18 - doi: 10.1093/femsyr/foy021.

6 Rosa AL, Miot-Sertier C, Laizet Y, Salin F, Sipiczki M, Bely M, Masneuf-Pomarede I, Albertin W. 2018. Draft genome sequence of the *Starmerella bacillaris* (syn., *Candida zemplinina*) type strain CBS 9494. Microbiol Resour Announc 7:e00872-18. https://doi.org/ 10.1128/MRA.00872-18.

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Recent publications.

1 Josey M, Maskell D, Speers RA. 2018. The impact of induced petites on lager fermentation. Submitted to J. ASBC.

Respiratory deficient cells or 'petites' are the most common type of mutation found in brewing yeast. Very high levels of petites are known to contribute to unwanted flavours in beer along with yeast flocculation problems during fermentation. However, little is known about the impact of petites when present at naturally occurring frequencies. Accordingly, this study investigated if petites – present at low frequencies – affect beer flavour and fermentation profiles. Laboratory [20 mL] fermentations were undertaken with yeast that contained a range of petite populations 3.7, 5.1, 8.7, and 10.8 %). During fermentation, the yeast in suspension, wort density, and alcohol were monitored. At the end of the fermentation, the beer was analysed for volatile flavor compounds. Correlations between petite levels nd levels of vicinal diketones, acetate esters and medium chain fatty acid (MCFA) ethyl esters existed. Higher alcohol levels were unchanged (propan-1-ol, 3-methyl butanol, 2-methyl butanol, and isobutanol) with increasing levels of petite concentrations. Similarly, the yeast in suspension behavior and the change in wort density attenuation between the control and petite enriched fermentations were insignificantly different ($p > 0.05$). This study suggests that low concentrations of petites in the pitched yeast would not be detectable in the final product.

2 Potter G, van Wyk PWJ, Duvenhage MM, Coetsee E, Swart HC, Budge SM, Speers RA. 2018. Compositional, ultrastructural and nanotechnological characterization of the SMA strain of *Saccharomyces pastorianus*. PLOS One. 13(7).

Nano scanning Auger microscopy (NanoSAM) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) have been used in materials science research for some time, but NanoSAM, in particular, has only recently been applied to biological specimens. Here, the first concurrent utilization of NanoSAM, TOF-SIMS and microscopic techniques for the examination of a standard beverage fermentation strain of *Saccharomyces pastorianus* uncovered the presence of intracellular networks of CO in fermenting cells. Respiring cells produced few bubbles and instead had large internal vacuolar structures. Transmission electron microscopy analysis also showed osmiophilic layers at the cell exterior of fermenting cells that became more prevalent with fermentation duration, while osmiophilic layers were largely absent in respiring cells. TOF-SIMS analysis showed a compositional difference at the exterior and interior of SMA cells and between fermenting and respiring cells. Fermenting cells also appeared to have different 3-OH oxylipin profiles compared to respiring cells based upon examination with immunofluorescence microscopy. The results of this work and further study using these materials science techniques will substantially enhance our understanding of the chemical, ultrastructural and metabolic changes that occur in fermentation yeasts.

3 Armstrong M, MacIntosh A, Josey M, Speers RA. 2018. Examination of premature yeast flocculation in UK malts. MBAA Tech Quarterly, 55:54-60.

Premature yeast flocculation (PYF) has been shown to induce early and enhanced yeast flocculation and can result in a myriad of production difficulties and delays. Researchers have demonstrated that PYF is linked to fungal infection of barley and/or malt. Therefore, one might suspect that PYF occurrence would increase in wet climates. To test this hypothesis, a control, a PYF-positive malt sample, and two Scottish- sourced malt varieties were compared with a control dataset of 77 fermentations. Miniature fermentation trials using the ASBC method Yeast-14 were performed on these samples. Miniature fermentation trials have been optimized to detect PYF-causing malt by directly measuring attenuation via extract readings and indirectly measuring yeast in suspension via absorbance. Nonlinear and tilted Gaussian regressions were fit to the data sets to obtain attenuation and absorbance curves, respectively. Results obtained indicated that the PYF-causing malt induced both early and enhanced flocculation, whereas the other three malts (including the Scottish malts) did not induce early or enhanced flocculation. All four samples were compared using the F test, and it was determined that the PYF-positive malt sample was significantly different than the other three malts ($P < 0.05$). Using previous results from 77 miniature fermentation trials on control malts, a novel 95% prediction band was developed. This band was applied and used to identify and decisively confirm the presence of PYF-causing malt as demonstrated herein.

4 Maskell PD, Speers RA, Maskell DL. 2017. Updating the accuracy of alcohol concentration determination for uncertainty of measurement calculations. Science and Justice. 57:321–330.

The Widmark equation is probably the most commonly used calculation for medico-legal purposes. Recently the National Research Council (USA) and the Forensic Science Regulator (UK) have called for the uncertainty of all results to be given with all forensic measurements and calculations. To improve the uncertainty of measurement of results from Widmark calculations we have concentrated on the uncertainties of measurement involved in the calculation of amount of alcohol, that of the volume of alcohol, the concentration of alcohol and the density of alcohol as previous studies have investigated some of the other factors involved. Using experimental studies, the scientific literature and legal statutes, we have determined revised and improved uncertainties of the concentration of ethanol for Widmark calculations for both the USA and UK. Based on the calculations that we have performed we recommend the use of Monte Carlo Simulation for the determination of uncertainty of measurement for Widmark Calculations.

5 Niu C, Zhu L, Hill A, Speers RA, Li Q. 2017. Construction of a highly thermostable 1,3-1,4-β-glucanase by combinational mutagenesis and its potential application in the brewing industry. Biotechnology Letters. 39:1-10.

Objectives - To improve the thermostability and catalytic property of a mesophilic 1,3-1,4-b-glucanase by combinational mutagenesis and to test its effect in Congress mashing. **Results** - A mutant b-glucanase (rE-BglTO) constructed by combinational mutagenesis showed a 25^0C increase in optimal temperature (to 70 ^{0C}) a 19.5^0C rise in T50 value and a 15.6^0C increase in melting temperature compared to wild-type enzyme. Its half-life values at 60 and 70^0C were 152 and 99 min, which were 370 and 800 % higher than those of wild-type enzyme. Besides, its specific activity and kcat value were 42,734 U mg-1 and 189 s-1 while its stability under acidic conditions was also improved. In flask fermentation, the catalytic activity of rE-BglTO reached 2381 U ml-1, which was 63 % higher than that of wild-type enzyme. The addition of rE-BglTO in Congress mashing decreased the filtration time and viscosity by 21.3 and 9.6 %, respectively. **Conclusions** - The mutant b-glucanase showed high catalytic activity and thermostability which indicated that rE-BglTO is a good candidate for application in the brewing industry.

6 Potter G, Xia W, Budge SM, Speers RA. 2017. Quantitative analysis of 3-OH oxylipins in fermentation yeast. Can J Microbiol 63: 100-109

Despite the ubiquitous distribution of oxylipins in plants, animals, and microbes, and the application of numerous analytical techniques to study these molecules, 3-OH oxylipins have never been quantitatively assayed in yeasts. The formation of heptafluorobutyrate methyl ester derivatives and subsequent analysis with gas chromatography – negative chemical ionization – mass spectrometry allowed for the first determination of yeast 3-OH oxylipins. The concentration of 3-OH 10:0 (0.68–4.82 ng/mg dry cell mass) in the SMA strain of Saccharomyces pastorianus grown in laboratory-scale beverage fermentations was elevated relative to oxylipin concentrations in plant tissues and macroalgae. In fermenting yeasts, the onset of 3-OH oxylipin formation has been related to fermentation progression and flocculation initiation. When the SMA strain was grown in laboratory-scale fermen- tations, the maximal sugar consumption rate preceded the lowest concentration of 3-OH 10:0 by 4.5 h and a distinct increase in 3-OH 10:0 concentration by 16.5 h.

Presentations.

7 Huismann M. Gormley F, Zait D, Speers RA, Maskell DL, 2019. Understanding aromatic stability in dry-hopped beer. Accepted. European Brewing Convention. Antwerp, BEL. June 2-6.

8 Reid S, Speers RA, Lumsden W, Maskell DL. 2018. Organic acid development during Scotch Whisky fermentations. Young Scientists Symposium on Malting, Brewing and Distilling. September 12-14. Bitberg/Trier, DEU.

9 Speers RA. 2018. Pasteurization. Invited Presentation. Qilu University of Technology. Jinan China. Aug. 22-24.

10 Speers RA. 2018. Yeast. Invited Presentation. Qilu University of Technology. Jinan China. Aug. 22-24.

11 Speers RA, Warrier R, Soroka A, Belanger K. 2018. Prediction of CO_2 purity in fermenter headspace gas. Presented at the ASBC/MBAA Brewing Summit. San Diego, CA. Aug.12-15.

12 Speers RA. 2018. Yeast flocculation – A review. Invited Presentation. Qilu University of Technology. Jinan China. May 21-25.

13 Speers RA. 2018. Premature yeast flocculation and modelling. Invited Presentation. Qilu University of Technology. Jinan China. May 21-25.

14 Speers RA, Josey M, Bryce J. 2018. Relationships between the speed of fermentation and levels of flavour compounds. Invited Presentation. Qilu University of Technology. Jinan China. May 21-25.

15 Speers RA. 2017. Cells, Colloids, Malt and Beer. Award of Merit Invited Presentation. MBAA Meeting, Atlanta, FL Oct. 12-14.

16 Roy L, MacIntosh AJ, Speers RA, Paulson AT. 2017. Brewing with 100% malted buckwheat: a gluten-free alternative incorporating extraneous enzymes. MBAA Meeting, Atlanta, FL. Oct. 12-14.

17 Huismann M, Gormley F, Maskell, DL, Speers RA. 2017. Ethanol's effect on terpene extraction. 2nd International Brewers Symposium- Hop Flavor and Aroma. Corvallis, OR, July 25-28.

18 Huismann M, Gormley F, Maskell DL, Speers RA. 2017. Kinetic modelling of terpenes in packaged beers. Presented at the ASBC meeting. Fort Myers, Fl. June 4-7.

19 Kaur M, Evans DE, Bowman JP, Speers RA, Stewart D, Koutoulis A. 2017. A rapid high throughput qPCR based diagnostic test for premature yeast flocculation (PYF) in malts. Presented at the Eur. Brew. Convention. Ljubljana, May 14-18.

XIII VTT Technical Research Centre of Finland Ltd, Tietotie 2, Espoo, FI-02044 VTT, Finland. Communicated by Brian Gibson <brian.gibson@vtt.fi> www.vttresearch.com.

Recent publications.

1 Rantasalo A, Landowski CP, Kuivanen J, Korppoo A, Reuter L, Koivistoinen O, Valkonen M, Penttilä M, Jäntti J, Mojzita D. 2018. A universal gene expression system for fungi. Nucleic Acids Res. 46(18):e111. doi: 10.1093/nar/gky558.

Biotechnological production of fuels, chemicals and proteins is dependent on efficient production systems, typically genetically engineered microorganisms. New genome editing methods are making it increasingly easy to introduce new genes and functionalities in a broad range of organisms. However, engineering of all these organisms is hampered by the lack of suitable gene expression tools. Here, we describe a synthetic expression system (SES) that is functional in a broad spectrum of fungal species without the need for host-dependent optimization. The SES consists of two expression cassettes, the first providing a weak, but constitutive level of a synthetic transcription factor (sTF), and the second enabling strong, at will tunable expression of the target gene via an sTF-dependent promoter. We validated the SES functionality in six yeast and two filamentous fungi species in which high (levels beyond organism-specific promoters) as well as adjustable expression levels of heterologous and native genes was demonstrated. The SES is an unprecedentedly broadly functional gene expression regulation method that enables significantly improved engineering of fungi. Importantly, the SES system makes it possible to take in use novel eukaryotic microbes for basic research and various biotechnological applications.

2 Rantasalo A, Kuivanen J, Penttilä M, Jäntti J, Mojzita D. 2018. Synthetic toolkit for complex genetic circuit engineering in *Saccharomyces cerevisiae*. ACS Synth Biol. 7(6):1573-1587. doi: 10.1021/acssynbio.8b00076.

Sustainable production of chemicals, materials, and pharmaceuticals is increasingly performed by genetically engineered cell factories. Engineering of complex metabolic routes or cell behavior control systems requires robust and predictable gene expression tools. In this challenging task, orthogonality is a fundamental prerequisite for such tools. In this study, we developed and characterized in depth a comprehensive gene expression toolkit that allows accurate control of gene expression in *Saccharomyces cerevisiae* without marked interference with native cellular regulation. The toolkit comprises a set of transcription factors, designed to function as synthetic activators or repressors, and transcription-factor-dependent promoters, which together provide a broad expression range surpassing, at high end, the strongest native promoters. Modularity of the developed tools is demonstrated by establishing a novel bistable genetic circuit with robust performance to control a heterologous metabolic pathway and enabling on-demand switching between two alternative metabolic branches.

3 Preiss, R Tyrawa, C Krogerus, K Garshol, L Van Der Merwe G. 2018. Traditional norwegian kveik are a genetically distinct group of domesticated *Saccharomyces cerevisiae* brewing yeasts. Frontiers in Microbiology. doi: 10.3389/fmicb.2018.02137

The widespread production of fermented food and beverages has resulted in the domestication of *Saccharomyces cerevisiae* yeasts specifically adapted to beer production. While there is evidence beer yeast domestication was accelerated by industrialization of beer, there also exists a farmhouse brewing culture in western Norway which has passed down yeasts referred to as kveik for generations. This practice has resulted in ale yeasts which are typically highly flocculant, phenolic off flavor negative (POF-), and exhibit a high rate of fermentation, similar to previously characterized lineages of domesticated yeast. Additionally, kveik yeasts are reportedly high-temperature tolerant, likely due to the traditional practice of pitching yeast into warm (>28°C) wort. Here, we characterize kveik yeasts from 9 different Norwegian sources via PCR fingerprinting, whole genome sequencing of selected strains, phenotypic screens, and lab-scale fermentations. Phylogenetic analysis suggests that kveik yeasts form a distinct group among beer yeasts. Additionally, we identify a novel POF- loss-of-function mutation, as well as SNPs

and CNVs potentially relevant to the thermotolerance, high ethanol tolerance, and high fermentation rate phenotypes of kveik strains. We also identify domestication markers related to flocculation in kveik. Taken together, the results suggest that Norwegian kveik yeasts are a genetically distinct group of domesticated beer yeasts with properties highly relevant to the brewing sector.

4 K Krogerus, R Preiss, B Gibson. 2018. A unique *Saccharomyces cerevisiae* × *Saccharomyces uvarum* hybrid isolated from Norwegian farmhouse beer: characterization and reconstruction. Frontiers in Microbiology. doi: 10.3389/fmicb.2018.02253

An unknown interspecies *Saccharomyces* hybrid, "Muri," was recently isolated from a "kveik" culture, a traditional Norwegian farmhouse brewing yeast culture (Preiss et al., 2018). Here we used whole genome sequencing to reveal the strain as an allo-diploid *Saccharomyces cerevisiae* × *Saccharomyces uvarum* hybrid. Phylogenetic analysis of its sub-genomes revealed that the *S. cerevisiae* and *S. uvarum* parent strains of Muri appear to be most closely related to English ale and Central European cider and wine strains, respectively. We then performed phenotypic analysis on a number of brewing-relevant traits in a range of *S. cerevisiae*, *S. uvarum* and hybrid strains closely related to the Muri hybrid. The Muri strain possesses a range of industrially desirable phenotypic properties, including broad temperature tolerance, good ethanol tolerance, and efficient carbohydrate use, therefore making it an interesting candidate for not only brewing applications, but potentially various other industrial fermentations, such as biofuel production and distilling. We identified the two *S. cerevisiae* and *S. uvarum* strains that were genetically and phenotypically most similar to the Muri hybrid, and then attempted to reconstruct the Muri hybrid by generating de novo interspecific hybrids between these two strains. The de novo hybrids were compared with the original Muri hybrid, and many appeared phenotypically more similar to Muri than either of the parent strains. This study introduces a novel approach to studying hybrid strains and strain development by combining genomic and phenotypic analysis to identify closely related parent strains for construction of de novo hybrids.

5 B Gibson, V Vidgren, G Peddinti, K Krogerus. 2018. Diacetyl control during brewery fermentation via adaptive laboratory engineering of the lager yeast *Saccharomyces pastorianus*. J Ind Microbiol Biotechnol.. https://doi.org/10.1007/s10295-018-2087-4

Diacetyl contributes to the flavor profile of many fermented products. Its typical buttery flavor is considered as an off flavor in lager-style beers, and its removal has a major impact on time and energy expenditure in breweries. Here, we investigated the possibility of lowering beer diacetyl levels through evolutionary engineering of lager yeast for altered synthesis of α-acetolactate, the precursor of diacetyl. Cells were exposed repeatedly to a sub-lethal level of chlorsulfuron, which inhibits the acetohydroxy acid synthase responsible for α-acetolactate production. Initial screening of 7 adapted isolates showed a lower level of diacetyl during wort fermentation and no apparent negative influence on fermentation rate or alcohol yield. Pilot-scale fermentation was carried out with one isolate and results confirmed the positive effect of chlorsulfuron adaptation. Diacetyl levels were over 60% lower at the end of primary fermentation relative to the non-adapted lager yeast and no significant change in fermentation performance or volatile flavor profile was observed due to the adaptation. Whole-genome sequencing revealed a non-synonymous SNP in the ILV2 gene of the adapted isolate. This mutation is known to confer general tolerance to sulfonylurea compounds, and is the most likely cause of the improved tolerance. Adaptive laboratory evolution appears to be a natural, simple and cost-effective strategy for diacetyl control in brewing.

6 Vidgren V Gibson B. 2018. Trans-regulation and localization of orthologous maltose transporters in the interspecies lager yeast hybrid. FEMS Yeast Res 18:foy065, https://doi.org/10.1093/femsyr/foy065

In the interspecies lager yeast hybrid there are MAL loci involved in maltose and maltotriose utilization derived from each parent (*Saccharomyces cerevisiae* and *Saccharomyces eubayanus*). We show that trans-regulation across hybrid subgenomes occurs for MAL genes. However, gene expression is less efficient with non-native activators (trans-activation) compared to native activators (cis-activation). MAL genes were induced by maltose and repressed by glucose irrespective of host. Despite the strong

expression of *S. cerevisiae*-type genes in the *S. eubayanus* host, a very low amount of transporter protein was actually observed in cells. This suggests that proper formation and configuration of the *S.cerevisiae* transporters is not efficient in *S. eubayanus*. The *S. eubayanus-type* Malx1 transporter was present in the plasma membrane in high amounts in all hosts (*S. cerevisiae*, *S. eubayanus* and *Saccharomyces pastorianus*) at all times. However, the *S. cerevisiae*-type transporters appeared sequentially in the plasma membrane; scMalx1 was localized in the plasma membrane during early to late linear growth and subsequently withdrawn to intracellular compartments. In contrast, the scAgt1 transporter was found in the plasma membrane mainly in the stationary phase of growth. Different localization patterns may explain why certain transporter orthologues in natural *S. pastorianus* strains were lost to mutation.

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Recently published contributions.

1 Walker GM, Walker RSK. 2018. Enhancing yeast alcoholic fermentations. Advances in Applied Microbiology 105. https://doi.org/10.1016/bs.aambs.2018.05.003

2 Black K, Walker G, White P, Squire G and Iannetta P. 2018. Intercropped barley for brewing & distilling. *In:* Distilled Spirits: Local Roots; Global Reach. Ed. F Jack, D Dabrowska, S Davies, M Garden, D Maskell and D Murray Context Products Ltd, Packington, UK. Chapter 9, pp45-48

3 Lachance MA, Walker GM. 2018. Yeasts. In: eLS: Citable Reviews in the Life Science. DOI: 10.1002/9780470015902.a0000380.pub3. https://doi.org/10.1002/9780470015902.a0000380.pub3

The following is an abstract of a presentation given at ISSY34 in Bariloche.

4 Walker G, Daute M. 2018. Non-*Saccharomyces* yeasts for alcohol fermentations: challenges and opportunities.

Although *Saccharomyces* spp. are the premier industrial microorganisms that are exploited in many traditional and modern biotechnologies, several diverse non-*Saccharomyces* yeast species have great potential in industrial fermentation technologies, particularly those involved in alcohol production. For example, in the fuel alcohol sector, several non-*Saccharomyces* yeasts are advantageous, not least due to their abilities to utilise pentose sugars in lignocellulosic hydrolysates for second-generation bioethanol production. Examples include *Scheffersomyces stipitis* that can ferment xylose and displays Crabtree-negative metabolic behaviour. Concerning beverage fermentations, several diverse yeast species are finding applications, including: *Brettanomyces* and *Saccharomycodes* spp. which are being exploited in production of speciality "sour" and low-alcohol beers, respectively; *Metchnikowia* and *Torulaspora* spp. which are being used in commercial wine production; and *Kluyveromyces* spp. which are used in cheese whey fermentations for production of distilled spirits. Such yeasts are employed due to their synthesis of interesting flavour congeners that contribute desirable organoleptic characteristics in fermented beverages. Currently, in production of whisky from cereal substrates, commercial strains of *S. cerevisiae* dominate in distillery fermentations, mainly due to tradition and for their rapid alcohol production properties, but there is scope to employ more flavoursome yeasts. Our research focuses on evaluating the potential of using non-*Saccharomyces* yeasts in Scotch whisky fermentations to impart desirable aromas and flavours in distilled spirits prior to maturation in oak wood casks. Results will be presented from trial fermentations using diverse species of yeast and subsequent distillations, including GC-MS analyses of major volatile congener chemicals together with sensory analyses of freshly distilled spirits. This presentation will highlight the opportunities of using non-*Saccharomyces* yeasts for production of distilled spirits and will discuss some of the fermentation challenges when compared with the use of more conventional distilling yeast strains. **Acknowledgements**: We thank colleagues at Abertay University (John Grigor and Peter Maskell) and at The Scotch Whisky Research Institute (Frances Jack, Jane Walker, Irene Baxter and Barry Harrison) for their invaluable collaboration on this project.

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The following have been published since the last issue of the Yeast Newsletter.

1 Lachance MA, Vale H, Sperandio E, Carvalho AOS, Santos ARO, Grondin C, Jacques N, Casaregola S, Rosa CA. 2018 *Wickerhamiella dianesei* f. a., sp. nov. and *Wickerhamiella kurtzmanii* f.a., sp. nov., two yeast species isolated from plants and insects. Int J syst Evol Microbiol 68:3351-3355

Six yeast strains representing two novel *Wickerhamiella* species were isolated from plants and insects collected in Costa Rica, Brazil, and French Guiana. They belong to a subclade containing *Wickerhamiella domercqiae* and *Wickerhamiella bombiphila*, and differ by approximately 12 % in the D1/D2 sequences of the large subunit rRNA gene from these species. The intergenic spacer (ITS) regions of the two novel species differ by around 19 and 27 %, respectively, from those of *W. domercqiae*. The novel species exhibit 5 % divergence in the D1/D2 sequences among them (around 4 % in the ITS). The names *Wickerhamiella dianesei* f.a., sp. nov. and *Wickerhamiella kurtzmanii* f.a., sp. nov. are proposed to accommodate these species, for which a sexual cycle has not been observed. *Wickerhamiella dianesei* was isolated from the stingless bee, *Trigona fulviventris*, collected in an Asteraceae flower in Costa Rica, and from leaves of *Sabicea brasiliensis* (Rubiaceae) and a flower of *Byrsonima crassifolia* (Malpighiaceae) in Brazil. *Wickerhamiellsa kurtzmanii* was isolated from a flower of *Ipomoea batatoides* (Convolvulaceae) in Costa Rica, the surface of a fruit of *B. crassifolia* in Brazil, and flowers in French Guiana. The type strains are *Wickerhamiella dianesei* UWOPS 00-107.1^{T} (=CBS 14185=NRRL Y-63789; Mycobank number MB 827008) and Wickerhamiella kurtzmanii UWOPS 00-192.1^{T} (=CBS 15383=NRRL Y-63979; MB 827011).

2 Lachance MA and Walker G 2018 Yeasts. Encyclopaedia of Life Sciences, Wiley.

Yeasts are a group of eukaryotic microfungi with a well-defined cell wall whose growth is either entirely unicellular or a combination of hyphal and unicellular reproduction. The approximately 1500 known yeast species belong to two distinct fungal phyla, the Ascomycota and the Basidiomycota. Within each of these phyla, yeasts can be found in several subphyla or classes, reflecting the enormous diversity of their evolutionary origins and biochemical properties. In nature, yeasts are found mainly in association with plants or animals but are also present in soil and aquatic environments. Yeasts grow rapidly and have simple nutritional requirements, for whieh reason they have been used as model systems in biochemistry, genetics and molecular biology. They were the first microorganisms to be domesticated for the production of beer, bread or wine, and they continue to be used for the benefit of humanity in the production of many important health care and industrial commodities, including recombinant proteins, biopharmaceuticals, biocontrol agents and biofuels. The best-known yeast is the species *Saccharomyces cerevisiae*, which may be regarded as the world's foremost industrial microbe.

The following article will be part of a special issue of *FEMS Yeast Research* honoring the memory of Clete Kurtzman.

3 Lachance MA 2018 C. P. Kurtzman's evolving concepts of species, genus, and higher categories. FEMS Yeast Res 18:foy103.

Cletus P. Kurtzman transformed the way yeast systematists practice their trade and how they perceive the yeast species. He redefined many genera of ascomycetous yeasts and provided a sound basis upon which to base higher taxonomic categories. Within his extraordinary corpus lies a trail of elements that can be used to reconstruct his evolving vision of the concepts that underlie the species and the genus, rarely set in a theoretical framework. While occasionally tipping his hat to the biological and phylogenetic species, Kurtzman espoused a concept founded primarily on genetic distance, even when claiming otherwise. In contrast, his notion of genus incorporated components of both genetic distance and phylogenetic structure, and possibly a size consideration. A phylogenetic approach predominated with higher taxa.

Conference presentation.

4 Lachance MA *Metschnikowia* mating habits. ISSY34 - International Specialized Symposium on Yeasts, Bariloche, Argentina.

Species of the genus *Metschnikowia* share the intriguing property of producing asci with only two ascospores, never more. These are genuine meiotic products. The spores are assembled in parallel with the first meiotic division, but the timing and localization of meiosis II has remained a mystery. An account of our progress towards determining the exact time course will be given. This involves labelling nuclei of complementary strains with different fluorescent proteins in species for which a genetic toolkit is not yet available. Many *Metschnikowia* species share a haplontic and heterothallic sexual cycle also found in the sister genus *Clavispora*, suggesting that this represents the ancestral condition, whereas later-emerging clades are mostly diplontic. The haplontic condition has made it easy to examine mating and ascus formation as indicators of species boundaries. Abundant mating takes place between closely related species, but fertile asci only arise in conspecific matings. We have mined draft genomes to improve our understanding of genes involved in mating type determination and cell-cell recognition within and across many species, including those assigned to the floricolous beetle-associated large-spored group. The mating type locus and its flanking genes are conserved across the family Metschnikowiaceae and bear some similarities with those of the neighbouring Debaryomycetaceae. Genes responsible for the synthesis of mating pheromones vary across species in number of loci and number of coding regions per locus. Pheromone sequences follow other genes phylogenetically and their similarity or divergence among species made it possible to predict mating compatibility in some, but not all cases. It is clear that speciation in haplontic *Metschnikowia* species begins with genetic divergence between isolated populations and is only accompanied by prezygotic isolation much later in evolutionary time.

Obituaries

Inna Pavlovna Babjeva (October 5 1927 – June 18 2018)

On June 18 2018, renowned Russian microbiologist Dr. Inna Pavlovna Babjeva passed away at 91. Babjeva made invaluable contributions to the study of yeast diversity and ecology. Three years after graduating at Lomonosov Moscow State University, in 1950, she obtained her PhD in microbiology. She became assistant professor at Lomonosov Moscow State University in 1961 and worked in this position until her retirement in 2001.

Inna Pavlovna was born in a family of agronomists and entertained a passion for soil through her scientific career, which started in 1953 when she joined the newly founded department of soil biology at Lomonosov Moscow State University, headed by prominent Soviet microbiologist Nikolai Krasilnikov. At that time, the faculty launched a large-scale study of soils across the Russian plain, and Babjeva was invited to lead the field microbiological research in these expeditions in the following 4 years. Krasilnikov, who studied soil microorganisms in the 1930s while searching for novel actinomycetes, suggested to investigate of soils as a source of yeasts even though the ability of yeasts to propagate in soils was repeatedly questioned at the time. Astonishingly, this daring trial became the basis for nearly 50 years research on yeasts inhabiting soils and other natural habitats.

Few studies of soil yeasts were available at that time, and so Babjeva and her long-term assistant Irina Sergeevna Reshetova began to develop and optimise methods for the isolation and characterisation of soil yeasts. These early works largely determined the directions of development of the laboratory, which was later known as the Soil yeasts laboratory. Interestingly, at around the same time soil yeasts become a focus of attention for Margaret E. di Menna, who worked on the other side of the Earth, in New Zealand. In 1950-60s, research of both laboratories often went in parallel whereby isolation techniques were developed, new soil-borne species were discovered, yeast adaptation to soils was studied, and the distribution of yeasts in different soil types was explored. Two studies that revolutionised views on soil yeasts, showing their

distribution across different soil types, were published by Babjeva and Golovleva and by di Menna in 1963 and 1965, respectively.

Species of the genus *Lipomyces* were among first yeasts isolated from soils by Babjeva. Her students and collaborators cultured members of the genus from the entire range of studied soils. The biology and distribution of indigenous soil yeasts was investigated over 30 years. A review of this knowledge was published in 1987 by Gorin and Babjeva. Interactions of yeasts with plants, algae, and fungi were studied by Babjeva and co-workers in 1960s. This topic was explored further in the 1980-1990s with studies of yeast killer toxins or mycocins by two students in the lab, Golubev and Vustin. Starting from the 1970s, the scientific scope of the laboratory diversified. The repeated isolation of basidiomycetous yeasts from soils and above ground substrates gave rise to numerous studies on the biology and systematics of these yeasts performed by Aksenov, Golubev, and Chernov. Yeasts associated with invertebrates (e.g. bark beetles, bees, ants, springtails, and earthworms) become another prominent topic of the laboratory. With the growing number of investigated soils, geography soon took on a greater importance in studies conducted in the Soil yeast laboratory. Although soils remained the primary topic for a long time, substrates adjacent to soils were routinely investigated by Babjeva and co-workers to distinguish true soil-borne yeasts from transient species. Later this approach drove the work from soils towards studies of entire biotopes, to elucidate the diversity of yeasts in other substrates such as plants (from juvenile to decomposing), invertebrates, and mushrooms. Representative cultures isolated during these surveys were preserved in the culture collection currently known under the acronym KBP MSU (WDCM 1173). In 1990-2000s a former student of Inna Pavlovna, Ivan Chernov, summarised results collected by the laboratory and performed multivariate analyses to detect trends in the distribution of soil yeasts as a function of soil properties and climate. He also investigated spatial and temporal trends in distribution of yeasts above and below ground.

Inna Pavlovna Babjeva published over 220 publications and 8 books, including practical guides and handbooks. She was a commissioner representing Soviet Union and later Russia in the International Commission on Yeasts. For many years, she remained one of very few woman commissioners. But her main legacy is her students - a total of 60 graduate students, of which 21 PhD students, passed through the soil yeast laboratory. Her students work in universities and research institutes worldwide. Her contribution to yeast science was recognised by colleagues who described two yeast genera and two yeast species in her honour, namely, *Babjevia*, *Babjeviella*, *Rhodotorula babjevae* and *Saccharomycopsis babjevae*.

I had the great pleasure of meeting Inna Pavlovna Babjeva when I started my education at Moscow State University and came to the Soil yeast laboratory in 1998, a few years before she retired. She supervised my very first student work. Among the most important skills I learned at that time, was to read scientific publications carefully and make proper notes during my experiments. She patiently taught her students to document results in lab books and to make good microscopic line drawings of yeasts – no yeast is round like a ball! These simple but useful skills always help me in my research.

Inna Pavlovna Babjeva will be missed by her two children, four grandchildren, ten great-grandchildren, her students, former colleagues, and many others.

Andrey Yurkov

Takashi Nakase (1939-2018)

Dr. Takashi Nakase, an eminent yeast taxonomist born April 11 1939, passed away on July 24 2018. His major contributions included the taxonomic significance of DNA G+C content, the application of molecular techniques to yeast taxonomy, studies on the species diversity of basidiomycetous yeasts, especially those that form ballistoconidia, and his involvement in an Asian microbial research network, featuring both yeasts and other microorganisms. He published over 320 articles, including original papers, reviews, books, book chapters, and proceedings.

Nakase graduated from the Faculty of Agriculture, Tottori University, Tottori Prefecture, Japan, in 1962, and started his carrier at the Central Research Laboratories, Ajinomoto Co., Inc., Japan. There, he met Dr. Kazuo Komagata, chief microbiologist, who was to hold an important place in his life. In later years, Komagata became Professor, then Professor Emeritus at the University of Tokyo, as well as the first Director of the Japan Collection of Microorganisms (JCM), which was established in 1981 at RIKEN (The Institute of Physical and Chemical Research at that time).

Nakase started his work on yeast taxonomy at Ajinomoto Co., Inc., under Komagata's supervision. He began to determine the mol% G+C (G+C content) of yeast nuclear DNA based on T*m*, and published the mol% G+C data of yeast species, one after another, from the genera *Hansenula*, *Hanseniaspora*, *Pichia*, *Debaryomyces*, and many others. As the number of species and genera constantly increased, his publications covered most of both ascomycetous and basidiomycetous yeast species. This comprehensive characterization of yeasts based on mol% G+C had a major impact of the yeast taxonomy worldwide. Nakase received his degree of Doctor of Agriculture (Ph. D.) from The University of Tokyo in 1979; the title of his thesis was 'Taxonomic studies of yeasts, especially of the taxonomic significance of G+C content' (in Japanese). In 1981, he was promoted to−Chief Scientist at the Research Institute for Life Sciences, Ajinomoto Co., Inc.

In September 1982, Nakase moved to the JCM at RIKEN. The Collection was still in its infancy, being only in its second year of activity. He initiated studies of ballistoconidium-forming yeasts on plant leaves and continued to develop the chemotaxonomic characterization of yeasts. Upon Komagata's retirement in March 1989, Nakase was appointed Director of JCM, and continued to be a driving force in the growth of JCM and the development of yeast taxonomic research in Japan for 19 years until his retirement from RIKEN in March 2000.

During this period, JCM witnessed two major advances: the first was a liquid nitrogen preservation facility, the JCM-Annex, erected next to the JCM building in 1993. With these

facilities, JCM was able to increase its deposition capacity considerably. Second, from 1995, Dr. Nakase led the international collaboration project "Asian Network on Microbial Researches, ANMR", supported by Special Coordination Funds for the promotion of Science and Technology, Japan. As part of the project, he nurtured JCM staff as group leaders (curators), by being involved in the entire process of culture collection work, namely accession, preservation, and various characterizations of taxa under their responsibility. Post-doctoral researchers were trained as taxonomists of their respective taxa. With the yeasts, he expanded his work on mol% G+C to complete chemotaxonomic characteristics such as the major ubiquinone and the carbohydrate composition of cell walls, as well as phylogenetic analyses based on partial rRNA gene sequences.

From 2002 to 2004, Nakase worked in Thailand, first at the National Science and Technology Development Agency (NSTDA) of the National Center for Genetic Engineering Biotechnology (BIOTEC), and later with funding from the JSPS Cooperative Research Fellowship. The project was entitled "Studies on the Species and Functional Diversity of Yeasts Living in the Natural Environment of Thailand". However, Nakase's accomplishments included the development of human resources in yeast taxonomy, the investigation of yeasts in natural environments in Thailand (isolation and identification), and support for management of the BIOTEC culture collection. Approximately 700 yeast strains were isolated and served as material for Nakase to train young yeast scientists. The program was not limited to BIOTEC researchers. It also involved those in other Thai organizations such as Kasetsart University, the Thailand Institute of Scientific and Technological Research (TISTR) as well as collaborations with Japan, China, and Taiwan, contributing to an expansion of the network.

From May 2005 to March 2011, Nakase joined the Biological Resource Center, National Institute of Technology and Evaluation, Japan, as an advisor, and continued to mentor yeast researchers/scientists in both Japan and other countries.

Nakase's research interests were broad. He characterized his work as "culture collection-oriented". This encompassed all aspects of taxonomy and systematics, including isolating novel yeast species, adding them to the catalog of JCM cultures, and making correctly identified strains available to users. Reliability was at the heart of his work. Although his studied both ascomycetous and basidiomycetous yeasts, his primary interest lay with ballistospore-forming yeasts. Mature asexual ballistospores are discharged from sterigmata, and Nakase used this property to develop an improved isolation method that made it easy to isolate ballistosporogenous yeasts from plant samples collected in Japan as well as overseas. He described several new genera initially named *Ballistosporomyces, Bensingtonia, Bullera, Dioszegia, Kockovaella, Sporobolomyces,* and *Udeniomyces*, although many have since been renamed as a result of recent developments in phylogenetic classification. His expertise led him to author monographs on all genera of ballistospore-forming yeasts in *The Yeasts, A Taxonomic Study*, 5th ed., (2011). Nowadays, ballistospore-forming yeasts are understood as epiphytic phylloplane yeasts, and some species are regarded as "hub" taxa that play important roles in the plant-microorganism relationship. Nakase's forty years of yeast isolation, identification, and description of novel taxa were a major element in the development of our knowledge of the biodiversity of phylloplane yeasts in the greater context of ecological and environmental studies.

Nakase served as the President (1993 - 1997) and board member of Japan Society for Culture Collections (currently Japan Society for Microbial Resources and Systematics) and was elected as an honorary member of its Society in 2009. In 2000, also, The Mycological Society of Japan (MSJ) Award was given to him for an outstanding mycological career and great contribution to yeast systematics.

Dr. Nakase was my immediate supervisor when he was at JCM, and he continued to be my mentor after he left the Collection. The yeast

taxonomy community and the Asian microbial research community were fostered by Dr. Nakase, and his influence continues to serve as a testament to his career. The Japan Collection of Microorganisms, now nearly 40 years old will contribute to the world microbiological community as a major Biological Resource Center.

I thank the following colleagues for their generous assistance in the preparation of this obituary: Marc-André Lachance, Kazuo Komagata, Junta Sugiyama, Ken-ichiro Suzuki, Astushi Yamazaki, Akira Nakagiri, and Sasitorn Jindamorakot.

Masako Takashima

Japan Collection of Microorganisms, RIKEN BioResource Research Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

Recent meetings

International Commission on Yeasts (ICY)
Mycology and Eukaryote Microbiology (MEM) Division
International Union of Microbiological Societies (IUMS)

ICY Commissioners Meeting, Wednesday, October 3, 2018
34th International Specialised Symposium on Yeasts
Manush Brewery Restaurant, Bariloche, Argentina

Minutes of Meeting

Present (35): Hiroshi Takagi (ICY Chair; Japan), Charles Abbas (ICY Vice-Chair; USA), Diego Libkind Frati (ISSY34 Chair; Argentina), Feng-Yan Bai (China), Javier Carvajal Barriga (Ecuador), Florian Franz Bauer (S. Africa), Peter Biely (Slovakia), Teun Boekhout (Netherlands), Kyria Boundy-Mills (USA), Pietro Buzzini (Italy), Heide-Marie Daniel (Belgium), Hüseyin Erten (Turkey), Patrick Fickers (Belgium), Ma Angelica Ganga (Chile), Ramon Gonzalez (Spain), Anne Gschaedler (Mexico), Lene Jespersen (Denmark), Marc-André Lachance (Canada), Leda Mendonça-Hagler (Brazil), Diethard Mattanovich (Austria), Vivien Measday (Canada), John Morrissey (Ireland), Liliana Godoy Olivares (Chile), Steve Oliver (UK), Patrícia Lappe Oliveras (Mexico), José Paulo Sampaio (Portugal), Rosane Freitas Schwan (Brazil), Matthias Sipiczk (Hungary), Masako Takashima (Japan), Johan Thevelein (Belgium), Graeme M. Walker (UK), Teresa Zoladek (Poland).

Apologies (36): Tiina Alamäe, Monique Bolotin-Fukuhara, Charoen Charoenchai, Ee-Sin Chen, Sylvie Dequin, Jean-Marie Francois, Brigitte Gasser, Lisa Granchi, Ivan Hapala, Matti Korhola, Thomas W. Jeffries, Vladimir Jiranek, Hyun Ah Kang, Yona Kassir, Huiqiang Lou, Anna Maraz, Sally Ann Meyer, Vladimir Mrša, Elena Naumova, Volkmar Passoth, Merja Penttilä, Gábor Péter, Rajendra Prasad, Isak S. Pretorius, Bernard Prior, Jack T. Pronk, Alexander Rapoport, Peter Raspor, Doris Rauhut, Patrizia Romano, Mathabatha Evodia Setati, Andriy Sibirny, Nitnipa Soontorngun, Jørgen Stenderup, Hana Sychrova, Ida J. van der Klei.

Meeting Agenda

Chair's Opening Remarks: Dr. Hiroshi Takagi welcomed the 35 delegates to the meeting. He thanked Dr. Diego Libkind and the Organizing Committees for the excellent job with ISSY34. He also presented the meeting agenda and confirmed the minutes of the previous meeting held in Rzeszów, Poland (May 17, 2018).

Tribute to Drs. Cletus P. Kurtzman, Gennadi Ivanovich Naumov and Inna Pavlovna Babjeva: Dr. Takagi recalled the sad news that our honorable Commissioners, Dr. Cletus P. Kurtzman (USA), Dr. Gennadi Ivanovich Naumov (Russia) and Dr. Inna Pavlovna Babjeva (Russia) passed away on November 27, 2017, May 6 and June 18, 2018, respectively. They were very active members of ICY and solid and reliable persons as excellent yeast scientists, particularly in the field of "yeast taxonomy" and "zymology". Commissioners mourned their death by holding one minute of silence. As sincerest condolences, Commissioners will dedicate memorial

addresses to honor their contribution to the community. Dr. Libkind kindly arranged a workshop on Taxonomy and Systematics of Yeasts for Dr. Kurtzman organized by Dr. Teun Boekhout and Dr. Marizeth Groenewald on the first day of ISSY34. Some memorial lectures for the two Russians colleagues, Dr. Naumov and Dr. Babjeva, will be included in the program of ISSY35 or ICY15 organized by Dr. Hüseyin Erten and Dr. Diethard Mattanovich, respectively.

Memorial for Other Yeast Colleagues: Two other distinguished yeast scientists, Dr. André Goffeau and Dr. Takashi Nakase, who were not ICY Commissioners, also passed away April 2 and July 24, 2018, respectively. Dr. Goffeau was considered the father of yeast genome research in Europe and served as an advisor to the Cornucopia seven-country EU project. Dr. Nakase was a respected Japanese taxonomist and was former Director of the Japan Collection of Microorganisms (JCM). Based on suggestions by Dr. Charles Abbas and Dr. Peter Raspor, Dr. Takagi proposed to honor our past colleagues by creating a special site at our official webpage that includes memorial items for each person, such as their CV, an overview of their research achievements, contributions to the yeast community, education, and pictures. Dr. Takagi will make a list of our colleagues who have recently passed away, such as Drs. Jure Piškur, Graham Fleet, Kurtzman, Naumov, and Babjeva, and will ask appropriate persons for individual colleagues to collect information and build the contents. Finally, he will ask Dr. Rob Samson, the secretary-general of IUMS, to upload the contents possibly by the end of this year.

New Commissioners: Dr. Takagi introduced three candidates for ICY membership. Dr. Andrey Yurkov is Curator for Fungi and Yeasts, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, and is an expert on taxonomy and ecology of yeasts, nominated by Dr. Eckhard Boles. Dr. Kenneth H. Wolfe is Professor of Genomic Evolution, University College Dublin, Dublin, Ireland, and is an expert on the evolution of eukaryotic genome organization, particularly in yeast species, nominated by Dr. John Morrissey. Dr. Rimantas Daugelavičius is Professor and the Head Biochemistry Department, Vytautas Magnus University, Kaunas, Lithuania, and is an expert on membrane transport phenomena in microorganisms, including uptake and excretion of metabolites, DNA and antibiotics in yeast cell, nominated by Dr. Andriy Sibirny. Each candidate provided his CV with a list of publications and two Letters of Recommendation from relevant National or International Societies and current members of ICY. These documents were spread electronically among Commissioners, who could express their view on the candidates. Drs. Yurkov and Wolfe got full support from 33 Commissioners with very few negative opinions. Commissioners unanimously elected Drs. Yurkov and Wolfe as new ICY members. Regarding Dr. Daugelavičius, Commissioners agreed to examine carefully his nomination in the light of his CV and letters of recommendation and to vote on a final decision before the ICY Commissioners' meeting held in Turkey next year.

Updates on Future ISSY/ICY Meetings: Each organizer provided a progress report on meeting preparation. Dr. Hüseyin Erten presented the update for ISSY35 that will be held in Turkey (October 21-25, 2019). Dr. Erten was requested to consider new speakers in addition to the confirmed speakers, who are the ICY Commissioners. Dr. Diethard Mattanovich also presented the update for ICY15 that will be held in Austria (August 23-27, 2020).

Suggestions for Future ISSY: Dr. Vivien Measday, who was recently approved as a new Canadian Commissioner, officially proposed to organize ISSY36, July 5-8, 2021, at the University of British Columbia, Vancouver, Canada. Commissioners accepted her proposal. Dr. Measday will start the preparation of ISSY36 including a proposed subtitle with the local/domestic/international organizing committee. For ISSY37 (2022), the candidate venue is Australia. Dr. Vladimir Jiranek and Dr. Sakkie Pretorius have started drafting a proposal for ISSY37 to be held in Adelaide, Australia, which last hosted ISY9 in 1996, organized by Dr. Graham Fleet. Dr. Takagi asked Dr. Jiranek to officially propose ISSY37 at the ICY Commissioners' meeting to be held in Turkey next year.

Miscellaneous information: Dr. Takagi and Dr. Pietro Buzzini introduced a recent e-mail and letter, respectively, from Dr. Patrizia Romano (Italy). Dr. Romano informed ICY Commissioners that the book "Yeasts in the production of wine", which is co-edited by Dr. Romano and Dr. Graham Fleet, is in the final phase of collecting the chapters from the different authors. The book will be dedicated to Dr. Fleet. Also, Dr. Teun Boekhaut shortly updated his "The Yeasts" project, which will become an open access, electronic and updating system for yeast biodiversity and function. Dr. Boekhaut introduced "The Yeasts

Foundation" <http://www.theyeasts.org/> founded on March 7, 2018, by three founders (Dr. Boekhout *et al*.), including aims, editors, expected releases, funding and benefits of sponsorship. Dr. Boekhaut asked the ICY Commissioners to suggest companies that potentially might be willing to sponsor his project. This foundation is endorsed by ICY and IUMS.

Boot of ICY Website: To share the information or to follow the past and upcoming meetings between us, Dr. Takagi has just started the website of ICY with the aid of Dr. Rob Samson, the secretary-general of IUMS, who is kindly assisting without any charges. The website in the IUMS webpage as follows:

https://www.iums.org/index.php/87-icy/138-international-commission-on-yeasts

Minutes presented by:
Dr. Hiroshi Takagi
ICY Chair

Dr. Charles Abbas
ICY Vice-Chair

Dr. Takagi is asking the past/future Chairs of ISSY/ICY to collect available "documents, program, PowerPoint presentation, resources, data and literature" to be uploaded. Also, regarding the "Yeast Newsletter", Dr. Marc André Lachance, who edits and manages the YNL, accepted to link its website <https://www.uwo.ca/biology/YeastNewsletter/Index.html> that includes the back issues of Yeast Newsletter to the ICY website.

Chair Closing Remarks: On behalf of ICY, Dr. Hiroshi Takagi expressed gratitude once again to Dr. Diego Libkind and his staff for an excellent meeting, with well-balanced and well-organized scientific and cultural programs. The ICY meeting was adjourned.

45th Annual Conference on Yeasts (45th ACY)

The 45th Annual Conference on Yeasts (45th ACY) was held 15-18 May 2018 at Smolenice Castle, Slovakia. Detailed information is available at http://yeastconference.sk/archive.

Forthcoming Meetings

46th Annual Conference on Yeasts (ACY)

The 46th Annual Conference on Yeasts (46th ACY) is still being planned for 7 - 10 May 2019 at Smolenice Castle, Slovakia. On-line registration will be opened on http://yeastconference.sk/ in December.

ISSY 35 – The 35th International Specialised Symposium on Yeasts
21-25 October 2019 - Amara Premier Palace, Antalya, Turkey

On behalf of the Organising Committee, I cordially invite you to attend the 35th International Specialized Symposium on Yeast (ISSY 35) that will be held in Antalya, Turkey on 21-25 of October 2019 at the Amara Premier Palace Hotel under the auspices of the International Commission on Yeasts (ICY) and Çukurova University (Turkey). The theme of the symposium is "Yeast Cornucopia: Yeast for Health and Wellbeing". The theme will encompass the use of yeasts in fermented foods and beverages, the role of spoilage yeasts and their control, yeasts as sources of ingredients and additives, yeasts as biocontrol agents, yeast taxonomy, ecology and biodiversity, yeasts in health and probiotics, and yeast genetics and genomics.

Antalya is a holiday paradise that is located on the south-west coast of Turkey, between the Taurus Mountains and the Mediterranean Sea. Today, Antalya is a world tourism centre, with over 25 million visitors to the city in 2018. The city has many historical sites and is surrounded by natural beauty with many luxury hotels along beaches that allow for relaxation and sun bathing.

Important dates

July 1st, 2019 Abstract Submission Deadline
July 1st, 2019 Early-bird Registration Deadline
October 15th, 2019 Full-Registration Deadline

Please check our website for detailed information: http://www.issy35.com/

Brief News Items

FEW Award of Excellence to Graeme Walker

Professor Graeme Walker, from the School of Science Engineering and Technology, at Abertay University (Dundee, Scotland) has been recognised by the Fuel Ethanol Workshop (FEW), with a FEW Award of Excellence at a ceremony in Omaha, Nebraska, USA on June 12. This Award recognizes the significant contributions an individual has made to the fuel ethanol industry through research, technical advisory or development activities. Walker was presented the award in recognition of his more than 40 years of work in the areas of yeast and fermentation.

Throughout his career, Walker has held academic positions and conducted research in the U.K., Denmark, New Zealand, Ireland, Canada and the U.S. He is currently a professor of zymology and director of the Yeast Research Group at Abertay University in Scotland. "The broad area of my research can be described as yeast physiology and biotechnology," Walker said. "This encompasses understanding more about yeast nutrition, growth, metabolism and stress responses in industrial fermentations. For bioethanol-related work, I have conducted research on bioconversion of diverse feedstocks to ethanol, including cheese whey, brewers/distillers spent grains, sorghum bagasse and macroalgae (seaweeds). For lignocellulose-derived ethanol we have also evaluated the potential of using ultrasound (high frequency sound waves) to assist the hydrolysis of feedstocks to fermentable sugars. Other aspects of my research over the years has involved yeast nutrition for the optimization of alcohol fermentations. This focus has been on minerals such as Mg, Ca and Zn which are often overlooked by industrial alcohol producers."

Walker notes his research has shown that the availability of key nutrients, such as minerals in fermentation media, plays and important role in dictating yeast fermentation performance. "For example, the levels of magnesium and zinc are especially important in optimizing alcohol production and in alleviating stress on yeast caused by high alcohol levels," he said. "Calcium levels are also important as high Ca levels can suppress yeast activity and alcohol fermentation, especially in molasses, and can additionally cause downstream processing difficulties (scaling on distillation systems)."

According to Walker, the efficient utilization of sustainable feedstocks for alcohol production is an area of continued research interest for him, particularly exploitation of residues from agricultural practices and food production. "In short, we still have a lot to learn about yeast growth, physiology and metabolism in fermentations for biofuel and beverage alcohol production," he said. When asked about the future of yeast development for the biofuels industry, Walker said "the really exciting areas for development currently lie in yeast strain engineering. For example, gene editing and synthetic biology techniques have potential to revolutionize yeast-based biotechnologies in the future." In his accepting his award at the FEW conference, Walker expressed appreciation and discussed Scotland's history in alcohol production. "It's a fantastic award and I'm extremely honored to accept this accolade," he said. "You might be asking yourself why is a Scotsman up here accepting this award? In fact, Scotland doesn't have a particularly active bioethanol industry but we have been making ethanol for over 500 years, the problem is we bottle it," Graeme said, creating a laugh throughout the crowd.

"I'd like to dedicate this to a lifelong friend and mentor," he continued. "In fact, you guys wouldn't be here if it wasn't for this particular individual. His name is *Saccharomyces cerevisiae*."

Lallemand Inc.'s Alcohol School for Plant Managers and Operators
W. M. Ingledew (BSc, PhD, UBC; Professor Emeritus, U. of Saskatchewan).

I have now retired from Ethanol Technology Institute's Alcohol School, a Lallemand subsidiary that educates employees of the fuel and beverage alcohol industries. I was involved with the this School for 33 years - first at Alltech Inc., and after University retirement in 2006, I served for six years as Scientific Director of Ethanol Technology Institute - in charge of the speakers and scientific content of the Alcohol Schools in Toulouse and Montreal - retiring from that position in 2011, but continuing to lecture there until this year. While at the University of Saskatchewan (1970-2006), my group published 175 refereed research papers/book chapters and presented over 276 presentations at scientific meetings and conferences around the world - the majority on alcohol production by yeast and microbiological problems in the brewing, winery and fuel alcohol industries. I had extensive industrial liaison and consulting experience, and trained more than 2,400 young industry employees at 42 industry short courses - including the years at Alcohol Schools in Lexington, Omaha, Montreal, and Toulouse. My group's specialization was very high gravity (VHG) fermentation - increasing fermentation rates and alcohol contents to as high as 23.8% v/v in fermentors - as well as determining the benefits of yeast foods, reducing yeast stresses caused by bacteria, wild yeasts (or their end products) and temperature - all benefiting industrial alcohol manufacture as employed in industry since 2000. I received the Alltech Medal of Excellence as well as the FEW Award of Excellence for 'outstanding research and technological advances' in industry, as well as an earned D.Sc. (2009) from the University of Saskatchewan based on a career in yeast research and development. I am an Honorary Life Member of the American Society of Brewing Chemists and was Editor of The American Society of Brewing Chemists Journal for 5 years. I reviewed more than 100 research papers submitted to a multitude of microbiology journals, and was the Senior Editor of The Alcohol Textbook 5th Edition (2009), and Co-editor of the 6th Alcohol Textbook (2017). I continue to reside in Parksville, B.C. and can be reached at:
mike.ingledew@usask.ca

Postdoctoral fellowship in Microbial Genomics
Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa

The Yeast Genomics Lab @ NOVA is seeking candidates for a postdoctoral fellow position. We use yeasts to investigate basic and applied aspects of microbe evolutionary biology and our strategies combine computational and experimental approaches. More specifically, we aim at integrating genomics with evolutionary genetics, ecology, microbial diversity and physiology. The candidate will be involved in a recently funded project entitled “Advancing wine yeast genomics –exploring the evolutionary dimensions of domestication and the emergence of virulence”. This project is focused on the domestication of the yeast *Saccharomyces cerevisiae* and will foster various genomic and laboratory analyses. Both novel strains and genome sequences will be obtained and they will be investigated together with a collection of strains / genomes already available in the lab and genomes retrieved from public databases. The main goals of the study concern understanding specific details of wine yeast domestication, like the occurrence of regional variants, and the fate of domesticated lineages. For this second topic, the occurrence of additional rounds of domestication (secondary domestication) and the emergence of pathogenic strains within the pool of domesticated lineages will be tested computationally and experimentally. Candidates should have a clear interest in genomics combined with interests in evolution and microbial ecology and physiology. While there are no strict requirements for the position, strong quantitative skills, programming experience, and a population or evolutionary genetics background are desirable. Contact:

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Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

January 1969 Volume XVII, Number 2

Mr. **D. Yarrow** of CBS communicated receipt of type strains of new species *Debaryomyces halotolerans, D. nepalensis, Pichia krusei, Rhodotorula vuilleminii,* and *Saccharomyces hispanica*. The 1968 CBS list of cultures was printed.

Three publications by Dr. **Shoji Goto** of Yamanishi University, Kofu, Japan described physiology and taxonomy of yeasts and molds isolated from animal dung and other habitats in upland Himalayan districts. Several new species included *Sporobolomyces coprophilus*.

Dr. **T. Nakase** of Ajinomoto Co., Inc., Kawasaki, Japan published two manuscripts on the taxonomic significance of the DNA base composition of yeast.

Dr. **D. G. Ahearn** of Georgia State College, Atlanta, Georgia, USA published papers on the ecology of yeasts from aquatic regions of South Florida, on the shape of ascospores of *Hanseniaspora uvarum*, and extracellular proteinases. Ahearn, S.P. Meyers, W.L. Cook, and Gayle Hansen studied the aquatic yeast flora of Lake Champlain at depths up to 115 meters.

Dr. **Leo Kaufman** of the National Communicable Disease Center, Atlanta, Georgia, published a paper in Applied Microbiology in which he and S. Blumer evaluated a number of serological tests for diagnosis of cryptococcosis. Maximal early and accurate diagnosis was accomplished using three tests: the latex agglutination (LA) test for the cryptococcal antigen, the indirect fluorescent antibody (IFA) test, and the tube agglutination (TA) procedure for *C. neoformans* antibodies. Analysis of cerebrospinal fluid vs. serum, possible cross-reactions, and quantification were briefly discussed.

The 1968 O.I.V. Prize in Enology was awarded to **E. Minárik** for the publication "Ecology of Natural wine Yeasts Species in Czechoslovakia". Publications included ecology of yeasts in the Tokay wine region. Visible islets of yeast were very common on cellar walls, floors and equipment but rare in the vineyard.

Two publications by Prof. **O. Verona** described the gastro-enteric yeast microflora of the wild edible dormouse and the snail.

Two studies were carried out in the laboratory of Dr. **M. Kozaki**. The first described yeasts isolated from swelled canned apple, which were exclusively *Saccharomyces italicus.* In the second, yeast flora of "Takuan-zuke", salted preserved radish prepared by two methods were examined. When packed in salted rice bran, *Debaryomyces* species were detected. When packed with dry salt, *Debaryomcyes, Hansenula* and *Saccharomyces* were isolated.

Dr. **Edward J. Buecher**, Jr. of the Clinical Pharmacology Research Institute, Berkeley, California, USA (a graduate of Herman Phaff's lab) compared the effects of extracts of *Saccharomycopsis guttulata* and Fleishmann's baker's yeast on growth of *Caenorhabditis briggsae*, in an effort to find alternatives to chick embryo or liver extracts. High populations of nematodes were obtained with a dialyzed baker's yeast extract.

Dr. **Samuel P. Meyers**, formerly of the Institute of Marine Sciences, University of Miami, became a professor in the Department of Food Science and Technology, Louisiana State University to develop a program on marine microbiological food research and sea grant estuarine programs. He shared the abstract of a publication on extracellular yeast proteinases, in which the proteolytic activity of 800 isolates of 70 species were compared.

Work in the lab of Dr. **Richard Snow** concerned regulation of acid phosphatase, fine structure and complementation patterns at the histidine-1 locus, and radiation-sensitive mutants.

Mr. P**. V. Patel** in the lab of Dr. **J. R. Johnston**, University of Strathclyde, Royal College, Glasgow, Scotland presented a paper at a symposium of the Botanical Society of Edinburgh about the genetics of nystatin resistance in yeast.

Prof. **N. Yanagishima** of Osaka City University, Japan studied control of growth of *S. cerevisiae* by the hormones auxin and gibberellin. Eleven publications described effects on yeast sporulation, effects of auxin on plant growth regulators, nucleic acid metabolism, heritable variants in yeast, and cell expanding effects.

Dr. **A. Sols** published papers describing the utilization of glycerol by *Candida utilis*, glycerol substrate specificity patterns of *C. mycoderma*, and the glycerol synthesis pathway of *S. cerevisiae*. They also developed a method to estimate penetration of non-metabolizable compounds into yeast protoplasts, to enable studies of hexose transport.

Dr. **Heikki Suomalainen** of the State Alcohol Monopoly of Helsinki, Finland shared summaries of eight publications related to alcohol production by yeast. Subjets included permeability characteristics of yeast, properties of the plasma membrane of dried yeast cells, locations of enzymes in the cell, the aroma composition and volatile fatty acids in distilled alcoholic beverages and beer, and morphology and nucleic acid content of baker's yeast.

Dr. **Pamela A. D. Rickard** of the University of New South Wales, Australia communicated the summary and conclusions of a submitted paper on the response of *Candida utilis* to controlled concentrations of oxygen and glucose. After a 10-hour lag, there was an inverse relationship between dissolved oxygen and cytochrome, and between glucose concentration and cytochrome.

Dr. **B. C. Rankine** of the Australian Wine Research Institute published a review on the importance of yeasts on composition and quality of wine. The death of AWRI Foundation Director J. C. M. Fornachon was also reported.

Work on effects on foam stability and maltotriose fermentation by brewing strains of *S. cerevisiae* was reported by Dr. **A. H. Cook**, Brewing Industry Research Foundation, Surrey, England.

Prof. Dr. **S. Windisch** listed three diversecpublications, covering use of Bronn's impinge to sample microbes in air, the need for collections of microorganisms, and breeding auxotrophic strains of *Saccharomyces*.

Dr. **A. Kockova-Kratochvilova** reported on the First International Conference on Culture Collections held in Tokyo October 7-12, 1968, which drew more than 332 participants from 27 countries to see 84 lectures. A committee was appointed to draw up a draft constitution an International Federation for Culture Collections.

Fifty scientists attended the Second International Symposium on Yeast Protoplasts, held August 1968 in Brno, Czechoslovakia.

Prof. **J. Santa Maria** published the description of *Saccharomyces hispanica,* a new "flor" yeast species.

Dr. **J. K. Bhattacharjee** listed several articles on lysine biosynthesis in yeast.

L. R. Hedrick published descriptions of *Trichosporon aquatile* and *T. oriense*, and an article on utilization of L-amino acids by *Hansenula* and *Trichosporon.*

Charles M. Bump of the New England Medical Center Hospitals, Boston, Massachusetts, USA published a paper on the routine identification of yeasts using molybdate-agar medium.

Dr. **H. J. Phaff**, department of Food Science and Technology, University of California Davis listed three publications on exo-beta-glucanases in yeast, DNA base composition in yeast, and sporulation in *Candida* and *Metschnikowia* yeasts. Dr. **A. Martini** of Perugia, Italy joined the lab to study DNA homologies as a taxonomic aid under a NATO fellowship. Drs. Miller, Phaff, Yoneyama and Soneda collected 700 yeasts from Alaska to California.

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