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### Editorials



#### Cletus P. Kurtzman (1938-2017)

The yeast researcher community is in shock with the loss our friend and colleague, Clete Kurtzman, from a heart attack, on November 27 2017. He is mourned by his wife, Mary Ann, three children, five grandchildren, and many others. Several obituaries are in preparation by various authors who were close to Clete. I hope to reproduce some of them in the Spring 2018 issue. At this time, it is appropriate to mention that his death provoked a flurry of reactions among members of the International Commission on Yeasts. All deplored the loss of a leading figure in yeast systematics, and all remarked on his quality as a true gentleman and mentor. He will be missed by all.

#### **ISSY 32 - Cork, Ireland**

Congratulations to John Morrissey and his local organization team for an excellent meeting at University College, Cork, in beautiful Ireland. I was impressed with the high degree of student participation and general involvement in the scientific dialogue. A superb concert of traditional Irish music was greatly appreciated by a lively crowd, as was an excellent banquet in a countryside venue.

#### June Issue of the Yeast Newsletter

It would appear that the low rate of response for communications to the June 2017 issue of the Yeast Newsletter was owed to a problem with my email software. I shall ensure that this does not happen again and I thank our readers for their patience with this matter.

I wish our readers an excellent, happy New Year!

MA Lachance, Editor

## I State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIgenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <<u>gnaumov@vahoo.com</u>>.

The following are papers for 2017 or in press.

- 1 Naumov GI, Naumova ES, Lee Ch-Fu. 2017. *Ogataea haglerorum* sp. nov., a novel member of the species complex, *Ogataea* (*Hansenula*) polymorpha. Int J Syst Evol Microbiol 67(7):2465–2469.
- 2 Naumova ES, Sadykova AZh, Martynenko NN, Naumov GI. 2017. Hybrid selection of Saccharomyces cerevisiae yeasts for thermotolerance and fermentation activity. Microbiology (Moscow) 87(2).

To find thermotolerant *S. cerevisiae* strains, we have conducted molecular-genetic screening of *Saccharomyces* yeasts, isolated from natural sources in different regions of the world with a hot climate: Africa, South America, Southeast and Central Asia. Based on physiological tests, four strains were selected that can grow at high temperatures ( $42^{\circ}$ C and  $43^{\circ}$ C) and have good fermentation activity: 7962-4B, 3529-7B, 52922-4-1-1A-1C and 87-2421.1-2A. Hybrids of monosporic culture of distiller's race XII (XII<sub>7</sub>-2) with

the thermotolerant strains were created. Unlike the strain XII<sub>7</sub>-2 unable to grow at above 39°C, all hybrids showed good growth at 42°C. Of the six hybrids analyzed two H2-1 (87-2421.1-2A x XII<sub>7</sub>-2) and H3-2 (7962-4B x XII<sub>7</sub>-2) showed higher fermentation activity than the parental strains. According to the results obtained, inter-strain hybridization is an effective method of creating *S. cerevisiae* strains, combining thermotolerance and high efficiency of alcoholic fermentation.

3 Shalamitskyi MYu, Naumov GI. 2017. Phylogenetic analysis of pectinases of ascomycetous yeasts. Biotekhnologiya (Moscow) 6 (in press).

A screening of PGU genes of ascomycetous yeasts deposited in the GenBank (http: //www.ncbi.nlm.nih. Gov/genbank/) and Sanger Institute databases (http://www.sanger.ac.uk) has been performed using the nucleotide sequence of the PGU1 gene of *S. cerevisiae* S288c as a query. Divergent pectinase genes were found out in the *Eremothecium*, *Galactomyces*, *Geotrichum*, *Kluyveromyces* and *Lachancea* genera. Within the genera, the following

similarity of the *PGU* nucleotide sequences was observed: 64.5–98.2% for *Kluyveromyces*, 72.7–81.3% for *Galactomyces* /*Geotrichum*, and 69–87.9% for *Eremothecium*. Polymeric *PGU* genes capable of interspecies transferring were documented in *Galactomyces citri-aurantii*, *Galactomyces candidus* and *Geotrichum klebahnii*. The importance of the PGU genes for the diagnosis and selection of yeasts is discussed.

4 Naumov GI, Naumova ES. 2017. *Saccharomyces bayanus* Saccardo is the intraspecies hybrid in accordance with genetic hybridization analysis. 33rd International Specialised Symposium on Yeasts Exploring and Engineering Yeasts for Industrial Application, 26–9 June 2017, Cork, Ireland, p.7.

Different word laboratories are currently involved in large-scale project on origin of European larger yeasts Saccharomyces pastorianus Hansen (syn. S. carlsbergensis Hansen). According to numerous molecular data, S. pastorianus is an allopolyploid containing genomes of the traditional yeast S. cerevisiae Meyen ex Hansen and recently discovered S. eubavanus Sampaio et al. In nature, S. eubavanus is documented in Argentina, North America, and China, however, the yeast has not been found in Europe yet. Great attention is also paid to the origin of recombinant yeasts S. bayanus var. bayanus isolated from human fermentations. They contain partial genomes of S. eubavanus and S. bavanus var. uvarum (Hansen) G. Naumov, and some S. cerevisiae subtelomeric sequences. The yeast S. bayanus var.

*uvarum* is associated with certain types of wines: Val de Loire's white, Sauternes, Alsatian and Cider (France), Tokaj (Hungary, Slovakia), Amarone (Italy), Txakoli (North Spain) and others. In order to assess the degree of genetic relatedness of S. eubayanus, S. bayanus var. bayanus and S. bayanus var. uvarum, we have conducted genetic hybridization analysis of these taxa. Based on ascospore viability and meiotic recombination of control parental markers of the hybrids, we have found that there is no complete interspecies post-zygotic isolation between the yeasts S. eubayanus, S. bayanus var. bayanus and S. bayanus var. uvarum. The genetic data obtained prove the belonging of the all three taxa to the same species. Taxonomic status of the yeast S. eubayanus is discussed.

II. 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 Paulovičová E, Paulovičová L, Hrubiško M, Krylov VB, Argunov DA & Nifantiev N E. 2017. Immunobiological activity of synthetically prepared immunodominant galactomannosides structurally mimicking *Aspergillus* galactomannan. Frontiers in Immunology 8 - Article 1273

The study is oriented at the in vitro evaluation of the immunobiological activity and efficacy of synthetically prepared isomeric pentasaccharides representing fragments of Aspergillus fumigatus cellwall galactomannan and containing  $\beta$ -(1 $\rightarrow$ 5)-linked tetra-galactofuranoside chain attached to O-6 (gM-1) or O-3 (gM-2) of a spacer-armed mannopyranoside residue. These compounds were studied as biotinylated conjugates which both demonstrated immunomodulatory activities on the RAW 264.7 cell line murine macrophages as in vitro innate immunity cell model. Immunobiological studies revealed time- and concentration-dependent efficient immunomodulation. The prolifer-ation of RAW 264.7 macrophages was induced at higher concentration (100 µg/mL) of studied glycoconjugates and longer exposure (48 h), with more pronounced efficacy for gM-1. The increase

of proliferation followed the previous increase of IL-2 production. The cytokine profile of the macrophages treated with the glycoconjugates was pre-dominantly pro-inflammatory Th1 type with significant increase of TNF $\alpha$ , IL-6, and IL-12 release for both glycoconjugates. The RAW 264.7 macrophages production of free radicals was not significantly affected by glycoconjugates stimulation. The phagocytic activity of RAW 264.7 cells was reduced following gM-1 treatment and was signif-icantly increased after 24 h stimulation with gM-2, contrary to 48 h stimulation. Moreover, the synthetically prepared galactomannoside derivatives have been evaluated as efficient serodiagnostic antigens recognized by specific Ig isotypes, and significant presence of specific IgM antibodies in serum of patients suffering from vulvovaginitis was observed.

2 R Vadkertiová, H Dudášová, M Balaščáková. 2017. Chapter 4. Yeasts in Agricultural and Managed Soils pp. 117-144. In: Yeasts in Natural Ecosystems: Diversity. Buzzini P, Lachance M.-A, Yurkov A. (Eds.) DOI 10.1007/978-3-319-62683-3\_4

All managed soils (agricultural soil, orchard soil, vineyard soil, pasture soil) are exposed to human intervention. These include regular tillage, crop or plant seeding, harvesting and the application of fertilizers, herbicides and pesticides. Yeasts are present in all types of managed soil; some of them are restricted to an individual season, soil horizon or locality, while others are present at all times in all soils. The abundance of yeasts depends on the availability of water, the type of soil and plant diversity. The composition and quantity of soil yeast communities are influenced by the yeasts originating from aerial parts of plants, which enter the soil during tillage or with decaying plant material. The size of the yeast population ranges from a few to several thousands of CFU per gram of soil. The diversity of ascomycetous yeasts present in agricultural soil includes fermentative species (e.g. Candida spp.,

Metschnikowia sp.), soil-related yeasts (e.g. Cyberlindnera saturnus and Lipomyces sp.), black yeasts (Exophiala sp., Aureobasidium sp.) and basidiomycetous yeasts, mainly species previously classified in the genera Cryptococcus and Trichosporon. Vineyard soils are inhabited by basidiomycetous yeasts (mainly Naganishia spp., Sollicoccozyma spp., Filobasidium sp.) and also by grape yeasts including Aureobasidium pullulans, Metschnikowia sp. and Hanseniaspora uvarum. In orchard soils, fruit-related yeasts H'spora uvarum and Metschnikowia pulcherrima are associated with the upper layer of soil. Species previously classified in the genera Cryptococcus and Trichosporon dominate the soil of citrus orchards. Grassland soils are mainly occupied by soil-related ascomycetous species Schwanniomyces capriotti, Barnettozyma vustinii and Cyberlindnera suaveolens.

#### III Department of Agricultural, Food and Environment al Sciences, Industrial Yeasts Collection DBVPG - www.dbvpg.unipg.it - Borgo XX Giugno 74, 06121, University of Perugia, Italy. Communicated by Pietro Buzzini pietro.buzzini@unipg.it>.

Recent publications.

1 Filippucci S, Tasselli G, Scardua A, Di Mauro S, Cramarossa MR, Perini D, Turchetti B, Onofri A, Forti L, Buzzini P. 2016. Study of *Holtermanniella wattica*, *Leucosporidium creatinivorum*, *Naganishia adeliensis*, *Solicoccozyma aeria* and *Solicoccozyma terricola* for their lipogenic aptitude from different carbon sources. 2016, Biotechnol. Biofuels 9:259.

Holtermanniella wattica, Leucosporidium creatinivorum, Naganishia adeliensis, Solicoccozyma aeria, and Solicoccozyma terricola strains were selected as a result of a large-scale screening on 706 yeasts (both Ascomycota and Basidiomycota). Lipid yields and fatty acid profiles of selected strains were evaluated at 20 and 25 °C on glucose, and on glycerol, xylose, galactose, sucrose, maltose, and cellobiose. A variable fatty acid profile was observed in dependence of both temperature and different carbon sources. On the whole, L. creatinivorum exhibited the highest performances: total lipid yield (YL) >7 g/l on glucose and glycerol, % of intracellular lipids on cell biomass (YL/DW) >70% at 20°C on glucose, lipid coefficient (YL/Glu) around 20% on glucose, and daily productivity (YL/d) on glucose and sucrose >1.6 g/(l\*d). This study provides some meaningful information about the lipogenic ability of some yeast species. Variable lipid yields and fatty acid profiles were observed in dependence of both temperature and different carbon sources. *L. creatinivorum* exhibited the highest lipogenic performances.

2 Forti L, Di Mauro S, Cramarossa MR, Filippucci S, Turchetti B, Buzzini P. 2015. Non-conventional yeasts whole cells as efficient biocatalysts for the production of flavors and fragrances. Molecules 20, 10377-10398.

The rising consumer requests for natural flavors and fragrances have generated great interest in the aroma industry to seek new methods to obtain fragrance and flavor compounds naturally. An alternative and attractive route for these compounds is based on bio-transformations. In this review, the application of biocatalysis by Non-Conventional Yeasts (NCYs) whole cells for the production of flavor and fragrances is illustrated by a discussion of the production of different class of compounds, namely Aldehydes, Ketones and related compounds, Alcohols, Lactones, Terpenes and Terpenoids, Alkenes, and Phenols.

3 França L, Sannino C, Turchetti B, Buzzini P, Margesin R. 2016. Seasonal and altitudinal changes of culturable bacterial and yeast diversity in Alpine forest soils. Extremophiles 20:855-873.

The effect of altitude and season on abundance and diversity of the culturable heterotrophic bacterial and yeast community was examined at four forest sites in the Italian Alps along an altitude gradient (545–2000 m). Independently of altitude, bacteria isolated at 0 °C (psychrophiles) were less numerous than those recovered at 20 °C. In autumn, psychrophilic bacterial population increased with altitude. The 1194 bacterial strains were primarily affiliated with the classes Alpha-, Beta-, Gammaproteobacteria, Spingobacteriia and Flavobacteriia. Fifty-seven of 112 operational taxonomic units represented potential novel species. Strains isolated at 20 °C had a higher diversity and showed similarities in taxa composition and abundance, regardless of altitude or season, while strains isolated at 0 °C showed differences in community composition at lower and higher altitudes. In contrast to bacteria, yeast diversity was seasondependent: site- and altitude-specific effects on yeast diversity were only detected in spring. Isolation temperature affected the relative proportions of yeast genera. Isolations recovered 719 strains, belonging to the classes Dothideomycetes, Saccharomycetes, Tremellomycetes and Mycrobotryomycetes. The presence of few dominant bacterial OTUs and yeast species indicated a resilient microbial population that is not affected by season or altitude. Soil nutrient contents influenced significantly abundance and diversity of culturable bacteria, but not of culturable yeasts. 4 Mannazzu I, Landolfo S, Lopes da Silva T, Buzzini P. 2015. Red yeasts and carotenoid production: outlining a future for non-conventional yeasts of biotechnological interest. World J. Microbiol. Biotechnol 31:1665-1673.

Carotenoids are one of the most common classes of pigments that occur in nature. Due to their biological properties, they are widely used in phytomedicine and in the chemical, pharmaceutical, cosmetic, food and feed industries. Accordingly, their global market is continuously growing, and it is expected to reach about US\$1.4 billion in 2018. Carotenoids can be easily produced by chemical synthesis, although their biotechnological production is rapidly becoming an appealing alternative to the chemical route, partly due to consumer concerns against synthetic pigments. Among the yeasts, and apart from the pigmented species *Phaffia rhodozyma* (and its teleomorph *Xanthophyllomyces dendrorhous*), a handful of species of the genera *Rhodosporidium*, *Rhodotorula*, *Sporobolomyces* and *Sporidiobolus* are well known carotenoid producers. These are known as 'red yeasts', and their ability to synthesize mixtures of carotenoids from low-cost carbon sources has been broadly studied recently. Here, in agreement with the renewed interest in microbial carotenoids, the recent literature is reviewed regarding the the stress factors that in uence their carotenogenesis, and the most advanced analytical tools for evaluation of carotenoid production. Moreover, a synopsis of the molecular and ''-omic'' tools available for elucidation of the metabolic pathways of the microbial carotenoids is reported.

5 Mokhtarnejad L, Arzanlou M, Babai-Ahari A, Di Mauro S, Onofri A, Buzzini P, Turchetti P. 2016. Characterization of basidiomycetous yeasts in hypersaline soils of the Urmia Lake National Park, Iran. Extremophiles 20:915-928.

Urmia Lake, located in northwest Iran, is an oligotrophic and extremely hypersaline habitat that supports diverse forms of life. Owing to its unique biodiversity and special environmental conditions, Urmia Lake National Park has been designated as one of the biosphere reserves by UNESCO. This study was aimed to characterize basidiomycetous yeasts in hypersaline soils surrounding the Urmia Lake National Park using a polyphasic combination of molecular and physiological data. Soil samples were collected from eight sites in Lake Basin and six islands insides the lake. Yeast strains were identified by sequencing the D1/D2 domains of the 26S rRNA gene. When D1/ D2

domain sequencing did not resolve the identity of the species, strain identification was obtained by ITS 1 & 2 sequencing. Twenty-one species belonging to the genera *Cystobasidium*, *Holtermanniella*, *Naganishia*, *Rhodotorula*, *Saitozyma*, *Solicoccozyma*, *Tausonia*, *Vanrija*, and *Vishniacozyma* were identified. *Solicoccozyma aeria* represented the dominant species. The ability of isolates to grow at 10 and 15 % of NaCl was checked; about two-thirds of the strains grew at 10%, while about 13% of the isolates grew in medium with 15% NaCl. this study is the first study on the culturable yeast diversity in hypersaline soils surrounding an Asian lake.

effects on quality and taste. Dry yeasts are also used in

agricultural animal feed. Another interesting

application of yeast dehydration is as an additional

stage in new methods for the stable immobilisation of

microorganisms, especially in cases when biotechnol-

ogically important strains have no af nity with the

carrier. Such immobilisation methods also provide a

new approach for the successful conservation of yeast

strains that are very sensitive to dehydration. In

addition, the application of dehydration procedures

6 Rapoport A, Turchetti B, Buzzini P. 2016, Application of anhydrobiosis and dehydration of yeasts for non-conventional biotechnological goals. World J. Microbiol. Biotechnol 32:104.

Dehydration of yeast cells causes them to enter a state of anhydrobiosis in which their metabolism is temporarily and reversibly suspended. This unique state among organisms is currently used in the production of active dry yeasts, mainly used in baking and winemaking. In recent decades non-conventional applications of yeast dehydration have been proposed for various modern biotechnologies. This mini-review briefly summarises current information on the application of dry yeasts in traditional and innovative elds. It has been shown that dry yeast preparations can be used for the ef cient protection, puri cation and bioremediation of the environment from heavy metals. The high sorption activity of dehydrated yeasts can be used as an interesting tool in winemaking due to their

opens up new possibilities for the use of yeast as a model system. Separate sections of this review also discuss possible uses of dry yeasts in biocontrol, bioprotection and biotransformations, in analytical methods as well as in some other areas. 7 Baeza M, Alcáino J, Cifuentes V, Turchetti B, Buzzini P. Cold-active enzymes from cold-adapted yeast. 2017. In: Biotechnology of Yeasts and Filamentous Fungi (Sibirny A.A. ed.), Springer, Berlin, pp. 297-324.

Cold-adapted yeasts include psychrophiles or psychrotolerant nonconventional species able to survive and grow at low temperatures. They represent an important source of biological diversity that has developed a set of structural and functional adaptation strategies to overcome the adverse effects of cold (sometimes associated with other limiting conditions). Among them, the production of cold-active enzymes is probably one of the most efficient adaptations of the eukaryotic physiology at low temperatures. Current literature reports that cold-active enzymes exhibit several advantages than their mesophilic and thermophilic homologues and may successfully replace traditional catalysts in a range of industrial applications carried out at low and moderate temperatures. Due to their singular traits, some coldactive hydrolases (i.e., lipases, amylases, and proteases,) isolated from cold-adapted yeasts have been studied since some decades, while some other, namely, xylanases, chitinases, pectinolytic enzymes, glycosidases, phytases, and invertases, have recently attracted a rising attention for their biotechnological potential from the academy and industry for both food and non-food exploitations.

8 Jagielski T, Bakuła Z, Di Mauro S, Casciari C, Cambiotti V, Krukowski H, Turchetti B, Ricchi M, Manuali E, Buzzini P. 2017. Comparative study of the *in vitro* activity of iodopropynyl butylcarbamate (IPBC) and amphotericin B (AMB) against *Prototheca* spp. isolates from European dairy herds. J Dairy Sci 100:7435-7445.

The objective of this study was to assess the in vitro effect of iodopropynyl butylcarbamate (IPBC) and amphotericin B (AMB) on the yeast-like Prototheca zopfii genotype 2 and Prototheca blaschkeae isolates recovered from dairy herds of Belgium, France, Italy, Germany, and Poland. The combination of IPBC with AMB on Prototheca isolates and toxicity of IPBC to the bovine mammary epithelial cells were also evaluated. Minimum inhibitory concentrations (MIC) and minimum algicidal concentrations (MAC) of IPBC and AMB were determined. To determine any synergistic, additive, or antagonistic effect of the combination of IPBC and AMB, 2-dimensional checkerboard combination tests were also performed to calculate fractional inhibitory concentrations. The MIC for 50 and 90% of isolates (MIC50 and MIC90, respectively)

for IPBC were 4 and 8 mg/L vs 0.5 and 1 mg/L for AMB, respectively. The MIC profiles differed between P. zopfii genotype 2 and P. blaschkeae, with the latter species being more susceptible to both compounds. The MIC50 and MIC90 of IPBC were 4 and 8 mg/L for P. zopfii genotype 2 and 1 and 2 mg/L for P. blaschkeae, respectively. The MIC50 and MIC90 of AMB were both 1 mg/L for P. zopfii genotype 2 and 0.25 and 1 mg/L for P. blaschkeae. respectively. Overall, the combined use of IPBC and AMB exhibited an increased algicidal effect, albeit the fractional inhibitory concentration index showed synergistic activity only against 3 P. zopfii genotype 2 isolates. The MTT assay results showed both IPBC and AMB, at the concentrations employed in the study, to be nontoxic to the epithelial mammary gland cells (cell viability >90%).

9 Selbman L, Onofri S, Coleine C, Buzzini P, Canini F, Zucconi L. 2017. Effect of environmental parameters on biodiversity of the fungal component in the lithic Antarctic communities. Extremophiles 21:1069-1080.

A wide sampling of rocks, colonized by microbial epi-endolithic communities, was performed along an altitudinal gradient from sea level to 3600 m asl and sea distance from the coast to 100 km inland along the Victoria Land Coast, Antarctica. Seventy-two rock samples of different typology, representative of the entire survey, were selected and studied using denaturing gradient gel electrophoresis to compare variation in fungal diversity according to environmental conditions along this altitudinal and sea distance transect. Lichenized fungi were largely

biodiversity was heavily influenced even by minimal local variations. The n-MDS analysis showed that altitude and sea distance affect fungal biodiversity, while sandstone allows the communities to maintain high biodiversity indices. The Pareto-Lorenz curves indicate that all the communities analyzed are highly adapted to extreme conditions but scarcely resilient, so any external perturbation may have irreversible effects on these fragile ecosystems.

predominant in all the samples studied and the

10 Tasselli G, Filippucci S, Sannino C, Turchetti B, Buzzini P. 2017. Cold-adapted basidiomycetous yeasts as a source of biochemicals. In: Psychrophiles: from Biodiversity to Biotechnology (Margesin R. ed.) Springer, Berlin, pp. 555-584.

Yeasts play a relevant role as starter cultures in traditional foods and beverages, as well as in innumerable biotechnological applications for obtaining highne biochemicals. Despite a value bulk and considerable number of studies on yeasts have been performed by using almost exclusively the species Saccharomyces cerevisiae (otherwise labelled as baker's yeast), the number of yeast species described so far accounts for more than 1600, belonging to over 130 ascomycetous and basidiomycetous genera. This huge yeast diversity includes many non-Saccharomyces species possessing useful, and sometimes uncommon, metabolic features potentially interesting for both food and non-food industries. Like other organisms, cold-adapted veasts include species able to survive and grow in cold environments. They are usually labelled as psychrophiles or psychrotolerants

11 Klassen R, Schaffrath R, Buzzini P, Ganter PF. 2017. Antagonistic interactions and killer yeasts. In: Buzzini P, Lachance M-A, Yurkov AM (eds) Yeasts in natural ecosystems: ecology. Springer, Heidelberg, pp 229-275.

Antagonistic interactions occur between yeasts and other competing microorganisms. These interactions may rely on non-proteinaceous compounds or proteins called killer toxins. A large variety of structurally and functionally diverse toxins released from killer yeasts are known. In addition to chromosomally encoded toxins, several wellcharacterized toxins are encoded by sel sh extrachromosomal DNA or RNA molecules of viral origin. Despite their structural diversity, only a handful of toxic strategies are utilized by structurally distinct killer toxins, and multistep modes of cell killing involve common steps, such as the binding of different cell wall receptors. In addition, distinct toxin types are known to rely on common mechanisms for maturation, structural stabilization, and release from producer cells. In case of the extrachromosomally encoded toxins, speci c immunity mechanisms are linked to toxin production. In these cases, toxins are assumed to

on the basis of their cardinal growth temperatures. Among them, yeasts belonging to the phylum Basidiomycota apparently exhibit a superior adaptation to cold. This apparent superiority, which could be the result of some metabolic strategies implemented for adapting life to different thermal conditions in order to overcome the adverse effect of cold, can be considered worthwhile for implementing their biotechnological application at low temperatures. Accordingly, cold-adapted basidiomycetous yeasts have attracted considerable attention for their biotechnological potential, because they have developed the ability to synthesize cold-active enzymes, as well as other important biochemicals, namely, cryoprotectant compounds, polymers, lipids, and other miscellaneous compounds.

provide a positive selection mechanism for the genetic system encoding both toxin and immunity. Hence, release of killer toxins might bene t both the toxin producer and the sel sh genetic element in the producer cell. Killer yeasts display broad taxonomic diversity, including basidiomycetes and ascomycetes. Target species may not only include yeasts of both fungal phyla but also other microorganisms such as bacteria or protozoa that may compete in certain natural habitats with the killer yeast. Although killer systems are assumed to be competitive mechanisms, their role in natural yeast communities is not yet well understood. Theoretical approaches have, in general, failed to predict the coexistence of killer, non-killer, and target strains that occurs with regularity in nature. The few empirical studies of natural killer systems have con rmed the ecological importance of killer toxins but have uncovered differences in the exact role the toxins play in yeast ecology.

12 Buzzini P, Turk M, Perini L, Turchetti B, Gunde-Cimerman N. 2017. Yeasts in polar and sub-polar habitats. In: Buzzini P, Lachance M-A, Yurkov AM (eds) Yeasts in natural ecosystems: diversity. Springer, Heidelberg, pp 331-365.

The yeasts that thrive in polar and subpolar areas have to be adapted to extreme environments with low temperatures and the consequential desiccation due to freezing of water into ice crystals, with relatively high

concentrations of ions, generally low levels of nutrients and, sometimes, high UV irradiation and hypoxia. Yeast communities in polar areas include circumpolar, endemic and cosmopolitan species. Although some endemic yeast species show psychrophilic behaviour, the majority of them are psychrotolerant yeasts that can adapt to growth across a wide range of temperatures. Most investigations on yeasts in polar and subpolar areas have remained limited to their biodiversity and the quanti cation of rare or new species. Comparative taxonomic studies of polar and subpolar habitats from Antarctic, Arctic and sub-Arctic have shown that the yeast communities belong prevalently to Basidiomycota, in contrast to the general fungal community distribution, which shows Ascomycota as the dominant phylum. The reviews on yeast diversity in cold habitats worldwide that have been published in recent years have reported the unambiguous prevalence of the former basidiomycetous genera *Cryptococcus* and *Rhodotorula*. But the recent taxonomic revision of the Pucciniomycotina and Tremellomycetes taxa positioned these polyphyletic genera into many new taxa, thus modifying the known taxonomical picture and ecological signi cance of the yeast distribution in polar and subpolar ecosystems. To overcome the problems associated with the quanti cation of unculturable microbial communities, the new high-throughput sequencing of both DNA and RNA is proving to be a valuable tool in deciphering the microbial diversity in cold environments.

13 Sannino C, Tasselli G, Filippucci S, Turchetti B, Buzzini P. 2017. Yeasts in non-polar cold habitats. In: Buzzini P, Lachance M-A, Yurkov AM (eds) Yeasts in natural ecosystems: diversity. Springer, Heidelberg, pp 367-396.

The main terrestrial cold areas (outside Antarctica and the Arctic and subarctic regions, viz., the Earth strip running approximately between 60°N and 60°S) are those covered by glaciers and permafrost soil, primarily con ned in the Himalayas, Andes, and European high mountains and only little portions in other parts of the globe. The study of microbial (prokaryotic and eukaryotic) biological diversity in nonpolar cold habitats represents a contribution to obtain a better de ned picture of fungal diversity (including veasts) inhabiting those ecosystems. The present chapter will provide an overview of both culturable and non-culturable yeast diversity found in nonpolar cold habitats. Yeasts found were identi ed as belonging to a number of ascomycetous and basidiomycetous species: among them, Basidiomycota

dominated yeast diversity; in particular, species of the genera Cysto lobasidium, Dioszegia, Filobasidium, Holtermanniella, Leucosporidium, Mrakia, Naganishia, Rhodotorula, Saitozyma, Solicoccozyma, and Vishniacozvma were the most abundant. A number of new species were also found. Most of the yeast species are apparently ubiquitous in different geographical locations and exhibit some adaptation of their physiology and metabolism that increase cell protection against the damaging effects of low temperatures. Due to those physiological and metabolic adaptations, they could play an ecological role in nonpolar cold ecosystems, especially in relation to the in situ hydrolysis of complex organic macromolecules connected with the mineralization of organic matter.

14 Libkind D, Buzzini P, Turchetti B, Rosa CA. 2017. Yeasts in continental and sea water. In: Buzzini P, Lachance M-A, Yurkov AM (eds) Yeasts in natural ecosystems: diversity. Springer, Heidelberg, pp 1-61.

Even though yeasts are normal inhabitants of almost any type of aquatic environment, in comparison to other type of substrates, relatively little research has been carried out on the factors affecting their biodiversity and distribution patterns. The distinction of a yeast species as transient or resident element of an aquatic habitat has long been challenging and has been one of the main dif culties in the study of yeast diversity in, for example, continental lakes and rivers. The present chapter will provide an overview of our current knowledge on yeast diversity and ecology in continental freshwater and marine environments; in particular habitats like tropical and temperate rivers

and lakes, seawater, and glacial melting water bodies will be reviewed. Water temperature and trophic state are major factors determining the yeast community composition in water bodies, and as they get more extreme due to the increase of stress factors such as cold temperatures, UV radiation, and scarce nutrient availability, the prevalence of basidiomycetous yeast gets more notorious. As a result of the evolutionary adaptation to extreme conditions, certain biotechnologically relevant traits became evident in extremophilic aquatic yeasts such as the production of carotenoid pigments, UV sunscreens, extracellular cold-adapted enzymes, etc.

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Recent publications.

1 Caridi A, Sidari R, Giuffrè AM, Pellicanò TM, Sicari V, Zappia C, Poiana M 2017. Test of four generations of *Saccharomyces cerevisiae* concerning their effect on antioxidant phenolic compounds in wine. European Food Research and Technology 243(7):1287-1294.

The aim of this research was to study the behaviour of 70 different *Saccharomyces cerevisiae* strains on the antioxidant compounds level in wines by RP-HPLC/DAD. Micro-winemaking was carried out in Cabernet Sauvignon grape must testing eight Italian wild strains, 12 derived monosporal cultures, 15 hybrids obtained by monosporal spore-to-spore conjugation, 34 monosporal cultures derived from the hybrids, and Zymaflore F15 as control strain. At the end of the winemaking, the wines show significant differences concerning their antioxidant levels in relation to the strain used. Catechin and epicatechin were the principal antioxidant compounds for all the samples. In particular, the catechin content varied from 0 to 79.53 mg/L, while epicatechin varied from 0 to 70.51 mg/L. The vanillic acid level varied from 3.10 to 12.71 mg/L. Gallic and caffeic acids varied, respectively, from 2.54 to 6.77 mg/L and from 0 to 10.63 mg/L. The rutin and quercetin content varied from 0 to 11.77 mg/L and from 0 to 2.09 mg/L, while trans-resveratrol level varied from 0 to 0.85 mg/L. Data validate the main role that wine yeast selection plays to enhance red wine content in antioxidant phenolic compounds.

2 Caridi A, Panebianco F, Labate MLC, Martorana A - Antifungal activity of lactic acid bacteria to control table olive production. VII International Conference on Environmental, Industrial and Applied Microbiology, BioMicroWorld2017, p.94, Madrid (Spain), 18-20 October 2017.

It is well known that sourdough lactic acid bacteria can produce compounds able to inhibit moulds related to bread spoilage [1]. Consequently, the addition of antifungal sourdough in the bakery industry is now a common practice having the potential to ensure the microbiological safety of bread [2,3]. Moreover, antifungal lactobacilli can be find in raw milk that appears to be a productive reservoir [4]. In table olive fermentation, fungal growth is a big problem and, at present, no biological control is adopted. Natamycin was proposed as a fungal control agent having, also, the potential to enhance the process by favouring the growth of the indigenous population of lactic acid bacteria against other competing microorganisms [5]. It has been reported that table olives act a suitable substrate for the production of citrinin mycotoxin; high hydrostatic pressure effects on citrinin and mould microflora has been evaluated [6]. Considering this state of the art, aim of the present work was to find antifungal strains of lactic acid bacteria among the almost 400 autochthonous strains present in the Collection of the Laboratory of Microbiology in order to control table olive fermentation. The strains were tested in Petri plates against two strains of moulds prevailing in two different olive brines. The strains exhibiting antifungal activity derived from olive brines, cheeses, and sourdoughs. Since each of them was able to inhibit only one of the tested moulds, the next step will be to carry out fermentation trials coupling the best strains as adjunct culture together with starter cultures. This study constitute a step towards the main goal to solve the problem of the fungal growth and the potential mycotoxin accumulation during the table olive fermentation.

This work was supported by *PON 03 PE\_00090\_2 -Modelli sostenibili e nuove tecnologie per la valorizzazione delle olive e dell'olio extra vergine di oliva prodotto in Calabria.* 

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[2] Ryan LAM, Dal Bello F, Arendt EK (2008) The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. Int J Food Microbiol 125:274-278.

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[4] Delavenne E, Mounier J, Déniel F, Barbier G, Le Blay G (2012) Biodiversity of antifungal lactic acid bacteria isolated from raw milk samples from cow, ewe and goat over one-year period. Int J Food Microbiol 155:185-190.

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3 Caridi A, Sidari R, Voce S - Genetic improvement for technological traits of non-*Saccharomyces* wine yeasts. VII International Conference on Environmental, Industrial and Applied Microbiology, BioMicroWorld2017, p.118, Madrid (Spain), 18-20 October 2017.

While Saccharomyces cerevisiae is the main species of wine production, other species have significant roles; interactions between the different species and their impacts on process efficiency and product quality need to be identified and evaluated [1]. At present, there is a growing demand for selected strains of non-Saccharomyces wine yeasts suitable to be used in association with Saccharomyces cerevisiae for grape juice fermentation [2]. Wine yeasts of the genus Hanseniaspora [3] and Schizosaccharomyces [4] perform alcoholic fermentation producing notably amounts of acetic acid and hydrogen sulphite and - for these attributes - usually they are not easily suitable in winemaking. Since they are ascosporogenous yeasts [5], as for Saccharomyces they could be processed via classical methods, such as micromanipulation, in order to improve their technological characteristics. The aim of this work was to consider the implications of a new technique - easy, cheap and fast - able to improve by genetic way technological traits in non-Saccharomyces ascospore-producing wine yeasts. The new technique essentially consists in: 1) inducing the production of ascospore using acetate agar. Gorodkowa agar, and Sabouraud agar; 2) inducing the lysis of the asci using zymolyase; 3) plating the serial dilutions in nutrient medium; 4) based on the morphological characteristics of colony and cell, collecting the descendants; 5) testing hydrogen sulphite and acetic acid production on BiGGY agar and Chalk agar; 6) selecting the best strains. A total of 12 strains of Schizosaccharomyces spp. and 35 strains of Hanseniaspora spp. were tested in order to identify strains able to produce enough

amount of ascospores. Then, they were processed using both the new technique and the micromanipulation technique. In details, using the new technique a total of 35 descendants of *Schizosaccharomyces* and 29 descendants of *Hanseniaspora* were collected. An interesting biodiversity was observed both on BiGGY agar and Chalk agar, so validating the effectiveness of the proposed technique.

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[4] Suárez-Lepe JA, Palomero F, Benito S, Calderón F, Morata A (2012) Oenological versatility of *Schizosaccharomyces* spp. Eur Food Res Technol 235:375-383.

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4 Sidari R, Martorana A, Caridi A. 2017. Effect of pH and salinity on biofilm-like phenotypes of yeasts isolated from fermented olives. 33<sup>rd</sup> ISSY, International Specialised Symposium on Yeasts, Exploring and Engineering Yeasts for Industrial Application, University College Cork, Ireland, 26-29 June 2017, Poster Abstract Book, P88, p. 90.

The yeast biofilm-like phenotypes such as media invasiveness and mat colony are considered mechanisms to colonize and persist in environmental niches. In table olive fermentation, pH and salinity are two hurdles that yeasts have to overcome to succeed in developing a stable population. Eight yeast strains among which *Candida*, *Pichia*, and *Wickerhamomyces* genera - isolated from table olive fermentations, and the two control strains of *Saccharomyces cerevisiae*  $\Sigma$ 1278b and BY4742 were tested in YPD agar either carbon-rich (glucose 2%) or -deficient (glucose 0.1%) media also modified both for pH (4.3) and salinity (NaCl 5%) to simulate the brine. Conventional (2%) and low (0.3%) agar concentrations were used to study invasiveness and mat colony formation, respectively. The majority of the strains showed bigger mat colonies with 2% of glucose than 0.1%. Three strains exhibited an increase in area colonies growing in the modified media. The pH and salinity modifications determined

invasive growth for six and two strains in the presence of either 2% or 0.1% of glucose, respectively. Two strains were unable to invade media. A wide diversity was observed among the strains and media; moreover, some of the strains displayed the two biofilm-like phenotypes in dissociated way. The observed phenotypic diversity could confer strain advantage during the olive fermentation process. Our results may be taking into account to select strains to drive fermentation process.

5 Sidari R, Martorana A, Caridi A 2017. Microbiota associated to fermented table olive of the cultivar *Carolea* assessed by molecular methods. MD2017, Microbial Diversity 2017, Drivers of microbial diversity, PS3-18, p. 373-374, Bari (Italy), 24-26 October 2017, ISBN 978-88-943010-0-7.

Natural fermentation is one of the most used methods to process the table olives; final product characteristics are determined by the specific microbiota of the olive cultivars. Aim of the work was to assess the microbiota of the cultivar Carolea olives that were naturally fermented using the following brines: 1) 8% NaCl (w/v), 2) 8% NaCl acidified to pH 4.30, 3) 5% NaCl for 20 days and then brought to 8% NaCl, 4) 5% NaCl for 20 days, then brought to 8% NaCl and acidified to pH 4.30. Lactic acid bacteria (LAB) and yeasts were analysed at the 240 days using both culture-dependent and culture-independent approach. LAB and yeasts were isolated, according to the colonies features, and stored at -80°C in the Microbial Collection of the Laboratory of Microbiology. The LAB identification was performed

by multiplex PCR assay of the recA gene while the yeasts were identified by PCR-ITS and RFLP analysis of ITS-5.8S rRNA region. Total DNA was extracted from the brines and subjected to PCR-DGGE. LAB and yeasts representative strains and amplicons of bands excised from polyacrylamide gels were sequenced. The microbial load ranged from 5.18 to 5.41 Log CFU/ml and from 5.18 to 5.80 Log CFU/ml for LAB and yeasts, respectively. Lactobacillus plantarum was predominant in all the trials while among the yeasts Pichia kudriavzevii, Candida boidinii, and Wickerhamomyces anomalus were detected. The DGGE analysis confirmed the presence of L. plantarum for the LAB population in all the trials and of C. boidinii in the trials 1), 3), 4), and W. anomalus in the trial 2) for the yeasts.

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Here are our most recent publications.

Sarilar V, Sterck L, Matsumoto S, Jacques N, Neuvéglise C, Tinsley CR, Sicard D, Casaregola S 2017. Genome sequence of the type strain CLIB 1764T (= CBS 14374T) of the yeast species *Kazachstania* saulgeensis isolated from French organic sourdough. Genom Data 13:41-43. doi: 10.1016/j.gdata.2017.07.003

*Kazachstania saulgeensis* is a recently described species isolated from French organic sourdough. Here, we report the high quality genome sequence of a monosporic segregant of the type strain of this species, CLIB 1764T (= CBS 14374T). The genome has a total length of 12.9 Mb and contains 5326 putative protein-

coding genes, excluding pseudogenes and transposons. The nucleotide sequences were deposited into the European Nucleotide Archive under the genome assembly accession numbers FXLY01000001-FXLY01000017.

2 Ribeiro LR, Santos ARO, Groenewald M, Smith MTH, Lara CA, Góes-Neto A, Jacques N, Grondin C, Casaregola S, Lachance MA, Rosa CA. 2017. Description of *Hyphopichia buzzinii* f.a., sp. nov. and *Hyphopichia homilentoma* comb. nov., the teleomorph of *Candida homilentoma*. Antonie Van Leeuwenhoek. **110**:985-994. doi: 10.1007/s10482-017-0870-2.

During studies of the yeast diversity associated with rotting wood in Brazil and fruits, plants and insects in French Guiana, three strains of a new species were isolated. Analysis of the sequences of the internal transcribed spacer (ITS)-5.8S and D1/D2 domains of the large subunit of the rRNA gene showed that this species belongs to the genus *Hyphopichia* and its closest relative is *Candida homilentoma*. These species differ by 44 nucleotide substitutions in D1/D2 sequences. A new species *Hyphopichia buzzinii* f. a., sp. nov., is proposed to accommodate these isolates. The type strain of *Hyphopichia buzzinii* sp. nov. is CLIB 1739T (=CBS 14300T = UFMG-CM-Y6121T; MycoBank number is MB 815609). In addition, we

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Jacques N, Mallet S, Laaghouiti F, Tinsley CR, Casaregola S. 2017. Specific populations of the yeast *Geotrichum candidum* revealed by molecular typing. Yeast **34**:165-178 - doi: 10.1002/yea.3223

Geotrichum candidum is a ubiquitous yeast and an essential component in the production of many soft cheeses. We developed a multilocus sequence typing (MLST) scheme with five retained loci (NUP116, URA1, URA3, SAPT4 and PLB3) which were sufficiently divergent to distinguish 40 sequence types (STs) among the 67 G. candidum strains tested. Phylogenetic analyses defined five main clades; one clade was restricted to environmental isolates, three other clades included distinct environmental isolates and dairy strains, while the fifth clade comprised 34 strains (13 STs), among which all but two were isolated from milk, cheese or the dairy environment. These findings suggest an adaptation to the dairy ecosystems by a group of specialized European G. candidum strains. In addition, we developed a isolated 11 strains of *C. homilentoma* from rotting wood, leaf surfaces, and water bodies in Brazil, and these strains when crossed among one another and with the type strain (CBS 6312T) of this species, produced hat-shaped ascospores typical of the genus *Hyphopichia*. We describe the teleomorph of *C. homilentoma* as a new combination, *Hyphopichia homilentoma* comb. nov. (type strain CBS 6312T; MycoBank number is MB 820009). We also propose to transfer the other six *Candida* species of the *Hyphopichia homilentoma* produced ethanol and xylitol from D-xylose whereas H. buzzinii was only able to convert this pentose to xylitol.

polymerase chain reaction inter-long terminal repeat scheme, a fast and reproducible random amplification of polymorphic DNA-like method for *G. candidum*, to type the closely related dairy strains, which could not be distinguished by MLST. Overall, our findings distinguished two types of dairy strains, one forming a homogeneous group with little genetic diversity, and the other more closely related to environmental isolates. Neither regional nor cheese specificity was observed in the dairy *G. candidum* strains analysed. This present study sheds light on the genetic diversity of both dairy and environmental strains of *G. candidum* and thus extends previous characterizations that have focused on the cheese isolates of this species.

4 Jacques N, Sarilar V, Urien C, Lopes MR, Morais CG, Uetanabaro AP, Tinsley CR, Rosa CA, Sicard D, Casaregola S. 2016. Three novel ascomycetous yeast species of the *Kazachstania* clade, *Kazachstania saulgeensis* sp. nov., *Kazachstania serrabonitensis* sp. nov. and *Kazachstania australis* sp. nov. Reassignment of *Candida humilis* to *Kazachstania humilis* f.a. comb. nov. and *Candida pseudohumilis* to *Kazachstania pseudohumilis* f.a. comb. nov. Int J Syst Evol Microbiol. 2016 66:5192-5200 - doi: 10.1099/ijsem.0.001495.

Five ascosporogenous yeast strains related to the genus *Kazachstania* were isolated. Two strains (CLIB 1764T and CLIB 1780) were isolated from French sourdoughs; three others (UFMG-CM-Y273T, UFMG-CM-Y451 and UFMG-CM-Y452) were from rotting wood in Brazil. The sequences of the French and Brazilian strains differed by one and three substitutions, respectively, in the D1/D2 large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS). The D1/D2 LSU rRNA sequence of these strains differed by 0.5 and 0.7 % from *Kazachstania* 

*exigua*, but their ITS sequences diverged by 8.1 and 8.3 %, respectively, from that of the closest described species *Kazachstania barnettii*. Analysis of protein coding sequences of RPB1, RPB2 and EF-1α distinguished the French from the Brazilian strains, with respectively 3.3, 6 and 11.7 % substitutions. Two novel species are described to accommodate these newly isolated strains: *Kazachstania saulgeensis* sp. nov. (type strain CLIB 1764T=CBS 14374T) and *Kazachstania serrabonitensis* sp. nov. (type strain UFMG-CM-Y273T=CLIB 1783T=CBS 14236T).

Further analysis of culture collections revealed a strain previously assigned to the *K. exigua* species, but having 3.8 % difference (22 substitutions and 2 indels) in its ITS with respect to *K. exigua*. Hence, we describe a new taxon, *Kazachstania australis* sp. nov. (type strain CLIB 162T=CBS 2141T), to accommodate this strain. Finally, *Candida humilis* and *Candida pseudohumilis* are reassigned to the genus *Kazachstania* as new combinations. On the basis of sequence analysis, we also propose that *Candida milleri* and *Kazachstania humilis* comb. nov. are conspecific.

5 Burgaud G, Coton M, Jacques N, Debaets S, Maciel NO, Rosa CA, Gadanho M, Sampaio JP, Casaregola S. 2016. *Yamadazyma barbieri* f.a. sp. nov., an ascomycetous anamorphic yeast isolated from a Mid-Atlantic Ridge hydrothermal site (-2300 m) and marine coastal waters. Int J Syst Evol Microbiol 66:3600-3606 - doi: 10.1099/ijsem.0.001239.

Two yeast strains that are members of the same species were isolated from different marine habitats, i.e. one from Mid-Atlantic Ridge ocean water samples located in the direct vicinity of black smokers near the Rainbow deep-sea hydrothermal vent and one from Brazilian marine water samples off the Ipanema beach. Strains CLIB 1964T and CLIB 1965 are anamorphic ascomycetous yeasts affiliated to the *Yamadazyma* clade of Saccharomycetales. Interestingly, these strains were phylogenetically and distinctly positioned into a group of species comprising all species of the genus *Yamadazyma* isolated from marine habitats including deep-sea hydrothermal vents, i.e. *Candida atmosphaerica*, *C. spencermartinsiae*, *C. atlantica*, *C. oceani* and *C. taylorii*. These strains differed

significantly in their D1/D2 domain sequences of the LSU rRNA gene from the closely related species mentioned above, by 2.6, 3.0, 3.4, 3.8 and 6.0 %, respectively. Internal transcribed spacer region sequence divergence was also significant and corresponded to 4.6, 4.7, 4.7, 12.0 and 24.7 % with *C. atlantica, C. atmosphaerica, C. spencermartinsiae, C. oceani* and *C. taylorii*, respectively. Phenotypically, strains CLIB 1964T and CLIB 1965 could be distinguished from closely related species by their inability to assimilate 1-sorbose. CLIB 1964T (=CBS 14301T=UBOCC-A-214001T) is the designated type strain for *Yamadazyma barbieri* sp. nov. The MycoBank number is MB 815884.

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- 1 Ritala A, Häkkinen S, Toivari M, Wiebe MG. 2017. Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. Front. Microbiol. <u>https://doi.org/10.3389/fmicb.2017.02009</u>

By 2050, the world would need to produce 1,250 million tonnes of meat and dairy per year to meet global demand for animal-derived protein at current consumption levels. However, growing demand for protein will not be met sustainably by increasing meat and dairy production because of the low efficiency of converting feed to meat and dairy products. New solutions are needed. Single cell protein (SCP), i.e., protein produced in microbial and algal cells, is an option with potential. Much of the recent interest in SCP has focused on the valorisation of side streams by using microorganisms to improve their protein content,

which can then be used in animal feed. There is also increased use of mixed populations, rather than pure strains in the production of SCP. In addition, the use of methane as a carbon source for SCP is reaching commercial scales and more protein-rich products are being derived from algae for both food and feed. The following review addresses the latest developments in SCP production from various organisms, giving an overview of commercial exploitation, a review of recent advances in the patent landscape (2001–2016) and a list of industrial players in the SCP field.

2 David Ribas, Joana Sá-Pessoa, Isabel Soares-Silva, Sandra Paiva, Yvonne Nygård, Laura Ruohonen, Merja Penttilä, Margarida Casal. 2017. Yeast as a tool to express sugar acid transporters with biotechnological interest FEMS Yeast Research. Volume 17: fox005.

Sugar acids can be used as platform chemicals to generate primary building blocks of industrially

relevant products. Microbial production of these organic compounds at high yields requires the

engineering of the enzymatic machinery and the presence of plasma membrane transporters able to export them outside the cells. In this study, several yeast carboxylic acid transporters belonging to the Jen family were screened for the transport of biotechnologically relevant sugar acids, namely gluconic, saccharic, mucic, xylaric and xylonic acid, and functionally characterised in *Saccharomyces cerevisiae*. We show that Jen permeases are capable of transporting most of these sugar acids, although with different specificities. Saccharate is a substrate of the transporters ScJen1-S271Q and KlJen2, gluconate of CaJen2 and KlJen2, and xylarate and mucate of CaJen2. A molecular docking approach of these transporters identified the residues that play a major role in the substrate binding of these sugar acids, namely R188 (ScJen1), R122 (CaJen2) and R127 (KlJen2), all equivalent residues (TMS II). The identification of Jen members as sugar acid transporters can contribute to engineering efficient microbial cell factories with increased sugar acid production, as the ScJen1 is able to promote substrate efflux.

3 Markus Nikinmaa, Syed Ariful Alam, Mari Raulio, Kati Katina, Ilkka Kajala, Emilia Nordlund, Nesli Sozer. 2017. LWT - Bioprocessing of bran with exopolysaccharide producing microorganisms as a tool to improve expansion and textural properties of extruded cereal foams with high dietary fibre content. Food Science and Technology 77:170-177.

High dietary fibre levels, especially insoluble that is typical for cereal bran, have been associated with poor structural, textural and sensory properties in extruded products. The effect of fermentation with baker's yeast, fermentation with a mixture of *Kazachstania exigua* and *Lactobacillus brevis* with and without added hydrolytic enzymes, as well as with *Weissella confusa*, on the structural and textural properties of high dietary fibre extrudates (ca. 6–14 g/100 g added fibre) in extrusion was studied. Superior structural and textural extrudate characteristics were achieved by fermentation of bran with dextran producing *W. confusa*. At 40 g/100 g

addition of *W. confusa* –treated bran (12 g/100 g fibre content) radial expansion was the same as for the pure rye endosperm flour control, while density was 35% lower. Hardness (54.1  $\rightarrow$  16.3 N) and crispiness work (4.11  $\rightarrow$  0.45 Nmm) were reduced (P < 0.05), while the crispiness index was significantly higher (0.002  $\rightarrow$  0.05) than that of the control extrudate. Bioprocessing with mixed fermentation, containing *K. exigua* and *L. brevis*, together with hydrolytic enzymes also improved the structural and textural characteristics of the bran in extrusion, while baker's yeast fermentation did not significantly affect these characteristics.

4 Brickwedde A, van den Broek M, Geertman JMA, Magalhães F, Kuijpers NGA, Gibson B, Pronk JT, Daran JMG. 2017. Evolutionary engineering in chemostat cultures for improved maltotriose fermentation kinetics in *Saccharomyces pastorianus* lager brewing yeast. Frontiers in Microbiology 8:1690.

The lager brewing yeast Saccharomyces pastorianus, an interspecies hybrid of S. eubayanus and S. cerevisiae, ferments maltotriose, maltose, sucrose, glucose and fructose in wort to ethanol and carbon dioxide. Complete and timely conversion ('attenuation') of maltotriose by industrial S. pastorianus strains is a key requirement for process intensification. This study explores a new evolutionary engineering strategy for improving maltotriose fermentation kinetics. Prolonged carbon-limited, anaerobic chemostat cultivation of the reference strain S. pastorianus CBS1483 on a maltotriose-enriched sugar mixture was used to select for spontaneous mutants with improved affinity for maltotriose.

residual maltotriose concentration and a higher ethanol concentration than the parental strain. Uptake studies with <sup>14</sup>C-labelled sugars revealed an up to 4.75-fold higher transport capacity for maltotriose in evolved strains. In laboratory batch cultures on wort, evolved strains showed improved attenuation and higher ethanol concentrations. These improvements were also observed in pilot fermentations at 1000-L scale with high-gravity wort. Although the evolved strain exhibited multiple chromosomal copy number changes, analysis of beer made from pilot fermentations showed no negative effects on flavour compound profiles. These results demonstrate the potential of evolutionary engineering for strain improvement of hybrid, alloploid brewing strains. 6 Gibson B, Geertman JMA., Hittinger CT, Krogerus K, Libkind D, Louis EJ, Magalhães F, Sampaio JP. 2017. New yeasts - new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res 17.

The brewing industry is experiencing a period of change and experimentation largely driven by customer demand for product diversity. This has coincided with a greater appreciation of the role of yeast in determining the character of beer and the widespread availability of powerful tools for yeast research. Genome analysis in particular has helped clarify the processes leading to domestication of brewing yeast and has identified domestication signatures that may be exploited for further yeast development. The functional properties of nonconventional yeast (both *Saccharomyces* and non-*Saccharomyces*) are being assessed with a view to creating beers with new flavours as well as producing flavoursome non-alcoholic beers. The discovery of the psychrotolerant *S. eubayanus* has stimulated research on *de novo S. cerevisiae* x *S. eubayanus* hybrids for low-temperature lager brewing and has led to renewed interest in the functional importance of hybrid organisms and the mechanisms that determine hybrid genome function and stability. The greater diversity of yeast that can be applied in brewing, along with an improved understanding of yeasts' evolutionary history and biology, is expected have a significant and direct impact on the brewing industry, with potential for improved brewing efficiency, product diversity and, above all, customer satisfaction.

7 Nikulin J, Krogerus K, Gibson B. 2017. Alternative *Saccharomyces* interspecies hybrid combinations and their potential for low-temperature wort fermentation. Yeast - DOI: 10.1002/yea.3246.

The lager yeast hybrid (*S. cerevisiae x S. eubayanus*) possesses two key characteristics that are essential for lager brewing: efficient sugar utilization and cold tolerance. Here we explore the possibility that the lager yeast phenotype can be recreated by hybridizing *S. cerevisiae* ale yeast with a number of cold tolerant *Saccharomyces* species including *S. arboricola*, *S. eubayanus*, *S. mikatae and S. uvarum*. Interspecies hybrids performed better than parental strains in lager brewing conditions (12 °C and 12 °P wort), with the *S. mikatae* hybrid performing as well as the *S. eubayanus* hybrid. Where the *S. cerevisiae* parent was capable of utilizing maltotriose, this trait

- was inherited by the hybrids. A greater production of higher alcohols and esters by the hybrids resulted in production of more aromatic beers relative to the parents. Strong fermentation performance relative to the parents was dependent on ploidy, with polyploid hybrids (3n, 4n) performing better that diploid hybrids. All hybrids produced 4-vinyl guaiacol, a smoke/clove aroma generally considered an off flavour in lager beer. This characteristic could however be eliminated by isolating spore clones from a fertile hybrid of *S. cerevisiae* and *S. mikatae*. Results suggest that *S. eubayanus* is dispensable when constructing yeast hybrids that express the typical lager yeast phenotype.
- 8 Magalhães F, Krogerus K, Castillo S, Ortiz-Julien A, Dequin S, Gibson B. 2017. Exploring the potential of *Saccharomyces eubayanus* as a parent for new interspecies hybrid strains in winemaking. FEMS Yeast Res 17.

Yeast cryotolerance brings some advantages for wine fermentations including the improved aromatic complexity of white wines. Natural cold tolerant strains are generally less adept to wine fermentations but fermentative fitness can be improved through hybridization. Here we studied the potential of using hybrids involving *Saccharomyces eubayanus* and a *S. cerevisiae* wine strain for low temperature wine making. Through screening the performance in response to variable concentrations of sugar, nitrogen and temperature we isolated one hybrid strain that exhibited the superior performance. This hybrid strain was propagated and dried in laboratory scale and tested for the fermentation of Macabeu and Sauvignon blanc grape musts. We obtained highly viable active dry yeast, which was able to efficiently ferment the grape musts with superior production of aroma active volatiles in particular, 2-phenylethanol. The genome sequences of the hybrid strains revealed variable chromosome inheritance among hybrids, particularly within *S. cerevisiae* sub-genome. With the present paper, we expand the knowledge on the potentialities of using *S. eubayanus* hybrids in industrial fermentation other than lager beer.

9 Krogerus K, Seppänen-Laakso T, Castillo S. & Gibson B. 2017. Inheritance of brewing-relevant phenotypes in constructed *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* hybrids. Microbial Cell Factories 16:66.

Interspecific hybridization has proven to be a potentially valuable technique for generating *de novo* lager yeast strains that possess diverse and improved traits compared to their parent strains. To further enhance the value of hybridization for strain development, it would be desirable to combine phenotypic traits from more than two parent strains, as well as remove unwanted traits from hybrids. One such trait, that has limited the industrial use of *de novo* 

lager yeast hybrids, is their inherent tendency to produce phenolic off-flavours; an undesirable trait inherited from the *Saccharomyces eubayanus* parent. Trait removal and the addition of traits from a third strain could be achieved through sporulation and meiotic recombination or further mating. However, interspecies hybrids tend to be sterile, which impedes this opportunity.

10 Magalhães F, Krogerus K, Vidgren V, Sandell M. & Gibson B. 2017. Improved cider fermentation performance and quality with newly-generated *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* hybrids. J Indust Microbiol Biotechnol - DOI 10.1007/s10295-017-1947-7.

Yeast cryotolerance may be advantageous for cider making, where low temperatures are usually employed. Here we crossed the cryotolerant *S. eubayanus* with an *S. cerevisiae* wine strain and assessed the suitability of the hybrids for lowtemperature cider fermentation. All strains fermented the juice to 5% ABV, but at different rates; hybrid strains outperformed *S. cerevisiae*, which was sensitive to low temperatures. The best hybrid fermented similarly to *S. eubayanus*. *S. eubayanus* produced sulphurous off flavours which masked a high concentration of fruity ester notes. This phenotype was absent in the hybrid strains, resulting in distinctly fruitier ciders. Aroma was assessed by an independent consumer panel, which rated the hybrid ciders as identical to the wine strain cider. Both were significantly more pleasant than the *S. eubayanus* cider. Interspecific hybridization can apparently be used effectively to improve low-temperature fermentation performance without compromising product quality.

11 Krogerus K, Magalhães F, Vidgren V. & Gibson B. 2017. Novel brewing yeast hybrids: creation and application. Appl Microbiol Biotechnol 101:65-78.

Yeast plays a crucial role in beer production, and the modern brewing industry has come to rely on the interspecific yeast hybrid *Saccharomyces pastorianus* as the main workhorse\_for both historical and biological reasons. Hybrid species often exhibit superior phenotypic qualities over the parent strains, i.e. heterosis. The creation of *de novo* yeast hybrids through intra- and interspecific hybridization also offers great potential as a strain development tool for the brewing industry. Recent studies have revealed that a variety of traits desirable for brewing can be enhanced in *de novo* yeast hybrids. Here, we discuss how hybridization can be used as a strain development tool for brewing applications, with particular focus on the recent research that has been carried out on *de novo* lager yeast. These hybrids have been shown to outperform their parent strains in different aspects, including faster fermentation rates, better sugar utilization, greater stress tolerance, and increased aroma formation. Hybrid genome function and stability, as well as techniques for generating hybrids are also briefly discussed. Hybridization not only offers the possibility of generating novel non-GM brewing yeast strains with unique properties, but will aid in unraveling the complex evolutionary history of industrial lager yeast.

12 Djordjević V, Willaert R, Gibson B. & Nedović V. 2016. Immobilized yeast cells and secondary metabolites. In: *Fungal Metabolites*, JM Mérillon, KG. Ramawat (eds.). Springer International Publishing, Switzerland. DOI 10.1007/978-3-319-19456-1\_33-1.

The use of immobilized cell technology (ICT) is viewed as a promising biotechnological tool to achieve high volumetric productivities of yeast fermentation in bioindustry of alcoholic beverages. During this process a huge number of organic compounds are being formed as yeast secondary metabolites, among which volatile compounds, such as higher alcohols, esters and vicinal diketones are the most important flavoring compounds. The objective of this chapter is to summarize the knowledge on the origin of the flavoractive and non-volatile compounds synthesized by yeast and to describe how the composition of the medium, culture strain, process conditions (temperature, aeration etc.), bioreactor design, and other critical parameters influence the metabolic activities of yeast cultures. Despite the technological and economic advantages provided by ICT, commercialization of this technology experienced only limited success, mainly due to unpredictable effect of immobilization on yeast physiology. This chapter is an attempt to rationalize and make some conclusions about the impact of cell immobilization on yeast metabolism collected from empirical experiences in production of alcoholic beverages. The knowledge addressing this issue may be of particular benefit to the nascent bioflavor industry.

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

Recent publication.

1 Golubev WI. 2017. Mycocinogeny in yeast of the genus *Starmera*. Problems in Med Mycology 19(2):10-13 (in Russian).

Type strain of *Starmera quercuum* secrets fungicidal mycocin. The most activity it exhibits at pH 6.0 and in the presence in the medium of 2% NaCl. Ascomycetous (predominantly of the order Saccharomycetales) and basidiomycetous (mostly of the order Sporidiobolales) are sensitive to this mycocin. In addition, representatives of the orders Taphrinales and Schizosaccharomycetales as well as are sensitive.

#### VIII 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 <<u>cthittinger@wisc.edu</u>>.

Recent publications.

1 Hittinger CT, Steele JL, Ryder DS. 2017. Diverse yeasts for diverse fermented beverages and foods. Curr Opin Biotechnol - doi:10.1016/j.copbio.2017.10.004. (review)

Yeasts play vital roles in food biotechnology, especially in fermented products. Yeasts are monoculture bioprocessing agents, are members of complex microbial communities, and are even consumed directly. Advances in genetic technologies, such as whole genome and environmental DNA sequencing, have shed light on the diverse yeasts used in both traditional and industrialized processes. The yeast *Saccharomyces cerevisiae* plays an outsized role in fermented beverage and food production, but new research has revealed a cornucopia of yeast biodiversity that includes dozens of species. These often surprising studies have shown how yeasts are related, how they interact with other microbes, and how valuable traits are encoded in their genomes. This deeper understanding illuminates current practices in food biotechnology, while foreshadowing future innovation.

2 Peris D, Pérez-Torrado R, Hittinger CT, Barrio E, Querol A. On the origins and industrial applications of *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii* hybrids. Yeast - doi:10.1002/yea.3283. (review)

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.

3 Gibson B, Geertman JA, Hittinger CT, Krogerus K, Libkind D, Louis EJ, Magalhães F, Sampaio JP. 2017. New yeasts-new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res 17: fox038. (review)

The brewing industry is experiencing a period of change and experimentation largely driven by customer demand for product diversity. This has coincided with a greater appreciation of the role of yeast in determining the character of beer and the widespread availability of powerful tools for yeast research. Genome analysis in particular has helped clarify the processes leading to domestication of brewing yeast and has identified domestication signatures that may be exploited for further yeast development. The functional properties of nonconventional yeast (both *Saccharomyces* and non-*Saccharomyces*) are being assessed with a view to creating beers with new flavours as well as producing flavoursome non-alcoholic beers. The discovery of the psychrotolerant *S. eubayanus* has stimulated research on de novo *S. cerevisiae*  $\times$  *S. eubayanus* hybrids for low-temperature lager brewing and has led to renewed interest in the functional importance of hybrid organisms and the mechanisms that determine hybrid genome function and stability. The greater diversity of yeast that can be applied in brewing, along with an improved understanding of yeasts' evolutionary history and biology, is expected to have a significant and direct impact on the brewing industry, with potential for improved brewing efficiency, product diversity and, above all, customer satisfaction.

4 Shen XX, Zhou X, Kominek J, Kurtzman CP, Hittinger CT, Rokas A. 2016. Reconstructing the backbone of the Saccharomycotina yeast phylogeny using genome-scale data. G3 (Bethesda) 6:3927-3939.

Understanding the phylogenetic relationships among the yeasts of the subphylum Saccharomycotina is a prerequisite for understanding the evolution of their metabolisms and ecological lifestyles. In the last two decades, the use of rDNA and multi-locus data sets has greatly advanced our understanding of the yeast phylogeny, but many deep relationships remain unsupported. In contrast, phylogenomic analyses have involved relatively few taxa and lineages that were often selected with limited considerations for covering the breadth of yeast biodiversity. Here we used genome sequence data from 86 publicly available yeast genomes representing 9 of the 11 known major lineages and 10 non-yeast fungal outgroups to generate a 1,233-gene, 96-taxon data matrix. Species phylogenies reconstructed using two different methods (concatenation and coalescence) and two data matrices (amino acids or the first two codon positions) yielded identical and highly supported relationships between

the 9 major lineages. Aside from the lineage comprised by the family Pichiaceae, all other lineages were monophyletic. Most interrelationships among yeast species were robust across the two methods and data matrices. However, 8 of the 93 internodes conflicted between analyses or data sets, including the placements of: the clade defined by species that have reassigned the CUG codon to encode serine, instead of leucine; the clade defined by a whole genome duplication; and the species Ascoidea rubescens. These phylogenomic analyses provide a robust roadmap for future comparative work across the yeast subphylum in the disciplines of taxonomy, molecular genetics, evolutionary biology, ecology, and biotechnology. To further this end, we have also provided a BLAST server to query the 86 Saccharomycotina genomes, which can be found at http://y1000plus.org/blast.

5 Peris D, Moriarty RV, Alexander WG, Baker E, Sylvester K, Sardi M, Langdon QK, Libkind D, Wang QM, Bai FY, Leducq JB, Charron G, Landry CR, Sampaio JP, Gonçalves P, Hyma KE, Fay JC, Sato TK, Hittinger CT. 2017. Hybridization and adaptive evolution of diverse *Saccharomyces* species for cellulosic biofuel production. Biotechnol Biofuels 10:78.

Background: Lignocellulosic biomass is a common resource across the globe, and its fermentation offers a promising option for generating renewable liquid transportation fuels. The deconstruction of lignocellulosic biomass releases sugars that can be fermented by microbes, but these processes also produce fermentation inhibitors, such as aromatic acids and aldehydes. Several research projects have investigated lignocellulosic biomass fermentation by the baker's yeast Saccharomyces cerevisiae. Most projects have taken synthetic biological approaches or have explored naturally occurring diversity in S. cerevisiae to enhance stress tolerance, xylose consumption, or ethanol production. Despite these efforts, improved strains with new properties are needed. In other industrial processes, such as wine and beer fermentation, interspecies hybrids have combined important traits from multiple species, suggesting that interspecies hybridization may also offer potential for biofuel research. Results: To investigate the efficacy of this approach for traits relevant to lignocellulosic biofuel production, we generated synthetic hybrids by crossing engineered xylose-fermenting strains of S. cerevisiae with wild strains from various Saccharomyces species. These

interspecies hybrids retained important parental traits, such as xylose consumption and stress tolerance, while displaying intermediate kinetic parameters and, in some cases, heterosis (hybrid vigor). Next, we exposed them to adaptive evolution in ammonia fiber expansion-pretreated corn stover hydrolysate and recovered strains with improved fermentative traits. Genome sequencing showed that the genomes of these evolved synthetic hybrids underwent rearrangements, duplications, and deletions. To determine whether the genus Saccharomyces contains additional untapped potential, we screened a genetically diverse collection of more than 500 wild, non-engineered Saccharomyces isolates and uncovered a wide range of capabilities for traits relevant to cellulosic biofuel production. Notably, Saccharomyces mikataestrains have high innate tolerance to hydrolysate toxins, while some Saccharomyces species have a robust native capacity to consume xylose. Conclusions: This research demonstrates that hybridization is a viable method to combine industrially relevant traits from diverse yeast species and that members of the genus Saccharomyces beyond S. cerevisiae may offer advantageous genes and traits of interest to the lignocellulosic biofuel industry.

6 Shen XX, Hittinger CT, Rokas A. 2017. Contentious relationships in phylogenomic studies can be driven by a handful of genes. Nat Ecol Evol 1:0126.

Phylogenomic studies have resolved countless branches of the tree of life, but remain strongly contradictory on certain, contentious relationships. Here, we use a maximum likelihood framework to quantify the distribution of phylogenetic signal among genes and sites for 17 contentious branches and 6 wellestablished control branches in plant, animal and fungal phylogenomic data matrices. We find that resolution in some of these 17 branches rests on a single gene or a few sites, and that removal of a single gene in concatenation analyses or a single site from every gene in coalescence-based analyses diminishes support and can alter the inferred topology. These results suggest that tiny subsets of very large data matrices drive the resolution of specific internodes, providing a dissection of the distribution of support and observed incongruence in phylogenomic analyses. We submit that quantifying the distribution of phylogenetic signal in phylogenomic data is essential for evaluating whether branches, especially contentious ones, are truly resolved. Finally, we offer one detailed example of such an evaluation for the controversy regarding the earliest-branching metazoan phylum, for which examination of the distributions of gene-wise and site-wise phylogenetic signal across eight data matrices consistently supports ctenophores as the sister group to all other metazoans.

7 Haase MAB, Kominek J, Langdon QK, Kurtzman CP, Hittinger CT. 2017. Genome sequence and physiological analysis of *Yamadazyma laniorum* f.a. sp. nov. and a reevaluation of the apocryphal xylose fermentation of its sister species, *Candida tenuis*. FEMS Yeast Res 17:fox019.

Xylose fermentation is a rare trait that is immensely important to the cellulosic biofuel industry, and *Candida tenuis* is one of the few yeasts that has been reported with this trait. Here we report the isolation of two strains representing a candidate sister species to *C. tenuis*. Integrated analysis of genome sequence and physiology suggested the genetic basis of a number of traits, including variation between the novel species and *C. tenuis* in lactose metabolism due to the loss of genes encoding lactose permease and  $\beta$ -galactosidase in the former. Surprisingly, physiological characterization revealed that neither the type strain of *C. tenuis* nor this novel species fermented

- xylose in traditional assays. We reexamined three xylose-fermenting strains previously identified as *C. tenuis* and found that these strains belong to the genus *Scheffersomyces* and are not *C. tenuis*. We propose *Yamadazyma laniorum* f.a. sp. nov. to accommodate our new strains and designate its type strain as yHMH7 (=CBS 14780 = NRRL Y-63967T). Furthermore, we propose the transfer of *Candida tenuis* to the genus Yamadazyma as *Yamadazyma tenuis* comb. nov. This approach provides a roadmap for how integrated genome sequence and physiological analysis can yield insight into the mechanisms that generate yeast biodiversity.
- 8 Steffan SA, Dharampal PS, Diaz-Garcia L, Currie CR, Zalapa J, Hittinger CT. 2017. Empirical, metagenomic, and computational techniques illuminate the mechanisms by which fungicides compromise bee health. J Vis Exp 128:e54631.

Growers often use fungicide sprays during bloom to protect crops against disease, which exposes bees to fungicide residues. Although considered "beesafe," there is mounting evidence that fungicide residues in pollen are associated with bee declines (for both honey and bumble bee species). While the mechanisms remain relatively unknown, researchers have speculated that bee-microbe symbioses are involved. Microbes play a pivotal role in the preservation and/or processing of pollen, which serves as nutrition for larval bees. By altering the microbial community, it is likely that fungicides disrupt these microbe-mediated services, and thereby compromise bee health. This manuscript describes the protocols used to investigate the indirect mechanism(s) by which fungicides may be causing colony decline. Cage

experiments exposing bees to fungicide-treated flowers have already provided the first evidence that fungicides cause profound colony losses in a native bumble bee (Bombus impatiens). Using field-relevant doses of fungicides, a series of experiments have been developed to provide a finer description of microbial community dynamics of fungicide-exposed pollen. Shifts in the structural composition of fungal and bacterial assemblages within the pollen microbiome are investigated by next-generation sequencing and metagenomic analysis. Experiments developed herein have been designed to provide a mechanistic understanding of how fungicides affect the microbiome of pollen-provisions. Ultimately, these findings should shed light on the indirect pathway through which fungicides may be causing colony declines.

9 Morais CG, Batista TM, Kominek J, Borelli BM, Furtado C, Moreira RG, Franco GR, Rosa LH, Fonseca C, Hittinger CT, Lachance MA, Rosa CA. 2017. *Spathaspora boniae* sp. nov., a D-xylosefermenting species in the *Candida albicans/Lodderomyces* clade. Int J Syst Evol Microbiol 67:3798–805.

Two yeast isolates producing asci-containing elongate ascospores with curved ends typical of the genus *Spathaspora* were isolated from rotting wood samples collected in an Atlantic rainforest ecosystem in Brazil. Phylogenetic analysis of the LSU rRNA gene D1/D2 domain sequences demonstrated that the strains represent a new species and placed it next to *Candida blackwellae*, in a clade that also contains *Candida albicans* and *Candida dubliniensis*. Other sequences of the ribosomal gene cluster supported same placementin the same clade, and a phylogenomic analysis placed this new species in an early emerging position relative to the larger *C. albicans/Lodderomyces* clade. One interpretation is that the genus *Spathaspora* is, in fact, paraphyletic. In conformity with this view, we propose the novel species *Spathaspora boniae* sp. nov. to accommodate the isolates. The type strain of *Spathaspora boniae* sp. nov. is UFMG-CM-Y306<sup>T</sup> (=CBS 13262<sup>T</sup>). The MycoBank number is MB 821297. A detailed analysis of xylose metabolism was conducted for the new species.

10 Zhou X, Shen XX, Hittinger CT, Rokas A. 2017. Evaluating fast maximum likelihood-based phylogenetic programs using empirical phylogenomic data sets. Mol Biol Evol - doi:10.1101/142323.

Phylogenetics has witnessed dramatic increases in the sizes of data matrices assembled to resolve branches of the tree of life, motivating the development of programs for fast, yet accurate, inference. For example, several different fast programs have been developed in the very popular maximum likelihood framework, including RAxML/ExaML, PhyML, IQ-TREE, and FastTree. Although these four programs are widely used, a systematic evaluation and comparison of their performance using empirical genome-scale data matrices has so far been lacking. To address this question, we evaluated these four programs on 19 empirical phylogenomic data sets from diverse animal, plant, and fungal lineages with respect to likelihood maximization, tree topology, and computational speed. For single-gene tree inference, we found that the more exhaustive and slower strategies (ten searches per alignment) outperformed

faster strategies (one tree search per alignment) using RAxML, PhyML, or IQ-TREE. Interestingly, singlegene trees inferred by the three programs yielded comparable coalescent-based species tree estimations. For concatenation-based species tree inference, IQ-TREE consistently achieved the best-observed likelihoods for all data sets, and RAxML/ExaML was a close second. In contrast, PhyML often failed to complete concatenation-based analyses, whereas FastTree was the fastest but generated lower likelihood values and more dissimilar tree topologies in both types of analyses. Finally, data matrix properties, such as the number of taxa and the strength of phylogenetic signal, sometimes substantially influenced the relative performance of the programs. Our results provide realworld gene and species tree phylogenetic inference benchmarks to inform the design and execution of large-scale phylogenomic data analyses.

# IX Department of Soil Biology, Faculty of Soil Science, Lomonosov Moscow State University, 119991, Leninskie gory, 1/12, Moscow, Russia. Communicated by A.V. Kachalkin <<u>kachalkin\_a@mail.ru</u>>.

Recent publications.

1 Streletskii RA, Kachalkin AV, Glushakova AM, Demin VV, Chernov IYu. 2016. Quantitative determination of indole-3-acetic acid in yeasts using high performance liquid chromatography-tandem mass spectrometry. Microbiology. 85(6):727-736.

High performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) was applied to the comprehensive analysis of the ability of yeast strains to synthesize a plant hormone indole-3acetic acid (IAA). A total of 124 strains (37 species) of yeasts isolated from various regions and substrates were studied. Testing of IAA production showed that 92% strains were capable of IAA synthesis. The results indicated that, in general, ascomycetous yeasts were more active auxin producers than basidiomycetous ones. Geographically, strains from tropical regions were the most active IAA producers. Analysis of the substrate variability of the strains showed higher auxin production (on average) by the yeasts isolated from plants compared to the soil isolates, indicating a specific regulatory role of the plant yeast population.

#### 2 Glushakova AM, Kachalkin AV. 2017. Endophytic yeasts in leaf galls. Microbiology 86(2):250-256.

Yeast abundance and species diversity of endophytic complexes in galls (cecidia) formed on the leaves of *Salix fragilis, Salix caprea, Quercus robur, Tilia cordata*, and *Ulmus laevis* and the epiphytic yeast communities of undamaged leaves of these plants were studied. Dynamics of yeast abundance in the galls was significantly different from that of the epiphytic yeast communities. Maximum numbers of endophytic yeast cells in the galls (up to  $10^4$  CFU/g) were comparable to abundance of epiphytic yeasts. A total of 14 species of

endophytic yeasts were isolated from galls of different plants. Ascomycetous yeasts were found to predominate in the insect galls on willows and oak, while basidiomycetous yeasts dominated in mite galls on linden and elm, as well as on plant leaves. These results indicate that gall formation may be considered not only as a bidirectional pathological process of the interaction between plants and invertebrates, but also as a process in which the endophytic microbial population of the galls plays an important role. 3 Glushakova AM, Kachalkin AV. 2017. Endophytic yeasts in *Malus domestica* and *Pyrus communis* fruits under anthropogenic impact. Microbiology 86(1):128-135.

Yeast abundance and species diversity on the surface and in inner tissues of *Malus domestica* and *Pyrus communis* fruits under high anthropogenic impact in the city of Moscow (Russia) were studied. Results demonstrated that abundance of epiphytic yeasts on the fruits increased gradually, reaching the maximum of  $3.2 \text{ H } 10^{4} \text{ CFU/g}$  on mature fruits. During summer, abundance of endophytes did not change significantly (variation near  $2.5 \times 10^3 \text{ CFU/g}$ ) until complete maturation, while in September their numbers increased to  $10^4 \text{ CFU/g}$ . Basidiomycetous yeasts (*Filobasidium wieringae, F.magnum, Rhodo*-

torula glutinis, and Rhodospori-diobolus colostri) predominated on the fruit surface. Ascomycetous species were the most diverse group inside the fruits, which quantitatively increased through maturation. It was found that the share of opportunistic species *Candida parapsilosis* in internal tissues was significant during the entire period of fruit formation and development under anthropogenic impact in the city. Specific properties of epiphytic and endophytic yeast communities developing in natural ecological niches under synanthropic conditions and anthropogenic impact are discussed.

4 Glushakova AM., Kachalkin AV, Tiunov AV, Chernov IYu. 2017. Distribution of yeast complexes in the profiles of different soil types. Eurasian Soil Science 50(7):820-825.

The number and taxonomic structure of the yeast complexes were investigated in the full profiles of the soddy-podzolic soil (Central Forest State Nature Biosphere Reserve), dark gray forest soil (Kaluzhskie Zaseki Reserve), and chernozem (Privolzhskaya Forest-Steppe Reserve). In all these soils, the number of yeasts was maximal (10<sup>4</sup> CFU/g) directly under the litter; it drastically decreased with the depth. However, at the depth of 120-160 cm, the number of yeasts significantly increased in all the soils; their maximum was found in the illuvial horizon of the soddy-podzolic

5 Glushakova AM, Kachalkin AV. 2017. Yeasts of Nikitsky Botanical Garden plants. Microbiology 86(5):647-652.

Abundance and taxonomic structure of yeast communities on the surface and in the tissues of various plants of the Nikitsky Botanical Garden was studied. A total of 22 yeast species were isolated, including rare and new species. Yeast numbers on the studied plant substrates were varied within a broad range  $(8 \times 10^2 - 2.5 \times 10^7 \text{ CFU/g})$ , reaching the maximum in *Verbascum thapsus* flowers, on the surface

6 Kachalkin AV, Glushakova AM, Pankratov T.A. 2017. Yeast population of the Kindo Peninsula lichens. Microbiology 86(6):786-792.

Yeast abundance and species diversity in the lichens collected at the Kindo Peninsula (Karelia) were studied. A total of 14 lichen species analyzed belonged to the genera *Bryoria, Cladonia, Hypogymnia, Icmadophila, Nephroma, Peltigera*, and *Ramalina*. Abundance of cultured yeasts in lichens was intermediate between soil and phyllosphere. The average yeast number on lichens was  $\sim 2.5 \times 10^3$  CFU/g,

soil. Such a statistically significant increase in the number of yeasts at a considerable depth was found for the first time. Different groups of yeasts were present in the yeast communities of different soils. The species structure of yeast communities changed little in each soil: the same species were isolated both from the soil surface and from the depth of more than 2 m. The results showed that yeasts could be used for soil bioindication on the basis of specific yeast complexes in the profiles of different soil types rather than individual indicative species.

and inside the fruits of *Rubus* sp. and *Ficus carica*. Epiphytic and endophytic yeast communities of *Ficus carica* fruits were studied in dynamics, from fruit formation until their complete maturation and senescence. Specific properties of the yeast communities of the Nikitsky Botanical Garden plants and the features of the yeast communities from fig fruits are discussed in the paper.

while it exceeded  $8 \times 10^3$  CFU/g on plants and reached only  $1 \times 10^3$  CFU/g in soil. Yeast population of different parts of *Cladonia* lichens was found to vary significantly in abundance, species diversity, and community structure. The highest yeast abundance and diversity were revealed in the growth zone. Fifteen yeast species were isolated from lichens, including 6 basidiomycetous and 9 ascomycetous ones. Unlike soils and plants, yeast population of lichens consisted mainly of ascomycetous species, with predominance of *Candida sphagnicola* and anamorphous yeasts of the genus *Dothiora*. These results show that yeasts from different taxonomic and ecological groups are a necessary component of lichens; conditions favoring the preservation and development of specific yeast communities differing from the typical soil and phyllosphere yeast complexes are formed in the lichens of northern taiga forests.

### X 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 PhD, DSc Dorota Kregiel <<u>dorota.kregiel@p.lodz.pl</u>>.

The following papers have been published in indexed journals in 2016-2017.

1 Berlowska J, Pielech-Przybylska K, Balcerek M. Dziekonska-Kubczak U, Patelski P, Dziugan P, Kregiel D. 2016. Simultaneous saccharification and fermentation of sugar beet pulp for efficient bioethanol production. BioMed Research International (open access). Vol 2016, Article 3154929.

Sugar beet pulp, a byproduct of sugar beet processing, can be used as a feedstock in secondgeneration ethanol production. The objective of this study was to investigate the effects of pretreatment, of the dosage of cellulase and hemicellulase enzyme preparations used, and of aeration on the release of fermentable sugars and ethanol yield during simultaneous saccharification and fermentation (SSF) of sugar beet pulp-based worts. Pressure-thermal pretreatment was applied to sugar beet pulp suspended in 2% w/w sulphuric acid solution at a ratio providing 12% dry matter. Enzymatic hydrolysis was conducted using Viscozyme and Ultraflo Max (Novozymes) enzyme preparations (0.015-0.02 mL/g dry matter). Two yeast strains were used for fermentation: Ethanol Red (*S. cerevisiae*) (1 g/L) and *Pichia stipitis* (0.5 g/L), applied sequentially. The results show that efficient simultaneous saccharification and fermentation of sugar beet pulp was achieved. A 6 h interval for enzymatic activation between the application of enzyme preparations and inoculation with Ethanol Red further improved the fermentation performance, with the highest ethanol concentration reaching  $26.9 \pm 1.2$  g/L and  $86.5 \pm 2.1$  % fermentation efficiency relative to the theoretical yield.

2 Güneşer O, Karagül-Yüceer Y, Wilkowska A, Kregiel D. 2016. Volatile metabolites produced from agro-industrial wastes by Na-alginate entrapped *Kluyveromyces marxianus*. Brazilian J Microbiol 47:965-972.

The aim of this study was to evaluate the effects of alginate entrapment on fermentation metabolites of Kluyveromyces marxianus grown in agrowastes that served as the liquid culture media. K. marxianus cells entrapped in Na-alginate were prepared using the traditional liquid-droplet-forming method. Whey and pomaces from processed tomatoes, peppers, and grapes were used as the culture media. The changes in the concentrations of sugar, alcohol, organic acids, and flavor compounds were analyzed using gas chromatography-mass spectrometry (GC-MS) and high pressure liquid chromatography (HPLC). Both free and entrapped, K. marxianus were used individually to metabolize sugars, organic acids, alcohols, and flavor compounds in the tomato, pepper, grape, and acid whey based media. Marked changes in

the fermentation behaviors of entrapped and free *K. marxianus* were observed in each culture. A 1.45-log increase was observed in the cell numbers of free *K. marxianus* during fermentation. On the contrary, the cell numbers of entrapped *K. marxianus* remained the same. Both free and entrapped *K. marxianus* brought about the fermentation of sugars such as glucose, fructose, and lactose in the agrowaste cultures. The highest volume of ethanol was produced by *K. marxianus* in the whey based media. The concentrations of flavor compounds such as ethyl acetate, isoamyl alcohol, isoamyl acetate, 2-phenylethyl isobutyrate, phenylethyl acetate, and phenylethyl alcohol were higher in fermented agrowaste based media compared to the control.

3 Berlowska J, Pielech-Przybylska K, Balcerek M, Cieciura W, Borowski S, Kregiel D. 2017. Integrated bioethanol fermentation/anaerobic digestion for valorization of sugar beet pulp. Energies 10(9):1255.

Large amounts of waste biomass are generated in sugar factories from the processing of sugar beets. After diffusion with hot water to draw the sugar from the beet pieces, a wet material remains called pulp. In this study, waste sugar beet pulp biomass was enzymatically depolymerized, and the obtained hydrolyzates were subjected to fermentation processes. Bioethanol, biomethane, and biohydrogen were produced directly from the substrate or in combined mode. Stillage, a distillery by-product, was used as a feedstock for anaerobic digestion. During biosynthesis of ethanol, most of the carbohydrates released from the sugar beet pulp were utilized by a co-culture of *Saccharomyces cerevisiae* Ethanol Red, and

- Scheffersomyces stipitis LOCK0047 giving 12.6 g/L of ethanol. Stillage containing unfermented sugars (mainly arabinose, galactose and raffinose) was found to be a good substrate for methane production (444 dm<sup>3</sup> CH<sub>4</sub>/kg volatile solids (VS)). Better results were achieved with this medium than with enzymatic saccharified biomass. Thermal pre-treatment and adjusting the pH of the inoculum resulted in higher hydrogen production. The largest (p < 0.05) hydrogen yield (252 dm<sup>3</sup> H<sub>2</sub>/kg VS) was achieved with sugar beet stillage (SBS). In contrast, without pre-treatment the same medium yielded 35 dm<sup>3</sup> H<sub>2</sub>/kg VS. However, dark fermentation of biohydrogen was more efficient when sugar beet pulp hydrolyzate was used.
- 4 Berlowska J, Dudkiewicz-Kołodziejska M, Pawlikowska E, Pielech-Przybylska K, Balcerek M, Czyzowska A, Kregiel D. 2017. Utilization of post-fermentation yeasts for yeast extract production by autolysis: the effect of yeast strain and saponin from *Quillaja saponaria*. J Inst Brewing 123(3):396-401.

Spent yeasts, a co-product from fermentation processes, are a source for unconvential autolysis processes. In this study, five post-fermentation yeast strains that are often used in fermentation processes were used: *Saccharomyces cerevisiae* Ethanol Red (Lessafre), *Kluyveromyces marxianus* LOCK 0026, *K. marxianus* NCYC 179, *Scheffersomyces stipitis* NCYC 1541 and *Pichia angusta* NCYC 495. Autolysis was conducted at 50°C for 48 hours in the presence of saponins from *Quillaja saponaria*. The concentrations of proteins and free amino acids in the yeast autolysates were evaluated using IR spectroscopy and chromatography. The lysates were found to be good sources of essential amino acids, which constituted

between 29.3% (S. cerevisiae) and 40.7% (K. marxianus LOCK 0026) of the amino acid pools. The largest pools of free amino acids were found in autolysates of S. cerevisiae Ethanol Red (44.9 g/L) and P. angusta NCYC 495 (40.53 g/L). Saponin can be used as an auxiliary or alternative to conventional methods of cell lysis, especially since Q. saponaria extracts are approved for use in foods and could have significant health benefits. The usability of five post-fermentation yeast strains as a source of valuable nitrogen compounds in unconventional salt-free lysates was demonstrated for the first time in the present study.

5 Kregiel D, James SA, Rygala A, Berlowska J, Antolak H, Pawlikowska E. 2017. Consortia formed by yeasts and acetic acid bacteria *Asaia* spp. in soft drinks. Antonie van Leeuwenhoek - DOI: 10.1007/s10482-017-0959-7

Yeast strains and acetic acid bacteria were isolated from spoiled soft drinks with characteristic flocs as a visual defect. Polymerase chain reaction and amplification of a partial region of the 16S rRNA gene identified the bacteria as *Asaia* spp. Sequence analysis of the large subunit (LSU) rRNA gene in turn identified the yeast isolates as *Wickerhamomyces anomalus*, *Dekkera bruxellensis* and *Rhodotorula mucilaginosa*. The hydrophobicity and adhesion properties of the yeasts were evaluated in various culture media, taking into account the availability of nutrients and the carbon sources. The highest hydrophobicity and best adhesion properties were exhibited by the *R. mucilaginosa* cells. Our results suggest that *Asaia* spp. bacterial cells were responsible for the formation of flocs, while the presence of yeast cells may help to strengthen the structure of co-aggregates.

6 Pawlikowska E, Kręgiel D. 2017. Non-conventional yeast *Metschnikowia pulcherrima* and its application in biotechnology. Postepy Mikrobiologii (open access, in Polish) 56(4):405-415.

*Metschnikowia* spp. are extensively studied "nonconventional" yeasts. Strains belonging to these genera are considered as nonpathogenic and safe. The unique properties of *Metschnikowia* spp. allow us to look at these microorganisms as a promising subject for evolutionary genetics, taxonomy, ecology, as well as a natural biocontrol agent in biotechnology. This article provides a synthesis of the systematics, morphology, ecology and physiology of *Metschnikowia* spp., with special attention to *M. pulcherrima*. These yeasts are able to produce a number of important metabolites, including organic acids, aroma compounds, oil or pulcherrimic acid. In addition, this review discusses possible applications of these non-conventional yeasts in biotechnology.

#### XI Instituto Andino Patagónico de Tecnologías Biologicas y Geoambientales (IPATEC), CONICET – UNComahue, Bariloche, Argentina. Communicated by Diego Libkind libkindfd@comahue-conicet.gob.ar>.

Recent publications.

- 1 Colabella F & Libkind D. 2016. PCR based method for the rapid identification of astaxanthinaccumulating yeast isolates of the genus *Phaffia*. Revista Argentina de Microbiología 48(1):15-20 - doi: 10.1016/j.ram.2015.10.006
- 2 Russo G, Libkind D, Giraudo MR, Delgado O. 2016. Metal capture by autochthonous yeasts from a volcanic influenced environment of Patagonia. J Basic Microbiol 56(11):1203–1211 10.1002/jobm.201600048
- 3 Peris D, Langdon Q, Moriarty RV, Sylvester K, Bontrager M, Charron G, Leducq J-B, Landry CR, Libkind D, Hittinger CT. 2016. Complex origins of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. Plos Genetics 12(7):e1006155.
- 4 Bellora N, Moliné M, David-Palma M, Coelho MA, Hittinger CT, Sampaio JP, Libkind D. 2016. Comparative genomics provides new insights into the diversity, physiology, and sexuality of the only industrially exploited tremellomycete: *Phaffia rhodozyma*. BMC Genomics 17:901.
- 5 Gonçalves M, Pontes A, Almeida P, Barbosa R, Giza M, Libkind D, Hutzler M, Gonçalves P, Sampaio J.P. 2016. Distinct domestication trajectories in top-fermenting beer yeasts and wine yeasts. Current Biology 26(20):2750–2761 10.1016/j.cub.2016.08.040
- 6 Peris D, RV Moriarty, WG Alexander, E Baker, K Sylvester, M, Sardi, QK Langdon, D Libkind, Q Wang, F-Y Bai, JB. Leducq, G Charron, CR Landry, JP Sampaio, P Goncalves, KE Hyma, JC Fay, TK Sato, CT Hittinger. 2017. Hybridization and directed evolution of diverse *Saccharomyces* species for cellulosic biofuel production. *Biotechnology for Biofuels* 10:78 10.1186/s13068-017-0763-7
- 7 Brandão LR, AB Vaz, LC Espírito Santo, R.S Pimenta, PB Morais, D Libkind, LH Rosa, CA Rosa. 2017. Diversity and biogeography patterns of yeast communities from lakes of Antarctica, Argentinean Patagonia, and tropical Brazil. Fungal Ecology 28:33-43.
- 8 Gibson B, Geertman J-M, Hittinger C, Krogerus K, Libkind D, Louis E, Magalhães F, Sampaio JP. 2017. New yeasts - new brews: modern approaches to brewing yeast design and development. FEMS Yeast Research 17(4) - DOI:doi.org/10.1093/femsyr/fox038.
- 9 Trochine, A, Turchetti B, Vaz ABM, Brandao L, Rosa LH, Buzzini P, Rosa C, Libkind D. 2017. Description of *Dioszegia patagonica*, a novel carotenogenic yeast isolated from cold environments. Int J Syst Evol Microbiol - doi: 10.1099/ijsem.0.002211
- 10 Franco DL, Canessa P, Bellora N, Risau-Gusman S, Olivares-Yañez C, Libkind D, Larrondo LF, Marpegan L. 2017. Spontaneous circadian rhythms in a cold-adapted natural isolate of *Aureobasidium pullulans*. Scientific Reports 7, N°13837.

Book chapters.

- 11 Sequeiros C, Garcés ME, Fernández M, Martínez-Díaz SF, Libkind D, Olivera NL. 2016. Microorganisms from Patagonian Aquatic Environments for Use in Aquaculture. In: Biology and Biotechnology of Patagonian Microorganisms. Springer International Publishing. Print book: 978-3-319-42799-7. Ebook: 978-3-319-42801-7. pp 205-224.
- 12 Libkind D, Moline M, Bellora N, Trochine A, de García V. 2016. Patagonian yeasts of Biotechnological relevance. In: Biology and Biotechnology of Patagonian Microorganisms. Springer International Publishing. Print book: 978-3-319-42799-7. Ebook: 978-3-319-42801-7. pp 325-351.
- 13 Sampaio JP, Pontes A, Libkind D, Huztler M. 2017. Taxonomy, Diversity, and Typing of Brewing Yeasts. Bamforth & Bokulich (Eds.) In: Brewing Microbiology: Current Research, Omics and Microbial Ecology. Horizon Scientific Press. 978-1-910190-61-6. Pp.85-117.
- 14 Libkind D, Buzzini P, Turchetti B, Rosa CA. 2017. Yeasts in continental and sea water. In: Biodiversity and Ecology of Yeasts. Yurkov A & Buzzini P (Eds). Chapter 11. Springer. In press.
- 15 Libkind D, Moliné M, Colabella F. 2017. Isolation and Selection of New Astaxanthin Producing Strains of *Phaffia rhodozyma*. En: Microbial carotenoids: Methods and Protocols, Methods in Molecular Biology Series. Barredo JL (Ed.). Humana Press. *In press*.

Books edited.

- 16 Olivera N, Libkind D, Donati R. 2016. Biology and Biotechnology of Patagonian Microorganisms. Springer International Publishing. Print: 978-3-319-42799-7. Pp 360.
- XII 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> www.chromatinepigenetics.com

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

1 Bela Vasileva, Milena Georgieva, Dessislava Staneva, Plamen Zagorchev, George Miloshev. 2016. Chromatin modulates cellular response to UV light during the process of chronological ageing. Comptes rendus de l'Academie bulgare des Sciences 69(12):1595-1602.

All processes that involve the molecule of DNA are mainly regulated by chromatin remodelling complexes (CRCs). CRCs interact with chromatin, re- model its structure and thus allow access of transcription, repair and/or replication factors to DNA. The yeast Saccharomyces cerevisiae is a preferred model organism for studies regarding chromatin structure and is a brilliant model organism in biology of ageing. An important part of CRCs is the family of actin-related proteins (Arp's). It has been shown recently that Arp4p, the actin-related protein 4 homologue in *S. cerevisiae*, mediates the interaction be- tween the CRCs and the linker histone and thus influences chromatin structure and dynamics. The aim of this study is to reveal the significance of chromatin-remodelling complexes for cellular response to UV stress during chronological ageing. The results show the importance of chromatin organization for the preservation of genome stability during cellular ageing, and moreover, the role of the linker histone in the mediation of this cellular response to UV light irradiation.

2 Milena Georgieva, Bela Vasileva, Dessislava Staneva, Plamen Zagorchev and George Miloshev. 2017. An intricate interaction between linker histones and chromatin remodelling complexes maintain genome stability, directs cellular aging and retains longevity. IUBMB Focused Meeting on "Molecular aspects and longevity", October 16th - 19th 2017, Athens, Greece.

Higher-order chromatin organization is one of the most elusive epigenetic mechanisms which attracts attention and raises intriguing questions about the role of genome organization in cellular processes like aging. Chromatin is a nucleoprotein complex which main aim is to store genetic information but also to allow its proper functioning. These dual functions of chromatin make it one of the most difficult epigenetic mechanisms to be understood in living cells. Many factors like histones, other chromatin structural and functional proteins take essential part in genome organization. The intricate interactions among them are responsible for chromatin dynamics which in turn influence all processes in a cell. Linker histories are often omitted from the general picture of chromatin but they are essential players in chromatin homeostasis as they build and moreover maintain higher levels of chromatin compaction like chromatin loops,

transcription factors and chromosome territories. These higher levels of chromatin organization are a crucial epigenetic mechanism. Linker histones interact with many chromatin proteins and thus exert their refined and very important functions. Our results show that linker histones cooperate with actin-related protein 4 (Arp4) which is an essential counterpart of several chromatin remodelling complexes like INO80 and SWR1. This cooperation proves to be critical for maintaining genome stability and cellular sensitivity to different types of stress. Abolished interaction between the linker histones and Arp4 deprive cells of normal chromatin compaction and leads them to premature aging phenotypes. These results unambiguously prove the role of linker histones in aging by its ability to pertain genome stability and allow cells to adapt to stress by inducing dynamic changes in gene expression.

3 Milena Georgieva, Borislav Popov, Svetlana Georgieva, Dessislava Staneva, George Miloshev. 2017. Epigenetic role of higher-order chromatin organization in cellular aging. IUBMB Focused Meeting on "Molecular aspects and longevity", October 16th - 19th 2017, Athens, Greece.

Aging slowly, unavoidably and negatively impacts all processes in the living organisms. The features of approaching aging can be observed at all levels in the organism and make its marks on organs, systems, tissues, cells and DNA. Apparently, the process of getting old is induced by disturbance in functioning of the genome revealed as seriously dysregulated gene expression. As regulation of gene activity is accomplished by epigenetic mechanisms, clues of aging should be searched in the environmental traits which disturb epigenetic homeostasis. Chromatin organization and dynamics are the epigenetic milieu in which eukaryotic genome implements its respective functions. Our results obtained on cells in which the gene for one of the five histones - the linker histone H1, is deleted, demonstrate severe disruption of overall chromatin organization especially at its higher-

order levels of compaction. Notably, the disintegration of the higher-order chromatin organization in the mutant leads to earlier appearance of features resembling those of a prematurely aged cells. Furthermore, we have observed quite different sensitivity of the mutant's genome to gamma radiation, which was alleviated by rejuvenating extracts from a Balkan endemic plant Haberlea rhodopensis. Our results allow us to hypothesize a direct link between cellular aging and the higher-order chromatin organization, i.e. genome stability which is underlined and controlled by chromatin compaction. This link could be pertained and manipulated by certain biologically active substances which hold the potential to reduce age-associated higher-order chromatin reorganization and thus could slow cellular aging.

#### XIII Moorganisms Bank, Iranian Biological Resource Center (IBRC), ACECR, and Department of Microbial Biotechnology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran. Communicated by Shaghayegh Nasr <<u>shaghayegh2963@yahoo.com</u>>.

Recent publications.

1 Shaghayegh Nasr, Mona Mohammadimehr, Marzieh Geranpayeh Vaghei, Mohammad Ali Amoozegar, Seyed Abolhassan Shahzadeh Fazeli and Andrey Yurkov. 2017. *Jaminaea pallidilutea* sp. nov. (Microstromatales), a basidiomycetous yeast isolated from plant material of mangrove forests in Iran. Int J Syst Evol Microbiol - DOI 10.1099/ijsem.0.002302.

In the course of an ongoing study aiming to catalogue the natural yeast biodiversity of Iran, a number of yeasts were isolated from plant material collected from mangrove forests on the shoreline of Qeshm Island. Two strains were identified as members of order Microstromatales. Standard phenotypic, biochemical, physiological characterization and a phylogenetic analyses of the combined 26S rRNA

- gene (D1/D2 domains) and ITS region sequences showed the conspecificity of these isolates and suggest their placement in the genus Jaminaea, close to *Jaminaea lanaiensis* and *Jaminaea angkoriensis*. Here, we describe this species as *Jaminiaea pallidilutea* sp. nov. with IBRC-M 30284T=DSM 104392T=CBS 14684T as the type strain. The Mycobank accession number is MB 819618.
- 2 Hoda Nouri, Hamid Moghimi, Marzieh Geranpayeh Vaghei, Shaghayegh Nasr. 2017. *Blastobotrys persicus* sp. nov., an ascomycetous yeast species isolated from cave soil. Antonie van Leeuwenhoek DOI 10.1007/s10482-017-0972-x

Two strains (AHD129-1T and AHD129-2) of a new anamorphic yeast species were isolated from Mejare cave soil samples of Abdanan, Ilam, Iran. Nucleotide divergence in the D1/D2 domain of the large subunit (LSU) rRNA, and internal transcribed spacer (ITS) genes suggest that the two strains can be assigned to the *Trichomonascus/Blastobotrys* clade. A maximum likelihood tree based on sequences of the D1/D2 domain revealed that the new species is closely related to the species *Trichomonascus ciferrii*, *Candida allociferrii*, and *Candida mucifera*. The new species could be distinguished from the closely related species by its ability to grow at 42 C and the inability to assimilate D-arabinose and D-mannitol. The name *B. persicus* sp. nov. is proposed for the new anamorphic species. The type strain of *B. persicus* is AHD129-1T = IBRC-M30238T = CBS 14259T, and the Mycobank number is MB 819148.

XIV Plant Fungal Interactions Group, Plant Biology, Viikki Plant Science Centre, Department of Biosciences, University of Helsinki, PL 65, Viikinkaari 1, 00014 Helsinki, Finland. Communicated by Kirk Overmyer <<u>kirk.overmyer@helsinki.fi>.</u>

The following is the abstract of our recently published paper.

Kai Wang, Timo P. Sipilä & Kirk Overmyer. 2016. The isolation and characterization of resident yeasts from the phylloplane of Arabidopsis thaliana. Scientific Reports 6:39403
doi:10.1038/srep39403

The genetic model plant *Arabidopsis thaliana* (arabidopsis) has been instrumental to recent advances in our understanding of the molecular function of the plant immune system. However, this work has not yet included plant associated and phytopathogenic yeasts largely due to a lack of yeast species known to interact with arabidopsis. The plant phylloplane is a significant habitat for neutral-residents, plant-growth and health-promoting species, and latent-pathogenic species. However, yeast phylloplane residents of arabidopsis remain underexplored. To address this, resident yeasts from the phyllosphere of wild arabidopsis collected in

field conditions have been isolated and characterized. A total of 95 yeast strains representing 23 species in 9 genera were discovered, including potentially psychrophilic and pathogenic strains. Physiological characterization revealed thermotolerance profiles, sensitivity to the arabidopsis phytoalexin camalexin, the production of indolic compounds, and the ability to activate auxin responses in planta. These results indicate a rich diversity of yeasts present in the arabidopsis phylloplane and have created culture resources and information useful in the development of model systems for arabidopsis-yeast interactions.

XV Manaaki Whenua Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand. Communicated by Mahajabeen Padamsee <<u>PadamseeM@landcareresearch.co.nz</u>>.

#### New Zealand school students discover new species

School students from Rongomai Primary School, Kura Kaupapa Māori o Kaikohe, and Karamu High School have identified, named and published papers on three new yeast species in Fungal Planet. Each paper, one per yeast, highlights the origins of the students' chosen name for the yeast they found. Rongomai Primary students named their yeast *Candida rongomaipounamu*, which means 'treasure of Rongomai' when translated. The students at Kura Kaupapa Māori o Kaikohe named theirs *Rhodotorula ngohengohe*, which is based on their school motto E rere, kia koi, kia ngohengohe - 'Fly, be onto it, be humble in your success'. At Karamu High School pupils spotted a third yeast new to science but were stung by wasps when collecting it from the forest, hence they named it *Candida vespimorsuum* - Latin for 'wasp stings'. The students carried out this research as part of the Unlocking Curious Minds project Discover New Life, with help from mycologists at Manaaki Whenua Landcare Research.

- 1 Buchanan, PK, van der Spuy S, Padamsee M, Weir BS, Butler T, Haines L, Pattison N, Petterson ME, and Roberts A. 2017. *Candida rongomai-pounamu sp. nov, Candida vespimorsuum sp. nov*, and *Rhodotorula ngohengohe sp. nov*. Fungal Planet description sheets: 607–609. (Persoonia - Molecular Phylogeny and Evolution of Fungi, 38:240-384. ISSN 1878-9080.)
- XVI Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC) Catedrático Agustín Escardino, 9, 46980 Paterna (Valencia), Spain. Communicated by David Peris - www.uv.es/perisnay - <david.perisnayarro@gmail.com>.

I am now a Marie Sklodowska Curie Fellow at the Institute of Agrochemistry and Food Technology (IATA) of the Spanish National Research Council.

Peris D, Moriarty RV, Alexander WG, Baker E, Sylvester K, Sardi M, Langdon QK, Libkind D, Wang QM, Bai FY, Leducq JB, Charron G, Landry CR, Sampaio JP, Gonçalves P, Hyma KE, Fay JC, Sato TK, Hittinger C. 2017. Hybridization and directed evolution of diverse *Saccharomyces* species for cellulosic biofuel production. Biotechnology for Biofuels 10:78.

Background: Lignocellulosic biomass is a common resource across the globe, and its fermentation offers a promising option for generating renewable liquid transportation fuels. The deconstruction of lignocellulosic biomass releases sugars that can be fermented by microbes, but these processes also produce fermentation inhibitors, such as aromatic acids and aldehydes. Several research projects have investigated lignocellulosic biomass fermentation by the baker's yeast Saccharomyces cerevisiae. Most projects have taken synthetic biological approaches or have explored naturally occurring diversity in S. cerevisiae to enhance stress tolerance, xylose consumption, or ethanol production. Despite these efforts, improved strains with new properties are needed. In other industrial processes, such as wine and beer fermentation, interspecies hybrids have combined important traits from multiple species, suggesting that interspecies hybridization may also offer potential for biofuel research. Results: To investigate the efficacy of this approach for traits relevant to lignocellulosic biofuel production, we generated synthetic hybrids by crossing engineered xylose-fermenting strains of S. cerevisiae with wild strains from various Saccharomyces species. These

interspecies hybrids retained important parental traits, such as xylose consumption and stress tolerance, while displaying intermediate kinetic parameters and, in some cases, heterosis (hybrid vigor). Next, we exposed them to adaptive evolution in ammonia fiber expansion-pretreated corn stover hydrolysate and recovered strains with improved fermentative traits. Genome sequencing showed that the genomes of these evolved synthetic hybrids underwent rearrangements, duplications, and deletions. To determine whether the genus Saccharomyces contains additional untapped potential, we screened a genetically diverse collection of more than 500 wild, non-engineered Saccharomyces isolates and uncovered a wide range of capabilities for traits relevant to cellulosic biofuel production. Notably, Saccharomyces mikatae strains have high innate tolerance to hydrolysate toxins, while some Saccharomyces species have a robust native capacity to consume xylose. Conclusions: This research demonstrates that hybridization is a viable method to combine industrially relevant traits from diverse yeast species and that members of the genus Saccharomyces beyond S. cerevisiae may offer advantageous genes and traits of interest to the lignocellulosic biofuel industry.

2 Peris D, Arias A, Orlić S, Belloch C, Pérez-Través L, Querol A, Barrio E. 2017. Mitochondrial introgression suggests extensive ancestral hybridization events among *Saccharomyces* species. Mol Phylog Evol 108:49-60.

Horizontal gene transfer (HGT) in eukaryotic plastids and mitochondrial genomes is common, and plays an important role in organism evolution. In yeasts, recent mitochondrial HGT has been suggested between *S. cerevisiae* and *S. paradoxus*. However, few strains have been explored given the lack of accurate mitochondrial genome annotations. Mitochondrial genome sequences are important to understand how frequent these introgressions occur, and their role in cytonuclear incompatibilities and fitness. Indeed, most of the Bateson-Dobzhansky-Muller genetic incompatibilities described in yeasts are driven by cytonuclear incompatibilities. We herein explored the mitochondrial inheritance of several worldwide distributed wild Saccharomyces species and their hybrids isolated from different sources and geographic origins. We demonstrated the existence of several recombination points in mitochondrial region COX2-ORF1, likely mediated by either the activity of the protein encoded by the ORF1 (F-SceIII) gene, a freestanding homing endonuclease, or mostly facilitated by A+T tandem repeats and regions of integration of GC clusters. These introgressions were shown to occur among strains of the same species and among strains of different species, which suggests a complex model of Saccharomyces evolution that involves several ancestral hybridization events in wild environments.

New review.

3 Peris D, Pérez-Torrado R, Hittinger CT, Barrio E, Querol A. On the origins and industrial applications of *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii* hybrids. Yeast - In press.

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.

#### XVII Departamento de Biología, Universidade Federal de Lavras, Câmpus Universitário, Caixa Postal 3037, CEP 37200-000, Lavras, Minas Gerais, Brazil. Communicated by Rosane Freitas Schwan <<u>rschwan@dbi.ufla.br</u>>.

Recent publications.

1 Igor Magalhães da Veiga Moreira, Leonardo de Figueiredo Vilela, Maria Gabriela da Cruz Pedroso Miguel, Cledir Santos, Nelson Lima & Rosane Freitas Schwan. 2017. Impact of a microbial cocktail used as a starter culture on cocoa fermentation and chocolate flavor. Molecules 22(5):766 doi:10.3390/molecules22050766

Chocolate production suffered a vast impact with the emergence of the "witches' broom" disease in cocoa plants. To recover cocoa production, many disease-resistant hybrid plants have been developed. However, some different cocoa hybrids produce cocoa beans that generate chocolate with variable quality. Fermentation of cocoa beans is a microbiological process that can be applied for the production of chocolate flavor precursors, leading to overcoming the problem of variable chocolate quality. The aim of this work was to use a cocktail of microorganisms as a starter culture on the fermentation of the ripe cocoa pods from PH15 cocoa hybrid, and evaluate its influence on the microbial communities present on the fermentative process on the compounds involved during the fermentation, and to perform the chocolate sensorial characterization. According to the results obtained, different volatile compounds were identified in fermented beans and in the chocolate produced. Bitterness was the dominant taste found in non-inoculated chocolate, while chocolate made with inoculated beans showed bitter, sweet, and cocoa tastes. 2,3-Butanediol and 2,3-dimethylpyrazine were considered as volatile compounds making the difference on the flavor of both chocolates. *Saccharomyces cerevisiae* UFLA CCMA 0200, *Lactobacillus plantarum* CCMA 0238, and *Acetobacter pasteurianus* CCMA 0241 are proposed as starter cultures for cocoa fermentation.

2 Luciana Silva Ribeiro, Maria Gabriela da Cruz Pedrozo Miguel, Suzana Reis Evangelista, Pamela Mynsen Machado Martins, Joshua van Mullem, Maisa Honorio Belizario, Rosane Freitas Schwan. 2017. Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage. Food Res International 92:26-32

Pulped Mundo Novo and Ouro Amarelo coffee beans were inoculated with *Saccharomyces cerevisiae* (CCMA 0200 and CCMA 0543) during semi-dry coffee fermentation and compared with a non-inoculated control. Samples were collected throughout the fermentation process (12 days) to evaluate the persistence of the inoculum by Real-Time quantitative PCR (qPCR). Also, the chemical composition of the beans was determined by HPLC and GC–MS and the roasted beans were sensorial evaluated using the cupping test. *S. cerevisiae* CCMA 0543 had an average population of 5.6 log cell/g (Ouro Amarelo cultivar) and 5.5 log cell/g (Mundo Novo cultivar). Citric, malic, succinic and acetic acid were found in all samples, along with sucrose, fructose, and glucose. There were 104 volatile compounds detected: 49 and 55 in green and roasted coffee, respectively. All coffee samples scored over 80 points in the cupping test, indicating they were specialty-grade. Inoculation with the CCMA 0543 strain performed better than the CCMA 0200 strain. This is the first time that qPCR has been used to assess the persistence of the inoculated strains populations during coffee processing. Strain CCMA 0543 was the most suitable as an inoculant due to its enhanced persistence during the process and number of volatile compounds produced.

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

List of papers published in 20016-2017.

- 1 Karanyicz E, Antunovics Z, Kallai Z, Sipiczki M. 2017. Non-introgressive genome chimerisation by malsegregation in autodiploidised allotetraploids during meiosis of *Saccharomyces kudriavzevii* x *Saccharomyces uvarum* hybrids. Appl Microbiol Biotechnol. 101:4617-4633.
- 2 Gangl H, Tiefenbrunner W, Pfliegler WP, Sipiczi M, Leitner G, Tscheik G, Lopandic K. 2017. Influence of artificial interspecies yeast hybrids and their F1 offspring on the aroma profile of wine. Mitteilungen Klosterneuburg 67:68-83.
- 3 Pfliegler WP, Boros E, Pazmandi K, Jakab A, Zsuga I, Kovacs R, Urban E, Antunovics Z, Bacsi A, Sipiczki M, Majoros L, Pocsi I. 2017. Commercial strain-derived clinical *Saccharomyces cerevisiae* can evolve new phenotypes without higher pathogenicity. Mol. Nutr. Food. Res., (in press)
- 4 Pataki E, Sipiczki M, Miklos I. 2017. *Schizosaccharomyces pombe* rsv1 transcription factor and its putative homologues preserved their functional homology and are evolutionarily conserved. Curr Microbiol 74:710-717, 2017

- 5 Pfliegler WP, Sipiczki M. 2016. Does fingerprinting truly represent the diversity of wine yeasts? A case study with interdelta genotyping of *Saccharomyces cerevisiae* strains. Lett Appl Microbiol. 63:406-411.
- 6 Tap RM, Ho BLS, Ramli NY, Suppiah J, Hashim R, Sabaratnam P, Ginsapu SJ, Gowbei A, Razak MFA, Sipiczki M, Ahmad N. 2016. First isolation of *Candida wangnamkhiaoensis* from the blood of immunocompromised paediatric patient. Mycoses 59:734-741.
- 7 Nasr S, Nguyen HDT, Soudi MR, Fazeli SAHS, Sipiczki M. 2016. Wickerhamomyces orientalis f. a., sp. nov.: an ascomycetous yeast species belonging to the Wickerhamomyces clade. Int J System Evol Microbiol 66:2534-2539, 2016
- 8 Sipiczki M, Tap RM. 2016. *Candida vulturna* pro tempore sp. nov., a new dimorphic yeast species related to *Candida haemulonis* species complex isolated from flowers and clinical sample. Int J System Evol Microbiol 66:4009-4015.
- 9 Sipiczki M. 2016. Visualization of fission yeast cells by transmission electron microscopy. Methods Mol. Biol 1369:97-111 doi: 10.1007/978-1-4939-3145-3 8.
- 10 Sipiczki M. 2016. Overwintering of vineyard yeasts: survival of interacting yeast communities in grapes mummified on vines. Front Microbiol 7:212 doi: 10.3389/fmicb.2016.00212.
- 11 Papp L, Sipiczki M, Miklós I. 2016. Expression pattern and phenotypic characterization of the mutant strain reveals target genes and processes regulated by pka1 in the dimorphic fission yeast *Schizosaccharomyces japonicus*. Curr Genet 63:487-497.
- 12 Pataki E, Weisman R, Sipiczki M, Miklos I. 2016. 2016. *fhl1* gene of the fission yeast regulates transcription of meiotic genes and nitrogen starvation response, downstream of the TORC pathway. Curr Genet 63:91-101.
- 13 Lopandic K, Pfliegler WP, Tiefenbrunner W, Gangl H, Sipiczki M, Sterflinger K. 2016. Genotypic and phenotypic evolution of the yeast interspecies hybrids during the high-sugar fermentation. Appl Microbiol Biotechnol 100:6331-6343.
- 14 Bellasio M, Peymann A, Steiger M, Valli M, Sipiczki M, Sauer M, Graf A, Marx H, Mattanovich D. 2016. Complete genome sequence and transcriptome regulation of the pentose utilizing yeast *Sugiyamaella lignohabitans*. FEMS Yeast Res. 16.
- 15 Sipiczki M, Pfliegler WP, Safar SVB, Morais PB, Rosa CA 2016. *Metahyphopichia laotica* gen. nov., sp. nov., a novel polymorphic yeast related to *Hyphopichia*. Int J System Evol Microbiol 66:2550-2557.
- XIX Canadian Institute of Fermentation Technology, Dalhousie University, 1360 Barrington Street, Rm. D318 A.L MacDonald Building, Sexton Campus, Halifax, Nova Scotia, Canada. Communicated by Dr. Alex Speers <a href="mailto:aspeers@Dal.ca">aspeers@Dal.ca</a>>.

Our two-hectolitre brewery has arrived and is being installed. I am pleased to announce that I was appointed as Honorary Professor at Heriot-Watt University.

Ms. Maria Josey's Viva (doctoral thesis defence) set for Dec. 14, 2017 – Quantification and Understanding of the Fermentative Ability of Re-Pitched Yeast.

Journal articles.

1 Potter G, van Wyk PWJ, Duvenhage MM, Coetsee E, Swart HC, Budge SM & Speers RA. 2017. Compositional, ultrastructural and nanotechnological characterization of the SMA strain of *Saccharomyces pastorianus*. Submitted to J Appl Environ Microbiol.

- 2 Josey M, Maskell D. & Speers RA. 2017. The impact of induced petites on lager fermentation. J Inst Brewing.
- 3 Armstrong M, MacIntosh A, Josey M, Speers RA. 2017. Examination of premature yeast flocculation in UK malts. Submitted to Brewing Science Monatsschrift für Brauwissenschaft.
- 4 Maskell PD, Speers RA & Maskell DL. 2017. Updating the accuracy of alcohol concentration determination for uncertainty of measurement calculations. Science and Justice 57(3).
- 5 Huddleston RA & Speers RA. 2017. Natural selection brewing: how to brew a winner. MBAA Technical Quarterly 54:80-86.
- 6 Niu C, Zhu L, Hill A, Speers, RA & Li Q. 2017. Construction of a highly thermostable 1,3-1,4-βglucanase by combinational mutagenesis and its potential application in the brewing industry. Biotechnology Letters. 39:1-10.
- 7 Potter G, Xia W, Budge SM & Speers RA. 2017. Quantitative analysis of 3-OH oxylipins in fermentation yeast. Can Journal Microbiol 63(2):100-109.
- 8 MacIntosh AJ, Josey M & Speers RA. 2016. An examination of substrate and product kinetics during brewing fermentations J. ASBC, 74(4):250-257 - Selected by ASBC Editor as the 'Editors Pick' and winner of the 2016 Eric Kneen Award, Am Soc Brew Chem.

Scientific Presentations.

- 9 Speers RA. 2017. Cells, Colloids, Malt and Beer. Award of Merit Invited Presentation. MBAA Meeting, Atlanta, FL.
- 10 Roy L, MacIntosh AJ, Speers RA, Paulson AT. 2017. Brewing with 100% malted buckwheat: a glutenfree alternative incorporating extraneous enzymes. Accepted for MBAA Meeting, Atlanta, FL.
- 11 Huismann M, Gormley F, Maskell DL & Speers RA. 2017. Ethanol's Effect on Terpene Extraction. 2<sup>nd</sup> International Brewers Symposium- Hop Flavor and Aroma. Corvallis, OR, July 25-28.
- 12 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.
- 13 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.
- 14 Josey M, and Speers RA. 2016. Serial re-pitched fermentations: Two case studies. World Brewing Congress. Aug. 13-17. Denver. CO.
- 15 Potter G, Swart CW, Van Wyk PWJ, Swart HC, Budge SM, Speers RA. 2016. Compositional and ultrastructural characterization of the SMA strain of *Saccharomyces pastorianus*. World Brewing Congress. Aug. 13-17. Denver, CO.
- 16 Josey M, Speers RA. 2016. Petite Mutations and their Impact of Beer Flavours. IBD Asia-Pacific. March 14-18. Sydney, AUS.

XX Food Microbiology Laboratory, Department of Microbiology, School of Life Sciences, Sikkim University (National University), 6<sup>th</sup> Mile, Tadong, Gangtok 737102, India. Communicated by Professor Dr. Jyoti Prakash Tamang <<u>jyoti tamang@hotmail.com</u>>.

The following papers and book were published during 2016-17.

1 Shah SP, Jani K, Sharma A, Anupma A, Pradhan P, Shouche Y, Tamang JP. 2017. Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India. Scientific Reports - DOI: 10.1038/s41598-017-11609-y.

*Marcha* and *thiat* are traditionally prepared amylolytic starters use for production of various ethnic alcoholic beverages in Sikkim and Meghalaya states in India. In the present study we have tried to investigate the bacterial and fungal community composition of *marcha* and *thiat* by using high throughput sequencing. Characterization of bacterial community depicts phylum *Proteobacteria* is the most dominant in both *marcha* (91.4%) and *thiat* (53.8%), followed by Firmicutes, and Actinobacteria. Estimates of fungal community composition showed *Ascomycota* as the dominant phylum. Presence of *Zygomycota* in *marcha* distinguishes it from the *thiat*. The results of NGS analysis revealed dominance of yeasts in *marcha* whereas molds out numbers in case of *thiat*. This is the first report on microbial communities of traditionally prepared amylolytic starters of India using high throughput sequencing.

2 Sha SP, Anupma A, Pradhan P, Prasad GS, Tamang JP. 2016. Identification of yeasts by PCR-mediated DGGE in *marcha*, an ethnic amylolytic starter of India. J Ethnic Foods (Elsevier) 3:292-296.

*Marcha* is an ethnic amylolytic starter that is used to ferment boiled cereals to produce alcoholic drinks, commonly called jaanr, in the Himalayan Regions of Sikkim and Darjeeling of India. Methods: The aim of this study was to investigate yeast flora of marcha collected from Sikkim in India by phenotypic characterization and polymerase chain reactiondenaturing gradient gel electrophoresis (PCR-DGGE). The average load of yeast in *marcha* was 6 x 10<sup>8</sup> colony-forming units/g. The phenotypic characterization of yeast isolates from *marcha* showed the presence of *Candida*, *Pichia*, *Torulospora*, *Schizosaccharomyces*, *Kluveromyces*, *Issatchenki*, and *Saccharomycopsis*. The PCR-DGGE bands showed the dominance of *Wickerhamomyces anomalus* (72%) and *Pichia anomalus* (28%) in *marcha*. *W. anomalus* was reported for the first time from *marcha* using PCR-mediated DGGE.

3 Tamang JP, Shin DH, Jung SJ, Chae SW. 2016. Functional properties of microorganisms in fermented foods. Frontiers in Microbiology 7:578 - doi: 10.3389/fmicb.2016.00578

Fermented foods have unique functional properties imparting some health benefits to consumers due to presence of functional microorganisms, which possess probiotics properties, antimicrobial, antioxidant, peptide production, etc. Health benefits of some global fermented foods are synthesis of nutrients, prevention of cardiovascular disease, prevention of cancer, gastrointestinal disorders, allergic reactions, diabetes, among others. The present paper is aimed to review the information on some functional properties of the microorganisms associated with fermented foods and beverages, and their health-promoting benefits to consumers.

4 Tamang JP, Holzapfel WH, Watanabe K. 2016. Review: diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology 7:377 - doi: 10.3389/fmicb.2016.00377.

Culturalable and non-culturable microorganisms naturally ferment majority of global fermented foods and beverages. Traditional food fermentation represents an extremely valuable cultural heritage in most regions, and harbors a huge genetic potential of valuable but hitherto undiscovered strains. Holistic approaches for identification and complete profiling of both culturalable and non-culturable microorganisms in global fermented foods are of interest to food microbiologists. The application of cultureindependent technique has thrown new light on the diversity of a number of hitherto unknown and noncultural microorganisms in naturally fermented foods. Functional bacterial groups ("phylotypes") may be reflected by their mRNA expression in a particular substrate and not by mere DNA-level detection. An attempt has been made to review the microbiology of some fermented foods and alcoholic beverages of the world.

#### New books.

5 Tamang JP. 2016. Ethnic Fermented Foods and Alcoholic Beverages of Asia. Springer, New Delhi, 409 pp. - ISBN: 978-81-322-2798-4.

Asia has a long history of preparation and consumption of various types of ethnic fermented foods and alcoholic beverages based on available raw substrates of plant or animal sources and also depending on agroclimatic conditions of the regions. About 90% of the Asian ethnic fermented foods are naturally fermented by both culturable and unculturable microorganisms. Diversity of functional microorganisms in Asian ethnic fermented foods and alcoholic beverages consists of bacteria (lactic acid bacteria and Bacillus species, micrococci, etc.), amylolytic and alcohol-producing yeasts, and filamentous molds. Microorganisms establish on relevant substrates for survival and produce bioactive compounds that enrich the human diet, thereby promoting health benefits to consumers. This book has 15 chapters covering different types of ethnic fermented foods and alcoholic beverages of Asia. I tried to cover all Asian countries for this book, but could not get contributors for book chapters from many countries. However, I am grateful to all contributing authors who accepted our invitation to write this book. Some of them are well-known scientists and researchers with vast experiences in the field of fermented foods and beverages. We are happy to bring all of them in the same platform, bringing out

this book, and thanks to Prof. Tek Chand Bhalla, Dr. Namrata Thapa, and Dr. Savitri (India); Prof. Yearul Kabir and Dr. Mahmud Hossain (Bangladesh); Prof. Tika Karki, Dr. Pravin Ojha, and Dr. Om Prakash Panta (Nepal); Dr. Saeed Akhtar, Dr. Majid Hussain, Dr. Tariq Ismail, and Dr. Muhammad Riaz (Pakistan); Prof. Sagarika Ekanayake (Sri Lanka); Dr. Werasit Sanpamongkolchai (Thailand); Prof. Sh.Demberel, Dr. D. Narmandakh, and Dr. N. Davaatseren (Mongolia); Dr. Yoshiaki Kitamura, Dr. Ken-Ichi Kusumoto, Dr. Yukio Magariyama, Dr. Tetsuya Oguma, Dr. Toshiro Nagai, Dr. Soichi Furukawa, Dr. Chise Suzuki, Dr. Masataka Satomi, Dr. Kazunori Takamine, Dr. Hisanori Tamaki, and Dr. Sota Yamamoto (Japan); Prof. Dong-Hwa Shin, Prof. Cherl-Ho Lee, Dr. Young-Myoung Kim, Dr. Wan-Soo Park, Dr. Jae-Ho Kim, and Dr. Moonsil Lee Kim (South Korea); Dr. Maryam Tajabadi Ebrahimi, Dr. Neda Mollakhalili Meybodi, and Dr. Amir Mohammad Mortazavian (Iran); Dr. Francisco B. Elegado, Dr. Maria Teresa M. Perez, Dr. Shara Mae T. Colegio, Dr. Charina Grace B. Banaav, Dr. Bernadette C. Mendoza, Dr. Vanessa Marie T. Lim, Dr. Andrea Therese R. Gervasio, and Dr. Marilen P. Balolong (Philippines); Prof. Ingrid Survanti Surono (Indonesia); and Dr. Vu Nguyen Thanh and Dr. Nguyen Thi Viet Anh (Vietnam).

#### XXI School of Science, Engineering & Tech, Abertay University, Dundee DD1 1HG, Scotland, UK. Communicated by Graeme M Walker <<u>g.walker@abertay.ac.uk</u>>.

The following are recent publications.

Book.

1 Walker GM, Abbas C, Ingledew WM & Pilgrim C (Eds) 2017. The Alcohol Textbook, 6<sup>th</sup> Edition. Duluth, Georgia: Ethanol Technology Institute. 592 pp. ISBN: 978-0-692-93088-5 - Available through www.lbds.com

Special Edition journal article.

2 Walker GM and Stewart GG (Eds) 2016 Beverages. Special Issue on Saccharomyces cerevisiae. MDPI AG Publishers, Basel, Switzerland. http://www.mdpi.com/journal/beverages/special issues/saccharomyces cerevisiae Journal papers.

- 3 Nasidi M, Agu R, Deeni Y and Walker GM. 2016. Utilisation of whole sorghum crop residues for bioethanol production. J Inst Brewing 122:268-277.
- 4 Walker GM and Stewart GG. 2016. *Saccharomyces cerevisiae* in production of fermented beverages. Beverages 2(4):30 - doi:10.3390/beverages2040030
- 5 Walker GM and Hill A. 2016. *Saccharomyces cerevisiae* in production of whisky. Beverages 2(4):38 doi:10.3390/beverages2040038
- 6 Nayyar A, Walker GM, Canetta E, Wardrop F and Adya AK. 2017. Influence of cell surface and nanomechanical properties on the flocculation ability o industrial *Saccahromyces cerevisae* strains. J Food Res 6(5) - doi:10.5539/jfr.v6n5p1

Book Chapters:

- 7 Walker G. 2017. Physiology of ethanol-producing yeasts. In: The Alcohol Textbook, 6<sup>th</sup> Edition. Eds.: Walker GM, Abbas C, Ingledew WM & Pilgrim, C. Duluth, Georgia: Ethanol Technology Institute. Chapter 17, pp. 257-271.
- 8 Akunna, J. and Walker, G. 2017. Co-products from distilled spirit production and their utilisation. In: *The Alcohol Textbook*, 6<sup>th</sup> Edition. Eds: Walker, G.M, Abbas, C, Ingledew, WM & Pilgrim C. Duluth, Georgia: Ethanol Technology Institute. Chapter 34, pp. 529-537.

New PhD project (commencing October, 2017) - Martina Daute (MSc, Technical University of Munich). Collaboration between Abertay University and Scotch Whisky Research Institute. The aim is to investigate the potential of biologically diverse yeasts to create desirable flavours during whisky fermentation, while also giving the efficient alcohol production required by the industry.

Outline: Scotch Whisky legislation prohibits the addition of any flavourings, with all sensory characteristics (aroma and taste) being naturally generated during the production process. A wide range of flavour compounds, or congeners, are created during fermentation. Examples of these compounds include: higher alcohols (such as isoamyl alcohol), esters (such as ethyl acetate), acids (such as succinic acid), vicinal diketones (such as diacetyl), sulphur compounds (such as dimethyl sulphide), and phenolics (such as 4-vinyl guiaicol). The choice of yeast strain is crucially important in dictating the level of these congeners in the new-make spirit prior to whisky maturation. However, the industry is currently very conservative in terms of yeast use. A limited range of yeast supply companies provide the whisky production sector in Scotland with all Saccharomyces cerevisiae distilling yeasts. Nevertheless, whisky producers are increasingly interested in diversifying flavour in response to changing consumer demands, though production targets (alcohol yields) still need to be met.

This project will address industry aspirations by providing a fundamental understanding of the potential of a wide range of diverse yeast species to catalyse desirable flavour reactions. Work will focus on non-Saccharomyces yeasts, which have not previously been investigated in cereal-based, whisky fermentations. Such yeasts have been shown to produce a range of interesting flavour-active metabolites including esters, terpenols, lactones, higher alcohols and acids and some strains have applications in other fermented beverages (wine, rum etc). Based on findings from other industries, such as wine producers, it is anticipated that the project will identify several non-Saccharomyces yeasts with the potential to influence whisky flavour. This study will additionally explore the potential of using co-cultures (i.e. distilling strains of S. cerevisiae together with a non-Saccharomyces strain), either concurrently or sequentially, to give desired flavour in tandem with required alcohol vield.

New PhD Graduate - My son, Roy Walker, has successfully defended his thesis at Edinburgh University, October 11, 2017.

9 Walker RMK. De novo engineering of a tRNA neochromosome in yeast

#### XXII Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany - http://www.dsmz.de. Communicated by AM Yurkov <a href="mailto:andrey.yurkov@dsmz.de">andrey.yurkov@dsmz.de</a>>.

Recently published papers.

1 Nasr S, Mohammadimehr M, Vaghei MG, Amoozegar MA, Fazeli SAS, Yurkov A. 2017. *Jaminaea pallidilutea* sp. nov. (Microstromatales), a basidiomycetous yeast isolated from plant material of mangrove forests in Iran. Int J Syst Evol Microbiol 67:4405-4408.

In the course of an ongoing study aiming to catalogue the natural yeast biodiversity of Iran, a number of yeasts were isolated from plant material collected from mangrove forests on the shoreline of Qeshm Island. Two strains were identified as members of order Microstromatales. Standard phenotypic, biochemical, physiological characterization and a phylogenetic analyses of the combined 26S rRNA gene (D1/D2 domains) and ITS region sequences showed the conspecificity of these isolates and suggest their placement in the genus *Jaminaea*, close to *Jaminaea lanaiensis* and *Jaminaea angkoriensis*. Here, we describe this species as *Jaminiaea pallidilutea* sp. nov. with IBRC-M  $30284^{T} = DSM \ 104392^{T} = CBS$  $14684^{T}$  as the type strain. The Mycobank accession number is MB 819618.

2 Yurkov AM, Dlauchy D, Peter G. 2017. *Meyerozyma amylolytica* sp. nov. from temperate deciduous trees and the transfer of five *Candida* species to the genus *Meyerozyma*. Int J Syst Evol Microbiol 67:3977-3981.

In the course of two independent studies three veasts have been isolated from temperate deciduous trees in Hungary and Germany. Analyses of nucleotide sequences of D1/D2 domains of the 26S rRNA gene (LSU) suggested that these strains belong to the Meyerozyma clade in Debaryomycetaceae (Saccharomycetales). The phylogenetic analysis of a concatenated alignment of the ITS region and LSU gene sequences confirmed the placement of the three strains in the Meyerozyma clade close to Candida elateridarum. If mixed in proper combinations, the strains formed one to two hat shaped ascospores in deliquescent asci. In addition to the ascospore formation, the three studied strains differed from Candida elateridarum and other members of the Meyerozyma clade in terms of ribosomal gene

sequence and some physiological properties. To accommodate the above-noted strains, we describe the new species as Meverozvma amvlolvtica sp. nov. (holotype: DSM 27310<sup>T</sup>; ex-type cultures: NCAIM  $Y.02140^{T} = MUCL 56454^{T}$ , allotype: NCAIM Y.01955<sup>A</sup>; ex-allotype culture: DSM 27468), MB 821663. Additionally, we propose the transfer of five non-ascosporic members of the Meverozyma clade to the genus Meyerozyma as the following new taxonomic combinations Meyerozyma athensensis f.a., comb. nov. (MB 821664), Meyerozyma carpophila f.a., comb. nov. (MB 821665), Meyerozyma elateridarum f.a., comb. nov. (MB 821666), Meyerozyma neustonensis f.a., comb. nov. (MB 821667), and Meverozyma smithsonii f.a., comb. nov. (MB 821668).

3 Pontes A, Röhl O, Maldonado C, Yurkov AM, Sampaio JP. 2017. Cryptotrichosporon argae sp. nov., Cryptotrichosporon brontae sp. nov. and Cryptotrichosporon steropae sp. nov., isolated from forest soils. Int J Syst Evol Microbiol 67:3610-3614.

Yeast strains belonging to the basidiomycetous genus *Cryptotrichosporon* were isolated from forest soils in Serra da Arrábida Natural Park in Portugal. Similar to the already-known representatives of this genus, the new isolates formed pigmented colonies of a distinctive pale orange colour. Phylogenetic analyses employing concatenated sequences of the D1/D2 domains of the 26S (large subunit) rRNA gene and the internal transcribed spacer (ITS) region supported the recognition of three novel species: *Cryptotrichosporon*  argae sp. nov. (type strain CM  $19^{T}$  = CBS  $14376^{T}$  = PYCC  $7010^{T}$  = DSM  $104550^{T}$ ; MycoBank accession number MB 817168), *Cryptotrichosporon brontae* sp. nov. (type strain CM  $1562^{T}$  = CBS  $14303^{T}$  = PYCC  $7011^{T}$  = DSM  $104551^{T}$ ; MycoBank accession number MB 817077) and *Cryptotrichosporon steropae* sp. nov. (type strain OR  $395^{T}$  = CBS  $14302^{T}$  = PYCC  $7012^{T}$  = DSM  $104552^{T}$ ; MycoBank accession number MB 817078).

4 Schulz M, Sicker D, Schackow O, Hennig L, Yurkov A, Siebers M, Hofmann D, Disko U, Ganimede C, Mondani L, Tabaglio V, Marocco A. 2017. Interspecies-cooperations of Abutilon theophrasti with root colonizing microorganisms disarm BOA-OH allelochemicals. Plant Signaling & Behavior 12(8):e1358843.

microbial micro-community Α facultative, colonizing roots of Abutilon theophrasti Medik. supports the plant in detoxifying hydroxylated benzoxazolinones. The root micro-community is composed of several fungi and bacteria with Actinomucor elegans as a dominant species. The yeast Papiliotrema baii and the bacterium Pantoea ananatis are actively involved in the detoxification of hydroxylated benzoxazolinones by generating H<sub>2</sub>O<sub>2</sub>. At the root surface, laccases, peroxidases and polyphenol oxidases cooperate for initiating polymerization reactions, whereby enzyme combinations seem to differ depending on the hydroxylation position of BOA-OHs. A glucosyltransferase, able to glucosylate the natural

5 genera: Ascomycota Fungal Diversity 86(1):1-594.

Knowledge of the relationships and thus the classification of fungi, has developed rapidly with increasingly widespread use of molecular techniques, over the past 10-15 years, and continues to accelerate. Several genera have been found to be polyphyletic, and their generic concepts have subsequently been emended. New names have thus been introduced for species which are phylogenetically distinct from the type species of particular genera. The ending of the separate naming of morphs of the same species in 2011, has also caused changes in fungal generic names. In order to facilitate access to all important changes, it was desirable to compile these in a single document. The present article provides a list of generic names of Ascomycota (approximately 6500 accepted names published to the end of 2016), including those which are lichen-forming. Notes and summaries of the changes since the last edition of 'Ainsworth & Bisby's Dictionary of the Fungi' in 2008 are provided. The notes include the number of accepted species, classification, type species (with location of the type material), culture availability, life-styles, distribution, and selected publications that have appeared since

benzoxazolinone detoxification intermediates BOA-5and BOA-6-OH, is thought to reduce oxidative overshoots by damping BOA-OH induced H<sub>2</sub>O<sub>2</sub> generation. Due to this detoxification network, growth of Abutilon theophrasti seedlings is not suppressed by BOA-OHs. Polymer coats have no negative influence. Alternatively, quickly degradable 6-hydroxy-5nitrobenzo[d]oxazol-2(3H)-one can be produced by the micro-community member Pantoea ananatis at the root surfaces. The results indicate that Abutilon theophrasti has evolved an efficient strategy by recruiting soil microorganisms with special abilities for different detoxification reactions which are variable and may be triggered by the allelochemical's structure and by environmental conditions.

Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H et al. 2017. Notes for

2008. This work is intended to provide the foundation for updating the ascomycete component of the "Without prejudice list of generic names of Fungi" published in 2013, which will be developed into a list of protected generic names. This will be subjected to the XIXth International Botanical Congress in Shenzhen in July 2017 agreeing to a modification in the rules relating to protected lists, and scrutiny by procedures determined by the Nomenclature Committee for Fungi (NCF). The previously invalidly published generic names Barriopsis, Collophora (as Collophorina), Cryomyces, Dematiopleospora, Heterospora (as Heterosporicola), Lithophila, Palmomyces (as Palmaria) and Saxomyces are validated, as are two previously invalid family names, Bartaliniaceae and Wiesneriomvcetaceae. Four species of *Lalaria*, which were invalidly published are transferred to Taphrina and validated as new combinations. Catenomycopsis Tibell & Constant. is reduced under Chaenothecopsis Vain., while Dichomera Cooke is reduced under Botryosphaeria Ces. & De Not. (Art. 59).

6 Handel S, Wang T, Yurkov AM, König H. 2016. Sugiyamaella mastotermitidis, sp. nov. and Papiliotrema odontotermitidis f.a., sp. nov. from the gut of the termites Mastotermes darwiniensis and Odontotermes obesus. Int J Syst Evol Microbiol 66(11):4600-4608.

Two novel yeast species were isolated from the guts of two different termite species. A new member of the genus *Sugiyamaella* was isolated from the hindgut and nest material of the lower Australian termite *Mastotermes darwiniensis*. The second novel yeast species, isolated from the higher termite *Odontotermes obesus*, was identified as a member of the genus *Papiliotrema*. Both yeast species were able to hydrolyse xylan, methylumbelliferyl  $\beta$ -xylobiose and methylumbelliferyl  $\beta$ -xylotriose. The ability to debranch different hemicellulose side chains and growth without the addition of external vitamins was

- observed. A symbiotic role of the novel yeast species is indicated, especially in respect to xylan degradation and the production of vitamins. Here, we describe these species as *Sugiyamaella mastotermitis* sp. nov., MycoBank 816574 (type strain MD39V<sup>T</sup> = DSM  $100793^{T}$  = CBS 14182<sup>T</sup>), and *Papiliotrema odontotermitis* f.a., sp. nov., MycoBank 816575 (type strain  $OO5^{T}$  = DSM  $100791^{T}$  = CBS 14181<sup>T</sup>). Additionally, we transfer *Candida qingdaonensis* to the genus *Sugiyamaella* and propose the following combination: *Sugiyamaella qingdaonensis* f.a., comb. nov., MycoBank 816576.
- 7 Yurkov AM, Wehde T, Federici J, Schäfer AM, Ebinghaus M, Lotze-Engelhard S, Mittelbach, M, Prior R, Richter C, Röhl O, Begerow D. 2016. Yeast diversity and species recovery rates from beech forest soils. Mycological Progress 15(8):845-859.

Soil yeasts are globally diverse. They are found in almost all soil types, and the structure of soil yeast communities reflects aboveground vegetation properties. Cultivation techniques have often been successfully employed to study yeasts in forest soils. However, few studies have addressed the variation of soil yeast communities in space and time; especially, structural dynamics at a forest site between different seasons is unknown. Here, we analyse the results from our field experiments performed in 2008 and 2009. We reassess species inventory data and identify potential new species. Using improved species lists. we estimate the rate of species recovery from beech forest soils with a particular focus on repeated sampling. Our analyses showed that the number of observed yeast species was steadily increasing after one, two and three samplings. The observed diversity was likely approaching saturation after four samplings.

Additionally, we provide formal descriptions of new veast species isolated from forest soils in Germany during these studies, as 30 % of the observed species represented undescribed taxa. The following taxonomic novelties are proposed: Colacogloea demeterae Yurkov, Schäfer & Begerow sp. nov. (MB 816166), Slooffia velesii Federici, Röhl & Begerow sp. nov. (MB 816165), Hamamotoa cerberi Yurkov, Schäfer & Begerow sp. nov. (MB 816164), Hamamotoa telluris Yurkov, Schäfer & Begerow sp. nov. (MB 816163), Piskurozyma yama Richter, Mittelbach & Begerow, sp. nov. (MB 816162), Piskurozyma tuonelana Lotze-Engelhard, Richter & Begerow sp. nov. (MB 816161), Dioszegia dumuzii Ebinghaus, Prior & Begerow sp. nov. (MB 816160), and Chernovia houtui Federici, Yurkov & Begerow gen. nov. et sp. nov. (MB 816158, MB 816159).

8 Boundy-Mills KL, Glantschnig E, Roberts IN, Yurkov A, Casaregola S, Daniel HM, Groenewald M, Turchetti B. 2016. Yeast culture collections in the twenty-first century: New opportunities and challenges. Yeast 33(7): 243-260.

The twenty-first century has brought new opportunities and challenges to yeast culture collections, whether they are long-standing or recently established. Basic functions such as archiving, characterizing and distributing yeasts continue, but with expanded responsibilities and emerging opportunities. In addition to a number of well-known, large public repositories, there are dozens of smaller public collections that differ in the range of species and strains preserved, field of emphasis and services offered. Several collections have converted their catalogues to comprehensive databases and synchronize them continuously through public

services, making it easier for users worldwide to locate a suitable source for specific yeast strains and the data associated with these yeasts. In-house research such as yeast taxonomy continues to be important at culture collections. Because yeast culture collections preserve a broad diversity of species and strains within a species, they are able to make discoveries in many other areas as well, such as biotechnology, functional, comparative and evolution genomics, bioprocesses and novel products. Due to the implementation of the Convention of Biological Diversity (CBD) and the Nagoya Protocol (NP), there are new requirements for both depositors and users to ensure that yeasts were collected following proper procedures and to guarantee that the country of origin will be considered if benefits arise from a yeast's utilization. Intellectual property rights (IPRs) are extremely relevant to the current access and benefit-sharing (ABS) mechanisms; most research and development involving genetic resources and associated traditional knowledge will be subject to this topic.

9 Mittelbach M, Yurkov AM, Stoll R, Begerow D. 2016. Inoculation order of nectar-borne yeasts opens a door for transient species and changes nectar rewarded to pollinators. Fungal Ecology 22(8):90-97.

Nectar-borne yeast communities are species poor assemblages compring is a few specialized taxa (Saccharomycotina) and many transient species. Short flower lifetimes and harsh environmental conditions impose an enormous pressure on nectar-colonizers, which try to overcome these challenges through fast multiplication and osmotolerance. Since these traits are exclusively known for ascomycetes, the origin of multi-species communities is still poorly understood. We conducted field and laboratory experiments to analyze the competition between autochthonous pollinator-borne and transient yeast species in nectar.

- Subsequently we analyzed the impact of microbial growth on the environment. Our results endorse theories on priority effects and show that yeast incidences in natural flowers, cell densities in microcosms and the environmental impact strongly depend on the inoculation order of the respective yeast species. Transient species are more frequent in flowers visited only once, while specialists require several flower visits to establish common population structures most probably through tough inner-floral competition.
- 10 Pontes A, Röhl O, Carvalho C, Maldonado C, Yurkov AM, Sampaio JP. 2016. *Cystofilobasidium intermedium* sp. nov. and *Cystofilobasidium alribaticus* f.a. sp. nov. isolated from Mediterranean forest soils. Int J Syst Evol Microbiol 66:1058-1062.

Multiple isolates belonging to the basidiomycetous genus *Cystofilobasidium* were obtained from forest soils in Serra da Arrábida Natural Park in Portugal. Phylogenetic analyses employing concatenated sequences of the D1/D2 domain and ITS region support the recognition of two novel species: *Cystofilobasidium alribaticum* f.a., sp. nov. (type strain CBS 14164<sup>T</sup> = PYCC 6956<sup>T</sup> = DSM 101473<sup>T</sup>) and *Cystofilobasidium intermedium* sp. nov. (type strain CBS  $14089^{T}$  = PYCC  $6856^{T}$  = DSM  $101474^{T}$ ). Whereas *C. alribaticum* f. a. sp. nov. does not form hyphae, even when different strains are crossed, C. intermedium sp. nov. is self-fertile and forms mycelium with teliospores that upon germination give rise to slender basidia. The most remarkable physiological trait of the two novel species is their ability to grow at 35 °C, a property not observed for remaining species of the genus.

11 Yurkov AM., Röhl O, Pontes A, Carvalho C, Maldonado C, Sampaio JP. 2016. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. FEMS Yeast Research 16(2).

Soil yeasts represent a poorly known fraction of the soil microbiome due to limited ecological surveys. Here, we provide the first comprehensive inventory of cultivable soil yeasts in a Mediterranean ecosystem, which is the leading biodiversity hotspot for vascular plants and vertebrates in Europe. We isolated and identified soil yeasts from forested sites of Serra da Arrábida Natural Park (Portugal), representing the Mediterranean forests, woodlands and scrub biome. Both cultivation experiments and the subsequent species richness estimations suggest the highest species richness values reported to date, resulting in a total of 57 and 80 yeast taxa, respectively. These values far exceed those reported for other forest soils in Europe. Furthermore, we assessed the response of yeast diversity to microclimatic environmental factors in biotopes composed of the same plant species but showing a gradual change from humid broadleaf forests to dry maquis. We observed that forest properties constrained by precipitation level had strong impact on yeast diversity and on community structure and lower precipitation resulted in an increased number of rare species and decreased evenness values. In conclusion, the structure of soil yeast communities mirrors the environmental factors that affect aboveground phytocenoses, aboveground biomass and plant projective cover. Other publications.

12 Mittelbach M, Yurkov AM, Begerow D. 2016. Adaptive anonymity: Crypsis as an evolutionary trait of floral yeasts? bioRxiv 088179.

Nectar-dwelling yeast and bacteria are common inhabitants of flowers and evidently involved in pollination. The limited number of floral plantpollinator models studied to date reveal inconsistent conclusions on microbial effects, but coincide with respect to high microbial specificity: while bacteria reduce visitation frequencies of pollinators, nectarborne specialist yeasts (in contrast to allochthonous or transient species) impose none or even a beneficial effect on flower visitation. However, these findings are in conflict with the strong impact of these predominantly fermenting organisms on the nectar environment. In order to cope with the ultimate dependency of nectar-dwellers on repeated transportation by foragers as a result of early floral senescence, the modifications of nectar associated with specialist growth have been interpreted as adaptations that suit forager's preferences. But, the development of

foraging preferences to either axenic flowers or flowers colonized by specialist microorganisms would lead to a dead-end for nectar-dwellers, as the probability of inoculation into new suitable habitats would be reduced. Based on a critical survey of the available literature and an additional pollinator experiment where we find that the allochthonous species Cryptococcus victoriae negatively affects attraction and rewarding of floral visitors, while the specialist yeast Metschnikowia reukaufii does not, we propose the hypothesis that nectar-borne yeasts may have evolved to blend into their environment avoiding detection by pollinators, following the ecological concept of crypsis. Although, neither chemical nor olfactory crypsis has been reported for nectar-borne microorganisms, the attention to this mechanism in yeast dispersal needs to be directed in future studies.

Books.

- 13 Buzzini P, Lachance MA, Yurkov A. (eds.). 2017. Yeasts in natural ecosystems: Ecology. Springer, Heidelberg; eBook ISBN: 978-3-319-61575-2
- 14 Buzzini, P., Lachance, M.A., Yurkov, A. (eds.). 2017. Yeasts in natural ecosystems: Diversity. Springer, Heidelberg; eBook ISBN: 978-3-319-62683-3

Book chapters.

15 Yurkov A, Pozo MI. 2017. Yeast community composition and structure. Yeasts in Natural Ecosystems: Ecology pp. 73-100.

Yeasts are globally distributed, but different species occur in different climates and environments. With a few exceptions, yeasts do not occur in their natural environments as a pure culture but co-occur with other microscopic eukaryotes and prokaryotes and comprise microbial communities. The observed yeast diversity in natural environments is a combined result of the response of each species to habitat conditions, including arrival, growth, and further dispersal, and the biotic interactions among species. In this chapter, we review some recent concepts and tools developed in community ecology and discuss how they may help understand yeast diversity in nature. We address species recognition approaches and the effects of the intraspecific variation and application of molecular operational taxonomic units on the yeast community parameters. Community ecology tools discussed in this chapter include diversity (taxonomic and functional), quantity, priority effects, species richness estimators, and species-abundance distribution. Additionally, we compare the use of community composition and community structure parameters in the literature. Concepts such as frequent (vs. rare), autochthonous (vs. transient or allochthonous) and specialist (vs. generalist) yeast species are also discussed through this chapter.

16 Yurkov, A. 2017. Temporal and geographic patterns in yeast distribution. Yeasts in Natural Ecosystems: Ecology pp. 101-130.

The famous hypothesis formulated by Beijerinck and Baas Becking, 'Everything is everywhere, [but] the environment selects', has dominated microbiological research and directed it towards the search of ecological factors as the main determinants of microbial community composition. The apparent lack of geographic distribution patterns in microorganisms (ubiquity) is traditionally explained by their adaptive (physiological) flexibility and ease of dispersal. Strong disproof of yeast ubiquity comes from studies on yeasts associated with beetles, drosophilids, bees, and short-lived flowers. The current knowledge suggests that geographical barriers, insect vectors, and host plants are important factors determining distribution of

17 Begerow D, Kemler M, Feige A, Yurkov A. 2017. Parasitism in yeasts. Yeasts in Natural Ecosystems: Ecology pp. 179-210.

Yeasts are common in all habitats and interact with dead and living substrates such as plants, animals, and fungi. Besides their saprobic capabilities, parasitic interactions of yeasts and yeast-like organisms were brought into focus through enhanced/new species discovery that expanded our knowledge about phylogenetic relationships of yeasts and parasitic fungal lineages. Especially common dimorphism of many Basidiomycota goes along with an alternating saprobic yeast stage and parasitic filamentous stage. Interestingly, this seems to be a common feature not only for plant parasites but also for animal and fungal

18 Yurkov A. 2017. Yeasts in forest soils. Yeasts in Natural Ecosystems: Diversity, 87-116.

Soil yeasts are common inhabitants of various soils, including those in forest biotopes. Historically, yeasts were studied mainly in vineyard, orchard and agricultural soils. Due to limited ecological surveys, yeasts represent yet a poorly known fraction of the microorganisms in forest soils. Our knowledge of soil yeasts is biased towards temperate and boreal forests, whereas data from Africa, Americas and Asia is scarce. Forest soils in the Southern hemisphere are strongly undersampled. This chapter provides the first comprehensive review of yeasts in forest soils, their

19 Kemler M, Witfeld F, Begerow D, Yurkov A. 2017. Phylloplane yeasts in temperate climates. Yeasts in Natural Ecosystems: Diversity pp. 171-197.

Yeasts are integral parts of phylloplane communities of temperate regions, where ecosystems are not only influenced by short-term fluctuations in abiotic conditions, but additionally by cyclic seasonal changes. Phylloplane yeasts possess physiological adaptations, such as pigmentation and extracellular polysaccharides that enable them to resist harsh conditions encountered in these environments. Additionally, through production of plant hormonelike metabolites, they also might influence the behavior, fitness, and growth of their plant host. Here yeasts in their natural habitats. This chapter provides examples of the larger-scale distribution of yeasts in the environment, including endemism, latitudinal gradients, distance-decay relationships, and Holarctic and bipolar distributions. The influence of geographic factors on reproductive isolation in yeast populations is additionally addressed in this chapter. Temporal changes such as ecological successions and seasonal dynamics of yeast communities are also discussed.

parasites. Even some Ascomycota share this character. The chapter aims to provide an overview of the most relevant parasites among yeast species and lineages. For this we summarize the most recent literature to initiate further studies and to provide ideas for common patterns and strategies. As can be seen in this chapter, the knowledge differs between animal parasites, plant parasites, and mycoparasites leaving space for new research and hypotheses. However, it is apparent that the comparison of the three different host groups provides interesting insights of common features and concepts.

actions with other community members.

services. Basidiomycetes are dominant in forest soils, but ascomycetes genera, including several fermenting yeasts, are also permanent residents in the soil. A particular focus in the chapter is dedicated to the review of yeast diversity after reclassification of previously polyphyletic yeast genera *Cryptococcus*, *Rhodotorula* and *Trichosporon*. Factors influencing distribution of soils yeasts are also discussed in this chapter.

we review how the understanding of yeasts in this

environment has improved in the last years due to

discoveries in new habitats, new developments in

taxonomy, but also the application of environmental

sequencing and genomics. These new technologies, as

well as traditional approaches, have made it clear that yeasts are not only occupying this environment to gain

nutrients, but they are active participants that shape the

structure of microbial communities by diverse inter-

diversity, nutrition, traits and possible ecosystem

20 Groenewald M, Boundy-Mills K, Čadež N, Endoh R, Jindamorakot S, Pohl-Albertyn C, Rosa CA, Turchetti B, Yurkov A. 2017. Census of Yeasts Isolated from Natural Ecosystem and Conserved in Worldwide Collections. Yeasts in Natural Ecosystems: Diversity pp. 455-476.

There are many well-known public yeast repositories as well as a large number of smaller, lessknown collections worldwide; most of these are with the primary goal to preserve the yeast biodiversity in a specific region and the strains from a range of species that are important environmental strains, food spoilage organisms, or strains that play a role in food preparation and human or animal pathogens. In order to have an overview on how many yeast strains are isolated from natural ecosystems and are preserved in collections worldwide, curators of public and private fungal/yeast culture collections were contacted to participate in this survey. Curators of 41 collections from 27 countries supplied data representing a total of 58,095 strains. This includes information on the collection itself, type of environment the strains were isolated from, the countries of origin of the strains, and also the taxonomic information. The ecosystems that are well represented according to the data of preserved strains in the participating collections are plants, insects/invertebrates, aquatic habitats, soil, and extreme cold and extreme warm/dry habitats. The strains have been isolated from a large number of countries worldwide (countries of origin), but it is clear that many parts of the world's ecosystems are not yet well sampled for yeast diversity. A challenge during this survey was to list the genera and species due to the current and constant changes in taxonomic names. The outcome of this survey is discussed in this chapter.

#### Recent presentations.

21 Yurkov AM. 2016. Diversity, distribution and functions of yeasts in soils. 43<sup>rd</sup> Annual Conference on Yeasts, Smolenice, Slovakia, 10-13 May 2016.

Almost a half of the total terrestrial carbon is concentrated in boreal and temperate forests. These stocks are located in soils and dead plant material in form of complex substances, decomposed largely by fungi. Fungi living in soils can be divided in two functional groups: filamentous fungi that are multicellular and can form large mycelial networks and yeasts that are predominantly unicellular. Yeasts are an artificial group of fungi and belong to both the Asco- and Basidiomycota. While filamentous fungi may transport nutrients and water over long distances, yeasts are more locally dependent on environmental conditions but normally respond faster to environmental changes. Yeasts inhabit soils worldwide and basidiomycetous yeasts are among the dominating fungi in soil substrates as revealed by cultureindependent surveys. Since traditionally yeasts were considered as able to degrade only simple carbon compounds like sugars, their occurrence in soils was originally considered to be haphazard. However, unlike the typical saccharolytic phenotype often attributed to yeasts, basidiomycetous species are able to utilize a wide spectrum of carbon sources, including intermediates of lignin degradation, phenols and heterocyclic compounds. Assimilation of lignin and

cellulose derivatives, oligotrophy, and psychrotolerance imply that yeasts could play a role in the decomposition process, especially in cold regions or at low temperatures. Formation of polysaccharide capsules further helps yeasts to sequester and concentrate nutrients and sustain low water activity or freezing. These compounds also play a role in soil aggregation and stability. Recent studies on soil yeasts have shown that climate, basic soil parameters and rainfall determine the size, diversity and structure of the yeast community. However, in a single biome, forest properties and land management have a superior role over basic environmental factors. In forests, aboveground deadwood deposition support development of typical soil-borne species. Although soil-borne communities are usually species-poor, spatial and temporal fragmentation result in high diversity numbers with a remarkably high proportion of potential new yeast species. With a selection of case studies we will exemplify how biodiversity assessments are enlarging our knowledge of yeast inhabiting natural ecosystems. This work was supported in part by the Fundação para a Ciência e a Tecnologia (Portugal), projects PTDC/BIA-MIC/113051/2009, PTDC/BIA-BIC/4585/2012.

22 Yurkov AM, Guerreiro M, Pontes A, Fonseca Á, Sampaio JP. 2016. Multigene assessment of the species boundaries in Tremellomycetes. 14<sup>th</sup> International Congress on Yeasts (ICY14), Awaji Island, Japan, 11-15 September 2016.

Introduction: Sequencing of ribosomal gene regions has revolutionized identification of yeast species. However, a number of reports suggest that partial sequences of rRNA may not be sufficient to distinguish yeast species. Furthermore, very little is known on their intraspecific genetic variability or about the population structure of yeasts in Tremellomycetes, which are often considered as putatively asexual even though they are phylogenetically intermingled with members of sexual genera (Liu et al. 2015). Results and Discussion: Here, we studied strains from different locations and substrates, and used a multilocus sequencing (MLS) approach to reassess species boundaries in Papiliotrema (P. flavescens, P. terrestris), Vishniacozyma (V. victoriae, V. carnescens) and Solicoccozyma (S. aeria, S. terrea). We determined the discriminatory power of some frequently used loci: the LSU rRNA gene, the ITS region, the IGS1 spacer, and fragments of the genes RPB1, RPB2 and TEF1. We performed phylogenetic network analyses to detect recombining (potentially sexual) and clonal lineages. Additionally, we attempted to sequence mating type

(MAT) genes to verify the results of MLSA. In the genera *Papiliotrema* and *Solicoccozyma* the first universal fungal DNA-barcode (ITS) had a discriminatory power lower than LSU, suggesting a two-barcode system to be used in the future. We detected recombining and clonal lineages, and revealed several cryptic species within studied groups. The two MAT genes (*STE3* and *SXI1/SXI2*) sequenced for *P. flavescens* strains confirmed the potential for sexual reproduction and suggest the presence of a tetrapolar mating system with a biallelic pheromone/receptor locus and a multiallelic HD locus (Yurkov *et al.* 2015).

References:

- Liu XZ, Wang QM, Göker M, et al. 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. Studies in Mycology 81:85-147.
- Yurkov A, Guerreiro MA, Sharma L, et al. 2015. Multigene assessment of the species boundaries and sexual status of the basidiomycetous yeasts *Cryptococcus flavescens* and *C. terrestris* (Tremellales). PloS One 10: e0120400.

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

Recent publications.

- 1 Morais CG, Batista TM, Kominek J, Borelli M, Furtado C, Moreira RG, Franco GR, Rosa LH, Fonseca C, Hittinger CT, Lachance MA, Rosa CA. 2017. *Spathaspora boniae* sp. nov., a D-xylose-fermenting species in the *Candida albicans-Lodderomyces* clade. Int J Syst Evol Microbiol 67:3798-3805.
- 2 Vega C, Albaladejo RG, Guzmán B, Steenhuisen SL, Johnson SD, Herrera CM, Lachance MA. 2017. Flowers as a reservoir of yeast diversity: description of *Wickerhamiella nectarea* sp. f.a. nov., and *Wickerhamiella natalensis* sp. nov., f.a. from South African flowers and pollinators, and transfer of related *Candida* species to the genus *Wickerhamiella* as new combinations. FEMS Yeast Res - doi: 10.1093/femsyr/fox054.

Flowers offer favourable microenvironments for yeast growth, and are increasingly recognised as a rich source of novel yeast species. Independent surveys of yeasts associated with flowers and pollinators in South Africa led to the discovery of 38 strains of two new species. Physiological profiles and analysis of the internal transcribed spacer and the D1/D2 domains of the large subunit rRNA gene showed that they represent two novel species that belong to the *Wickerhamiella* clade. We describe the species as *Wickerhamiella* nectarea f.a. sp. nov. (type strain EBDCdVSA11-1T, CBS 14162T, NRRL Y-63791T) and *W. natalensis* f.a. sp. nov. (type strain EBD CdVSA7-1T, CBS 14161T, NRRL Y-63790T). We extend the known range of flower-associated *Wickerhamiella* species to South Africa and discuss the ecology and phylogenetic relationships of the clade in relation to its host species and biogeography. Examination of growth characteristics supports that the *Wickerhamiella* clade exhibits a high degree of evolutionary lability, and that specialisation to different niches may occur rapidly. We review the current status of floral yeast biodiversity and nectar as a reservoir of species diversity, and the importance of pollinators and biogeography. In addition, 18 species formerly assigned to the genus *Candida* are reassigned formally to the genus *Wickerhamiella*.

# **Recent meetings**

# 44<sup>th</sup> Annual Conference on Yeasts (44th ACY) Smolenice Castle, Slovakia, May 2-5 2017

The 44<sup>th</sup> Annual Conference on Yeasts (44th ACY) was held 2-5 May 2017 at Smolenice Castle, Slovakia. Detail information on the abstracts book are available at <u>http://yeastconference.sk/archive</u>.



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

ICY Commissioners Meeting, Tuesday, June 27, 2017 33<sup>rd</sup> International Specialised Symposium on Yeasts (ISSY33) University College Cork, Cork, Ireland



### **Minutes of Meeting**

Present (38): Hiroshi Takagi (ICY Chair), Charles Abbas (ICY Vice-Chair), John Morrissey (ISSY33 Chair), Florian Bauer, Teun Boekhout, Eckhard Boles, Pietro Buzzini, Neža Čadež, Sylvie Dequin, Hüseyin Erten, Patrick Fickers, Angelica Ganga, Lisa Granchi, Anne Gschaedler, Lene Jespersen, Vladimir Jiranek, Marc-André Lachance, Diego Libkind, Diethard Mattanovich, Leda Mendonca-Hagler, Vladimir Mrsa, Jens Nielsen, Steve Oliver, Volkmar Passoth, Merja Penttilä, Uros Petrovic, James du Preez, Bernard Prior, Jack Pronk, Amparo Querol, Alexander Rapoport, Ian Roberts, Rosane Schwan, Andriy Sibirny, Nitnipa Soontorngun, Hana Sychrová, Johan Thevelein, Graeme Walker.

Apologies (37): Tiina Alamäe, Jacobus Albertyn, Feng-Yan Bai, Javier Carvajal Barriga, Greg Bartosz, Peter Biely, Monique Bolotin, Kyria Boundy-Mills, Charoen Charoenchai, Heide-Marie Daniel, Li-Lin Du, Jean-Marie Francois, Liliana Godoy, Ji-Sook Hahn, Ivan Hapala, Thomas W. Jeffries, Hyun Ah Kang, Yona Kassir, Ida J. van der Klei, Akihiko Kondo, Matti Korhola, Cletus Kurtzman, Anna Maraz, Sally Ann Meyer, Gennadi Ivanovich Naumov, Elena Naumova, Patrícia Lappe Oliveras, Jose Martinez Peinado, Gabor Peter, Isak S. Pretorius, Peter Raspor, Doris Rauhut, Patrizia Romano, Jose Paulo Sampaio, Ana Clara Schenberg, Katherine Smart, Teresa Zoladek.

#### **Chair's Opening Remarks**

Dr. Hiroshi Takagi welcomed the delegates to the meeting and mentioned the apologies for those who could not attend. He thanked Dr. John Morrissey and the Organizing Committees for the excellent job regarding ISSY33 and presented the agenda. He also reported on ICY14 held in Japan in 2016. Surplus

funds from ICY14 have been made available for the forthcoming ISSY33-35 (2017-2019), ICY15 (2020), and the meeting on Non-Conventional Yeasts (2018).

#### New Commissioners

Dr. Takagi introduced three candidates for ICY membership: Dr. Brigitte Gasser, who is Assistant Professor of Microbial Systems Biotechnology, University of Natural Resources and Life Sciences. Vienna, Austria, and is an expert on protein production with Pichia pastors, nominated by Prof. Diethard Mattanovich; Dr. Ramón González, who is Full Professor at the Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño, Spain, and is an expert on wine yeast metabolism, genetics, and biotechnology, nominated by Prof. Amparo Querol; Dr. Evodia Setati, who is a Senior Researcher at the Institute for Wine Biotechnology at Stellenbosch University, South Africa, and is an expert on yeast relevant to wine science, nominated by Prof. Bernard Prior. Each candidate provided his/her CV with a list of publications and two Letters of Recommendation from a relevant National or International Society and current members of ICY. These documents were distributed electronically to Commissioners, who could express their opinions on candidates. All proposed candidates received full support from Commissioners. After a short discussion, the Commissioners unanimously elected Drs. Gasser, González and Setati as new ICY members.

The nomination process was reconfirmed as follows: the nominator will send the ICY Chair the necessary documents as attached files by email at least one month before an ICY meeting. The necessary documents are (i) a CV, (ii) a letter of recommendation from an appropriate local scientific society and (iii) a letter of recommendation from a Commissioner. The last two documents must appear on the letterhead of the society or the Commissioner's institution and include scanned signatures (Head or Secretary General of society and Commissioner). The ICY Chair will forward them to all Commissioners, who should read them before the ICY meeting. Diffusion of the documents should ideally be as a web-link (Dropbox) or a ZIP file, to avoid the flood of emails. Commissioners should communicate their opinion (pro and con) to the Chair and not by "Reply All" in advance of the meeting, at which time a vote will be held.

#### **ICY Membership Criteria**

Regarding membership, the Commissioners exchanged opinions and made a variety of proposals/ideas by email before ISSY 33. It is clear that Commissioners from different nations vary in their frequency of attendance at ICY/ISSY due to financial or other reasons. Based on several suggestions, Dr. Takagi proposed the following ICY membership criteria:

If a Commissioner cannot participate in an ICY meeting, he/she should send apologies and relevant proxy to the Chair. This would be enough to maintain one's status as Commissioner.

If a Commissioner has been silent for 4 years or has not given good reasons for not attending the ICY/ISSY, he/she should be replaced. If a Commissioner has been active in research and Commission work, his/her membership will continue.

After discussion, these criteria were accepted provisionally by attending Commissioners. Although the criteria became effective as of the meeting held at ISSY 33, further discussion is not excluded.

#### **Updates for Future ISSY/ICY**

Individual organizers provided progress reports on meeting preparation. First, Dr. Andriy Sibirny provided an update for Non-Conventional Yeasts meeting to be held in Poland (May 15-18, 2018). Next, Dr. Diego Libkind and Dr. Hüseyin Erten presented updates for ISSY34 and ISSY35 to be held in Argentina (October 1-4, 2018) and Turkey (October 6-10, 2019), respectively. Finally, Dr. Diethard Mattanovich presented an update on ICY15 to be held in Austria (August 23-27, 2020).

#### **Suggestions for a future ISSY**

Dr. Hiroshi Takagi reported that one of the candidate venues for ISSY36 in 2021 is North

America, as many recent ISSY have been held in Europe, namely Slovakia (2013), Slovenia (2014), Italy (2015) and Ireland (2017). Dr. Charles Abbas outlined the plan and concept for ISSY36 to be held in North America, possibly Vancouver (Canada), Seattle (Washington), or the Bay Area (California). The Commissioners endorsed Dr. Abbas's proposal. Dr. Vivien Measday (University of British Columbia, Vancouver) will be nominated and approved as a new Canadian Commissioner at the next meeting in Argentina, 2018. She has been approached and is willing to organize ISSY36.

#### **Boot of ICY Website**

Following a suggestion by Dr. Teun Boekhout, Dr. Hiroshi Takagi has contacted Dr. Rob Samson, the secretary-general of IUMS, who will assist to boot the official website of ICY without any charges. It would be best if several pages were made available for ICY in the IUMS website. Dr. Samson will need the text and other material (what is ICY, the current council and programs, and announcements of the next meetings) for setting them up. The Yeast Newsletter, prepared by Dr. Marc André Lachance, can be included at the website. Dr. Peter Raspor also proposed to upload the list of current commissioners, past commissioners, and supporters of the Yeast Commission. Dr. Takagi announced that the ICY website will be opened within the year.

#### **Relationship with IUMS**

ICY has been recognized under the umbrella of IUMS, which consists of three divisions, Bacteriology and Applied Microbiology (BAM), Mycology and Eukaryotic Microbiology (MEM), and Virology. ICY is one of five International Commissions within MEM. At the IUMS meeting in Montreal (2014), there was a suggested change to the naming of the Mycology Division to encompass other eukaryotic systems. ICY has considered a few options, namely 1) to continue under IUMS with the new change with a reduced focus on mycology (including yeasts), 2) to ask to be recognized as an independent entity within IUMS that is yeast focused, or 3) to establish ourselves independently of IUMS. Dr. Hiroshi Takagi expressed the opinion that it is good for ICY to be under the umbrella of IUMS in terms of beneficial exchange of science and personnel with other microbial societies.

#### Introduction of 'The Yeasts' Project

Dr. Teun Boekhout presented an interesting project for 'The Yeasts, a Taxonomic Study' to the commissioners, edited by him. The future version of "The Yeasts" will become an open access, electronic, continuously updated system for yeast biodiversity and function. The system will be built by the researcher community and has not identified a publisher at this stage. He also aims to diversify topics, with entries on food, clinical aspects, protocols, and also biotech. Many Commissioners have prominent positions in those fields and may contribute in the future. Dr. Boekhout wishes to begin with the biodiversity, but hopes to expand later to the other areas. Such a system may also be very useful for teaching, *i.e.*, Yeast Academy. After discussion, the Commissioners approved that 'The Yeasts' project be placed under

Minutes presented by:

Dr. Hiroshi Takagi ICY Chair ICY and IUMS. This moral support may also help to find corporate and other funding. When congresses make a profit in the future, some money will be made available for 'The Yeasts' as this can become a flagship understaking for ICY.

#### **Chair's Closing Remarks**

On behalf of the ICY, Dr. Hiroshi Takagi expressed his gratitude once again to Dr. John Morrissey and his staff for the excellent meeting, with a well balanced and well organized scientific and cultural program. The ICY meeting was closed.

Dr. Charles Abbas ICY Vice-Chair

# **Forthcoming Meetings**

## 45<sup>th</sup> Annual Conference on Yeasts (45th ACY) Smolenice Castle, Slovakia, May 15-18 2018

The 45<sup>th</sup> Annual Conference on Yeasts (45th ACY) is being planned for 15-18 May 2018 at Smolenice Castle, Slovakia. On-line registration will open at <u>http://yeastconference.sk/</u> in December. Emília Breierová

#### Non-conventional Yeasts: from Basic Research to Application Rzeszow University; Poland, 15-18 May 2018

This conference is organised by Andrei Sibirny and his colleagues and has a programme on nonconventional yeasts with the following sessions: (1) Genomics, Transcriptomics, Proteomics, and Systems Biology; (2) Systematics, Evolution and Ecology; (3) Sensing, Signaling, Stress; (4) Response and Membrane Transport; (5) Intracellular Traffic, Secretion, Organelles, Autophagy; (6) Production of Heterologous Proteins; (7) Metabolic Engineering for Food, Chemicals and Pharmaceuticals; (8) Nonconventional Yeasts for Biofuels (9) Host Design, Analytical and Other Applications. Please visit the conference website for more details <u>http://nonconventionalyeasts2018.pl</u> John Morrissey

#### 34th International Specialized Symposium on Yeast Yeast Odyssey: From Nature to Industry October 1-4 2018, Bariloche, Patagonia, Argentina



The meeting "Yeast Odyssey: From Nature to Industry" is the 34th International Specialized Symposium on Yeast (ISSY33), a conference series organized under the auspices of the International Commission on Yeasts (ICY) (www.issy34-bariloche.com). Organization of the meeting is being supported by several national and international scientific societies.

ISSY34 will take place in the beautiful city of Bariloche, in north-west Patagonia, Argentina. Bariloche is located at the base of the Andes, very close to Chile, and is served by an international airport located 10km from the city centre and conference venue. The meeting itself is being held at the recently

renovated NH Edelweiss Hotel, which is at walking distance from downtown (Link). The plenary sessions will be held in the Hotel Condor auditorium. Such a meeting has never before been held in Argentina during its 53 years history and it is an honour to be given the opportunity to host this event. In 2018, your favorite meeting will be held in a place you always wanted to visit: *Patagonia* 



The scientific focus of the meeting is "Yeast Odyssey: From Nature to Industry".

The topics of the meeting will cover (1) Ecology and biodiversity of yeasts; (2) Bioprospection of extremophilic yeasts; (3A) Yeasts in food and beverages: traditional fermentations; (3B) Yeasts in food and beverages: industrial fermentations; (4) Evolutionary genomics and domestication of yeasts; (5) Industrial applications of non-conventional yeasts; (6) Yeasts in Biorefineries (7) Natural variation and applied opportunities; (8) Genetic and metabolic improvement of yeasts.

Confirmed speakers: André Lachance, José Paulo Sampaio, Chris Hittinger, Luis Larrondo, Rosane Schwan, Nina Gunden-Cimerman, Carole Camarasa, Lene Jespersen, John Morrisey, Francisco Girio, Hiroshi Takagi and many others.

The location selected for ISSY34 provides a unique opportunity for experiencing Patagonia, by far one of the most appealing destinations worldwide.

**Pre and Post ISSY related events.** ISSY 34 will be connected with two important events to be held in the same city and venue: Jornadas Sudamericanas de Biologia y Biotecnología de Levaduras - Southamerican meeting in yeast Biology and Biotechnology (30th September 2018), and the International Workshop on Brewing Yeasts (5-6th October), which will be sponsored by ASBC. The latter event will feature the participation as speakers of Kevin Vestrepen, Mathias Huztler, Brian Gibson, and Chris Powell, among others. Sponsorship and exhibition opportunities for the latter are available too upon request.

For updates and other details, please consult our website: <u>www.issy34-bariloche.com</u>. Facebook: <u>https://www.facebook.com/issy34/</u>

Diego Libkind <<u>libkindfd@comahue-conicet.gob.ar</u>>

# **New Books**

Buzzini P, Lachance M-A & Yurkov A. 2017. Yeasts in Natural Ecosystems: Ecology, Springer, Heidelberg, Germany.

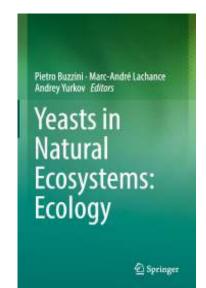
This book presents an up-to-date review of the ecology of yeast communities in natural ecosystems. It focuses on their biological interactions, including mutualism, parasitism, commensalism and antagonistic interactions, and is closely connected with the volume Yeasts in Natural Ecosystems: Diversity by the same editors. Yeasts are the smallest eukaryotic organisms successfully growing under a wide range of environmental conditions. They constantly modify the environment through their own metabolic activities. Although yeasts are

among the earlier colonizers of nutrient-rich substrates, their role in ecosystem processes is not limited to the consumption and transformation of simple sugars. They also engage in close relationships with animals, plants and other fungi in the environment as mutualists, competitors, parasites and pathogens. This book reviews the diversity of biological interactions and roles of yeasts in ecosystems and summarises recent concepts and tools developed in community ecology. All of the chapters were written by leading international yeast research experts, and will appeal to researchers and advanced students in the field of microbial ecology.

# **Table of Contents**

- Yeasts as Distinct Life Forms of Fungi
- Yeast Habitats: Different but Global
- Yeast Community Composition and Structure
- Temporal and Geographic Patterns in Yeast Distribution
- Biogeography and Ecology of the Genus Saccharomyces
- Mutualism in Yeasts
- Parasitism in Yeasts
- Commensalism: The Case of the Human Zymobiome
- Antagonistic Interactions and Killer Yeasts

http://www.springer.com/it/book/9783319615745



Buzzini P, Lachance M-A. Yurkov A. Yeasts in Natural Ecosystems: Diversity, Springer, Heidelberg, Germany, 2017.

This book focuses on the diversity of yeasts in aquatic and terrestrial ecosystems, including the association of yeasts with insects, invertebrate and vertebrate animals. It offers an overview of the knowledge accumulated in the course of more than 60 years of research and is closely connected with the volume Yeasts in Natural Ecosystems: Ecology by the same editors. In view of the rapid decline of many natural habitats due to anthropogenic activities and climate change, the need to study biodiversity is pressing. Rising temperatures threaten species inhabiting cold and aquatic environments, and species in terrestrial ecosystems are endangered by habitat fragmentation or loss. Most of our knowledge of intrinsic properties (autoecology) of yeasts reported throughout this book is derived from laboratory experiments with pure cultures. Accordingly, the importance of culture collections for ecological studies is highlighted by presenting an overview of worldwide available yeast strains and their origins. All of the chapters were written by leading international yeast research experts, and will appeal to researchers and advanced students in the field of microbial diversity.

# **Table of Contents**

- Yeasts in Continental and Seawater
- Yeasts in Aquatic Ecotone Habitats
- Yeasts in Forest Soils
- Yeasts in Agricultural and Managed Soils '
- Yeast in Anthropogenic and Polluted Environments
- Phylloplane Yeasts in Temperate Climates
- Phylloplane Yeasts in Tropical Climates
- Yeasts in Cacti and Tropical Fruit
- Yeasts Associated with Decomposing Plant Material and Rotting Wood
- Yeasts in Hypersaline Habitats
- Yeasts in Polar and Subpolar Habitats

Pietro Buzzini - Marc-André Lachance Andrey Yurkov *Editors* 

# Yeasts in Natural Ecosystems: Diversity

Springer

- Yeasts in Nonpolar Cold Habitats
- Yeasts in Insects and Other Invertebrates
- Yeasts in Birds
- Census of Yeasts Isolated from Natural Ecosystem and Conserved in Worldwide Collections

http://www.springer.com/it/book/9783319626826

# 50 Years Ago

Y E A S T A News Letter for Persons Interested in Yeast January 1968 Volume XVI, Number 2 Editor Herman J. Phaff, University of California, Davis, California Associate Editor Leslie R. Hedrick, Illinois Institute of Technology, Chicago, Illinois Associate Editor F. M. Clark, University of Illinois, Urbana, Illinois Associate Editor Cecil G. Dunn, Massachusetts Institute of Technology, Cambridge, Massachusetts

**Dr. D. Yarrow** of CBS, the Netherlands, reported that type strains of fifteen new yeast species were deposited in the collection: *Candida koshuensis*, *C. shehatae*, *C. tepae*, *Cryptococcus dimmenae*, *C. kutzingii*, *C. lactativorus*, *C. albidus* var. *ovalis*, *Endomycopsis fukushimae*, *Endomycopsis musicola*, *Pichia zaruensis*, *P. stipitis*, *Saccharomyces cerevisiae* var. *terrestris*, *S. silvestris*, *Sporobolomyces coprophilus*, and *Torulopsis peltata*.

**Dr. M. C. Pignal**, Université de Lyon, France published four papers related to classification of yeasts in the genera *Pichia* and *Kluyveromyces*, and new species of *Candida*. Also, Dr. Kockova-Kratocvilova visited the facility in September 1967.

**Dr. H. J. Phaff** of the University of California Davis shared recent publications. Miller et al. (1967 J. Bacteriol. 94: 258-259) used microdissections of asci to show that most species of *Metschnikowia* contain two needle-shaped spores instead of one, as formerly believed. A manuscript was submitted to Mycologia on the taxonomy of *Metschnikowia*. Sporulation and mating of several species of *Candida* and *Chlamydozyma* was achieved, leading to transfer of several species to *Metschnikowia* and description of *M. pulcherrima* and *M. reukaufii*. Fell and Phaff co-authored the description of *Cryptococcus dimennae*, *C. kutzingii* and *C. lactativorus* in Antonie van Leeuwenhoek (1967). Miss Manuela Vidal Leiria completed her M.S. work on yeast relationships by cell wall analysis.

**Dr. A. Stenderup** of the University of Aarhus, Denmark presented a paper at the 4<sup>th</sup> Meeting of the International Society for Human and Animal Mycology on deoxyribonucleic acid-base composition of *Candida* species. The melting temperature and G+C content of several *Candida* species were listed.

**Dr. H. Gutz** of the Southwest Center for Advanced Studies, Division of Biology, Dallas, Texas USA published in Science (1967, 158:796) an unexpected phenomenon he called "twin meiosis": asci formed from two diploid strains of *Schizosaccharomyces pombe* of compatible mating type contain eight spores rather than the normal four.

**Dr. A. P. James** of Chalk River Nuclear Laboratories, Ontario, Canada published seven papers related to DNA repair after radiation-induced damage.

**Dr. J. G. Kleyn** of the University of Puget Sound, Tacoma, Washington USA described the new \$4 million science complex, Thompson Hall, which houses microbiology teaching and research facilities.

**Professor S. P. Meyers** of the University of Miami, Florida, USA published a study of yeasts isolated from twelve sites in the North Sea from 1964 to 1966. Dominant yeasts were *Debaryomyces hansenii*, *Rhodotorula rubra* and *Candida diddensii*. Populations varied from <10 to>3000 viable cells/L, with higher concentrations in summer months.

**Dr. L. R. Hedrick** of the Illinous Institute of Technology, Chicago, IL, USA published a description of yeasts and molds in water and sediments in Lake Ontario in the Proceedings, Tenth Conference on Great Lakes Research, 1967.

Dr. Mercedes R. Edwards published work on the micromorphology of Cryptococcus neoformans.

**Professor Hiroshi Iizuka** is preparing for publication the 200-page "JFCC Catalogue of Cultures", the Japanese Federation of Culture Collections of Microorganisms. Iizuka and Iida presented a report on decane oxidation by *Candida rugose* at the Seventh International Congress of Biochemistry in Tokyo.

**Dr. H. Onishi**'s laboratory at the Noda Institute for Scientific Research, Japan studied aerobic dissimilation of carbohydrate materials by yeasts. Polyalcohol production by yeasts included conversion of D-xylose to xylitol, L-arabinose to L-arabinitol and D-ribose to ribitol by *Candida polymorpha*.

**Dr. Z. Böszörmenyi** of Eötvös University, Budapest communicated that the Czechoslovakian and Hungarian Academies of Sciences held a round-table discusson in Balatonálmadi (Hungary) on "Transport processes in microbial, plant and animal cells" in October 1967. A. Kotyk (Institute of Microbiology, Prague) reviewed the kinetics of sugar transport in yeast. M. Höfer discussed energetics of transport processes in *Rhodotorula gracilis*, and T. Deák (Budapest) reported on transport of monosaccharides in *Hansenula subpelliculosa*.

**Drs. J. F. T. Spencer** and **A. J. Gorin** (Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada) found differences in the structure of mannans extractable from several *Trichosporon* species.

**Dr. Heikki Suomalainen** (Research Laboratories of the State Alcohol Monopoly, Helsinki, Finland) listed four publications related to preparation of mitochondria and protoplasts, and free nucleotides in baker's yeast.

**Dr. B. C. Rankine** of the Australian Wine Research Institute listed eight publications about AWRI's examination of products of fermentation by a large number of yeasts. Products include hydrogen sulphide, pyruvic acid, fusel oils. Recent additions to the AWRI yeast collection were also described.

**Dr. E. Minarik** (Institut de Recherches Viti-Vinicoles, Bratislava, Czechoslovakia) summarized a recently published paper about biologic destruction of malic acid in fermenting grape juice by *Schizosaccharomyces* species. The Committee for Yeasts of the Czechoslovak Microbiological Society held their First Annual Meeting December 7-8, 1967 in Bratislava.

**Dr. Morio Akaki** (University of Mie, Japan) published a paper on the relationship between growth of *Saccharomyces sake* and aeration and agitation conditions in a 240-liter tank, related to manufacturing yeast for sake production.

**Dr. T. O. Wiken** announced the upcoming Third International Symposium on Yeasts, Delft, The Netherlands, June 2-7, 1969. Topics include taxonomy, cytology, genetics, ecology, pathology and immunology, technology, nutrition and growth, metabolism, and enzymology.

The International Conference on Culture Collections, sponsored by UNESCO, was scheduled for October 7-12, 1968 in Tokyo, Japan. The aims of the conference were to review present conditions of the field, and contribute to the formation of an international network of culture collections.

The Second International Symposium on Yeast Protoplasts was scheduled for late August 1968 in Brno, Czechoslovakia.

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