

Yeast

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

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Associate Editors

Patrizia Romano
Universitas Mercatorum
Piazza Mattei 10
00186 Rome
Italy

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

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Editorial

Lex Scheffers

We recently learnt of the death of our esteemed colleague Lex Sheffers at age 95. A generous contributor to the Yeast Commission, Lex organized a successful general Yeast Symposium at the Papendal Sport Centre in Arnhem in 2000. An enthusiastic supporter of the integration of all aspects of yeast research and the founding editor of *FEMS Yeast Research*, Lex was an excellent yeast researcher and a gentleman. Jack Pronk kindly authorized me to reproduce his message to the yeast researcher community.

Is *Debaryomyces* a yeast?

A recent *Science* article* reported that *Debaryomyces hansenii* may play a role in Crohn's disease, as it interferes with the repair of injured intestinal tissue in mice. Crohn's is a debilitating condition that has hitherto resisted treatment. The identification of a yeast as a potential contributing agent may represent a breakthrough in understanding the elusive disorder. One wonders, however, whether a yeast will get the recognition (fame or infamy) that it deserves, as the article omits any allusion to the fact that *Debaryomyces* is a genus of yeasts, referring to it only as a fungus, which is of course not incorrect. In the same article, *Saccharomyces cerevisiae* is honoured as a yeast that is "commonly found in human gut microbiota", but *Candida tropicalis* and *Candida albicans* both join *Debaryomyces* as mere fungi. *Candida famata* is cited as a synonym of *D. hansenii*, whose presence in cheese is regarded as noteworthy.

*Jain *et al.* 2021. *Debaryomyces* is enriched in Crohn's disease intestinal tissue and impairs healing in mice. *Science* 371:1154-1159 - doi: 10.1126/science.abd0919

M.A. Lachance, Editor

The following papers have been published.

- 1 Pawlikowska E, Kolesińska B, Nowacka M, Kregiel D. 2020. A new approach to producing high yields of pulcherrimin from *Metschnikowia* yeasts. *Fermentation* 6:114
<https://doi.org/10.3390/fermentation6040114>

Pulcherrimin, a red iron chelate, is produced by some yeasts and bacteria. It plays important ecological roles in many ecosystems, including growth control, biofilm inhibition and photoprotection. In this study, fifteen yeast strains of the genus *Metschnikowia* were characterized based on their production of pulcherrimin. Yeast pulcherrimin was isolated and its purity assessed using ¹H nuclear magnetic resonance

spectroscopy. Under experimental conditions, pulcherrimin formation varied depending on both the tested strains and culture media. The best producers formed up to 240 mg/L of pulcherrimin in minimal medium with glucose as the carbon source, supplemented with 0.05% FeCl₃ and 0.1% Tween 80. This study presents a new approach to producing high yields of pulcherrimin from yeasts.

- 2 Liszkowska W, Berlowska J. 2021. Yeast Fermentation at low temperatures: adaptation to changing environmental conditions and formation of volatile compounds (review). *Molecules* 26:1035.
<https://doi.org/10.3390/molecules26041035>

Yeast plays a key role in the production of fermented foods and beverages, such as bread, wine, and other alcoholic beverages. They are able to produce and release from the fermentation environment large numbers of volatile organic compounds (VOCs). This is the reason for the great interest in the possibility of adapting these microorganisms to fermentation at reduced temperatures. By doing this, it would be possible to obtain better sensory profiles of the final products. It can reduce the addition of artificial flavors and

enhancements to food products and influence other important factors of fermented food production. Here, we reviewed the genetic and physiological mechanisms by which yeasts adapt to low temperatures. Next, we discussed the importance of VOCs for the food industry, their biosynthesis, and the most common volatiles in fermented foods and described the beneficial impact of decreased temperature as a factor that contributes to improving the composition of the sensory profiles of fermented foods.

Recent publication.

- 1 Golubev WI. 2021. Mycocinotyping of *Cyberlindnera* species. *Microbiology (Moscow)* 90:223-225.

According to their sensitivity to *Wickerhamomyces anomalus* mycocins, *Cyberlindnera spp.* may be subdivided into two groups, one comprising heterothallic species with hat-shaped ascospores and

another containing homothallic ones with Saturn-shaped ascospores. The type strain of a species, its synonyms and anamorphs have identical reactions to mycocins.

Recent publications.

- 1 Shen XX, Li Y, Hittinger CT, Chen XX, Rokas A. 2020. An investigation of irreproducibility in maximum likelihood phylogenetic inference. *Nat Commun* 11:6096
DOI: 10.1038/s41467-020-20005-6

Phylogenetic trees are essential for studying biology, but their reproducibility under identical parameter settings remains unexplored. Here, we find that 3515 (18.11%) IQ-TREE-inferred and 1813 (9.34%) RAxML-NG-inferred maximum likelihood (ML) gene trees are topologically irreproducible when executing two replicates (Run1 and Run2) for each of 19,414 gene alignments in 15 animal, plant, and fungal phylogenomic datasets. Notably, coalescent-based ASTRAL species phylogenies inferred from Run1 and Run2 sets of individual gene trees are topologically irreproducible for 9/15 phylogenomic datasets, whereas concatenation-based phylogenies inferred

twice from the same supermatrix are reproducible. Our simulations further show that irreproducible phylogenies are more likely to be incorrect than reproducible phylogenies. These results suggest that a considerable fraction of single-gene ML trees may be irreproducible. Increasing reproducibility in ML inference will benefit from providing analyses' log files, which contain typically reported parameters (e.g., program, substitution model, number of tree searches) but also typically unreported ones (e.g., random starting seed number, number of threads, processor type).

- 2 Haase MAB, Kominek J, Opulente DA, Shen XX, LaBella AL, Zhou X, DeVirgilio J, Hulfachor AB, Kurtzman CP, Rokas A, Hittinger CT. 2021. Repeated horizontal gene transfer of *GAL*actose metabolism genes violates Dollo's law of irreversible loss. *Genetics* 217:iyaa012
DOI: 10.1093/genetics/iyaa012

Dollo's law posits that evolutionary losses are irreversible, thereby narrowing the potential paths of evolutionary change. While phenotypic reversals to ancestral states have been observed, little is known about their underlying genetic causes. The genomes of budding yeasts have been shaped by extensive reductive evolution, such as reduced genome sizes and the losses of metabolic capabilities. However, the extent and mechanisms of trait reacquisition after gene loss in yeasts have not been thoroughly studied. Here, through phylogenomic analyses, we reconstructed the evolutionary history of the yeast galactose utilization pathway and observed widespread and repeated losses of the ability to utilize galactose, which occurred

concurrently with the losses of *GAL*actose (*GAL*) utilization genes. Unexpectedly, we detected multiple galactose-utilizing lineages that were deeply embedded within clades that underwent ancient losses of galactose utilization. We show that at least two, and possibly three, lineages reacquired the *GAL* pathway via yeast-to-yeast horizontal gene transfer. Our results show how trait reacquisition can occur tens of millions of years after an initial loss via horizontal gene transfer from distant relatives. These findings demonstrate that the losses of complex traits and even whole pathways are not always evolutionary dead-ends, highlighting how reversals to ancestral states can occur.

- 3 O'Brien CE, Oliveira-Pacheco J, Ó Cinnéide E, Haase MAB, Hittinger CT, Rogers TR, Zaragoza O, Bond U, Butler G. 2021. Population genomics of the pathogenic yeast *Candida tropicalis* identifies hybrid isolates in environmental samples. *PLoS Pathog* 17:e1009138
DOI: 10.1371/journal.ppat.1009138

Candida tropicalis is a human pathogen that primarily infects the immunocompromised. Whereas the genome of one isolate, *C. tropicalis* MYA-3404,

was originally sequenced in 2009, there have been no large-scale, multi-isolate studies of the genetic and phenotypic diversity of this species. Here, we used

whole genome sequencing and phenotyping to characterize 77 isolates of *C. tropicalis* from clinical and environmental sources from a variety of locations. We show that most *C. tropicalis* isolates are diploids with approximately 2-6 heterozygous variants per kilobase. The genomes are relatively stable, with few aneuploidies. However, we identified one highly homozygous isolate and six isolates of *C. tropicalis* with much higher heterozygosity levels ranging from 36-49 heterozygous variants per kilobase. Our analyses show that the heterozygous isolates represent two different hybrid lineages, where the hybrids share one parent (A) with most other *C. tropicalis* isolates, but the second parent (B or C) differs by at least 4% at

the genome level. Four of the sequenced isolates descend from an AB hybridization, and two from an AC hybridization. The hybrids are *MTLa/α* heterozygotes. Hybridization, or mating, between different parents is therefore common in the evolutionary history of *C. tropicalis*. The new hybrids were predominantly found in environmental niches, including from soil. Hybridization is therefore unlikely to be associated with virulence. In addition, we used genotype-phenotype correlation and CRISPR-Cas9 editing to identify a genome variant that results in the inability of one isolate to utilize certain branched-chain amino acids as a sole nitrogen source.

- 4 LaBella AL, Opulente DA, Steenwyk JL, Hittinger CT, Rokas A. 2021. Signatures of optimal codon usage in metabolic genes inform budding yeast ecology. PLoS Biol 19:e3001185
DOI: 10.1371/journal.pbio.3001185

Reverse ecology is the inference of ecological information from patterns of genomic variation. One rich, heretofore underutilized, source of ecologically relevant genomic information is codon optimality or adaptation. Bias toward codons that match the tRNA pool is robustly associated with high gene expression in diverse organisms, suggesting that codon optimization could be used in a reverse ecology framework to identify highly expressed, ecologically relevant genes. To test this hypothesis, we examined the relationship between optimal codon usage in the classic galactose metabolism (*GAL*) pathway and known ecological niches for 329 species of budding yeasts, a diverse subphylum of fungi. We find that optimal codon usage in the *GAL* pathway is positively correlated with quantitative growth on galactose, suggesting that *GAL* codon optimization reflects increased capacity to grow on galactose. Optimal codon usage in the *GAL* pathway is also positively

correlated with human-associated ecological niches in yeasts of the CUG-Ser1 clade and with dairy-associated ecological niches in the family Saccharomycetaceae. For example, optimal codon usage of *GAL* genes is greater than 85% of all genes in the genome of the major human pathogen *Candida albicans* (CUG-Ser1 clade) and greater than 75% of genes in the genome of the dairy yeast *Kluyveromyces lactis* (family Saccharomycetaceae). We further find a correlation between optimization in the *GAL*actose pathway genes and several genes associated with nutrient sensing and metabolism. This work suggests that codon optimization harbors information about the metabolic ecology of microbial eukaryotes. This information may be particularly useful for studying fungal dark matter-species that have yet to be cultured in the lab or have only been identified by genomic material.

- 5 Li Y, Steenwyk JL, Chang Y, Wang Y, James TY, Stajich JE, Spatafora JW, Groenewald M, Dunn CW, Hittinger CT, Shen XX, Rokas A. 2021. A genome-scale phylogeny of the kingdom Fungi. Curr Biol 31:1653-65 - DOI: 10.1016/j.cub.2021.01.074

Phylogenomic studies using genome-scale amounts of data have greatly improved understanding of the tree of life. Despite the diversity, ecological significance, and biomedical and industrial importance of fungi, evolutionary relationships among several major lineages remain poorly resolved, especially those near the base of the fungal phylogeny. To examine poorly resolved relationships and assess progress toward a genome-scale phylogeny of the fungal kingdom, we compiled a phylogenomic data

matrix of 290 genes from the genomes of 1,644 species that includes representatives from most major fungal lineages. We also compiled 11 data matrices by subsampling genes or taxa from the full data matrix based on filtering criteria previously shown to improve phylogenomic inference. Analyses of these 12 data matrices using concatenation- and coalescent-based approaches yielded a robust phylogeny of the fungal kingdom, in which ~85% of internal branches were congruent across data matrices and approaches used.

We found support for several historically poorly resolved relationships as well as evidence for polytomies likely stemming from episodes of ancient diversification. By examining the relative evolutionary divergence of taxonomic groups of equivalent rank, we found that fungal taxonomy is broadly aligned with both genome sequence divergence and divergence

time but also identified lineages where current taxonomic circumscription does not reflect their levels of evolutionary divergence. Our results provide a robust phylogenomic framework to explore the tempo and mode of fungal evolution and offer directions for future fungal phylogenetic and taxonomic studies.

IV Department of AGRARIA, “Mediterranea” University of Reggio Calabria, Via Feo di Vito, I-89122 Reggio Calabria, Italy. Communicated by Andrea Caridi <acaridi@unirc.it>

Recent publications.

- 1 Caridi A, Sidari R, Pulvirenti A, Blaiotta G. 2020. Genetic improvement of wine yeasts for opposite adsorption activity of phenolics and ochratoxin A during red winemaking. *Food Biotechnol* 34:352-370 <https://doi.org/10.1080/08905436.2020.1850472>.

The aim of this research was to acquire new strains of *Saccharomyces cerevisiae* exhibiting opposite characteristics of cell wall adsorption: very high adsorption activity toward the ochratoxin A, very low adsorption activity toward the pigmented phenolic compounds contained in musts from black grapes. For this purpose, starting from 313 strains of *Saccharomyces cerevisiae*, 12 strains were pre-selected and used to obtain 27 intraspecific

hybrids. Eleven crosses out of 27 were validated as hybrids; the best five hybrids were used in guided winemaking at four Calabrian wineries. The employed experimental protocol has allowed to select yeast strains for their different adsorption activity, improving the strains by spore clone selection and construction of intraspecific hybrids. These results suggest an efficacious way to improve the characteristics of interest in wine yeasts.

- 2 Caridi A. 2021. Physiological characterisation of Calabrian dairy yeasts and their possible use as adjunct cultures for cheese making. *Acta Alimentaria*, in press.

V Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, 47907, USA. Communicated by M. Catherine Aime <maime@purdue.edu>

Recent publications.

- 1 Haelwaters D, Urbina H, Brown S, Newerth-Hansen S, Aime MC. 2021. Isolation and molecular characterization of the romaine lettuce phylloplane mycobiome. *J Fungi* 7:277 - doi: 10.3390/jof7040277.
- 2 Haelwaters D, Toome-Heller M, Albu S, Aime MC. 2020. Red yeasts from leaf surfaces and other habitats: three new species and a new combination of *Symmetrospora* (Pucciniomycotina, Cystobasidiomycetes). *Fungal Syst Evol* 5:187-196 - doi: 10.3114/fuse.2020.05.12
- 3 Kijpornyongpan T, Aime MC. 2020. Investigating the smuts: Common cues, signaling pathways, and the role of MAT in dimorphic switching and pathogenesis. *J Fungi* 6:368 - doi: 10.3390/jof6040368
- 4 Rush TA, Albu S, Kijpornyongpan T, Aime MC. 2020. *Farysia magdalena* sp. nov. and description of the anamorph of *Anthracoystis heteropogonicola* from the Americas. *Mycol Prog* 19:921-934. doi: 0.1007/s11557-020-01610-7?

Recent publications.

- 1 Caporusso A, Capece A, De Bari I. 2021. Oleaginous yeasts as cell factories for the sustainable production of microbial lipids by the valorization of agri-food wastes. *Fermentation* 7:50. doi.org/10.3390/fermentation7020050

The agri-food industry annually produces huge amounts of crops residues and wastes, the suitable management of these products is important to increase the sustainability of agro-industrial production by optimizing the entire value chain. This is also in line with the driving principles of the circular economy, according to which residues can become feedstocks for novel processes. Oleaginous yeasts represent a versatile tool to produce biobased chemicals and intermediates. They are flexible microbial factories able to grow on different side-stream

carbon sources such as those deriving from agri-food wastes, and this characteristic makes them excellent candidates for integrated biorefinery processes through the production of microbial lipids, known as single cell oils (SCOs), for different applications. This review aims to present an extensive overview of research progress on the production and use of oleaginous yeasts and present discussions on the current bottlenecks and perspectives of their exploitation in different sectors, such as foods, biofuels and fine chemicals.

- 2 Capece A, De Fusco D, Pietrafesa R, Siesto G, Romano P. 2021. Performance of wild non-conventional yeasts in fermentation of wort based on different malt extracts to select novel starters for low-alcohol beers. *Appl Sci (Switzerland)* 11:801 - doi:10.3390/app11020801

Nowadays, the increasing interest in new market demand for alcoholic beverages has stimulated the research on useful strategies to reduce the ethanol content in beer. In this context, the use of non-*Saccharomyces* yeasts to produce low-alcohol or alcohol-free beer may provide an innovative approach for the beer market. In our study, four wild non-*Saccharomyces* yeasts, belonging to *Torulaspora delbrueckii*, *Candida zemplinina* and *Zygosaccharomyces bailii* species, were tested in mixed fermentation with a wild selected *Saccharomyces cerevisiae* strain as starters for fermentation of different commercial substrates used for production of different beer styles (Pilsner, Weizen and Amber) to evaluate the influence of the fermentative

medium on starter behaviour. The results obtained showed the influence of non-*Saccharomyces* strains on the ethanol content and organoleptic quality of the final beers and a significant wort-starter interaction. In particular, each starter showed a different sugar utilization rate in each substrate, in consequence of uptake efficiency correlated to the strain-specific metabolic pathway and substrate composition. The most suitable mixed starter was P4-CZ3 (*S. cerevisiae*-*C. zemplinina*), which is a promising starter for the production of low-alcohol beers with pleasant organoleptic characteristics in all the tested fermentation media.

- 3 Capece A, Romaniello R, Scrano L, Siesto G, Romano P. 2018. Yeast starter as a biotechnological tool for reducing copper content in wine. *Front Microbiol* 8:2632 - doi: 10.3389/fmicb.2017.02632.

Copper is widely used in agriculture as a traditional fungicide in organic farming to control downy mildew on grapes, consequently it is possible to find this metal during all stages of the vinification process. Low amounts of copper play a key role on the function of key cell enzymes, whereas excess quantities can exert amount-dependent cytotoxicity, resulting in general cellular damage. Nowadays the excessive copper ions in wines is removed by addition of adsorbents, but these additives can influence the sensory characteristics of wine, as well as detrimental to the health of consumers. It is well known that high concentrations of Cu^{2+} can be toxic to yeasts,

inhibiting growth and activity, causing sluggish fermentation and reducing alcohol production. In this study, 47 *S. cerevisiae* strains were tested for copper tolerance by two different tests, growth on copper added medium and fermentative activity in copper added grape must. The results obtained by the two different tests were comparable and the high strain variability found was used to select four wild strains, possessing this characteristic at the highest (PP1-13 and A20) and the lowest level (MPR2-24 and A13). The selected strains were tested in synthetic and natural grape must fermentation for ability to reduce copper content in wine. The determination of copper

content in wines and yeast cells revealed that at the lowest copper residual in wine corresponded the highest content in yeast cells, indicating a strong strain ability to reduce the copper content in wine. This effect was inversely correlated with strain copper resistance and the most powerful strain in copper reduction was the most sensitive strain, MPR2-24. This wild strain was finally tested as starter culture in cellar pilot scale fermentation in comparison to a com-

mmercial starter, confirming the behavior exhibited at lab scale. The use of this wild strain to complete the alcoholic fermentation and remove the copper from wine represents a biotechnological sustainable approach, as alternative to the chemical-physical methods, ensuring at the same time a completed alcoholic fermentation and organoleptic quality of wine.

VII Technical Research Centre of Finland Ltd, Tietotie 2, Espoo, FI-02044 VTT, Finland. Communicated by Brian Gibson <brian.gibson@tu-berlin.de>

I have recently taken up a position as Chair of Brewing and Beverage Technology at the Institute for Food Technology and Food Chemistry, Technology Technical University Berlin, Ackerstr. 76, 13355 Berlin, Germany. The publications to follow report on work done while still as VTT.

- 1 Catallo M, Lattici F, Randazzo CL, Caggia C, Krogerus K, Magalhães F, Gibson B, Solieri, S. 2021. Hybridization of *Saccharomyces cerevisiae* sourdough strains with cryotolerant *Saccharomyces bayanus* NBRC1948 as a strategy to increase diversity of strains available for lager beer fermentation. *Microorganisms* 9:514.
- 2 Hutzler, M, Michel, M, Kunz, O, Kuusisto, T, Magalhães, F, Krogerus, K, Gibson, B. 2021. Unique brewing-relevant properties of the non-domesticated yeast *Saccharomyces jurei* isolated from ash (*Fraxinus excelsior*). *Front Microbiol* - doi: 10.3389/fmicb.2021.645271.
- 3 Johansson, L, Nikulin, J, Juvonen, R, Krogerus, K, Magalhães, F, Mikkelsen, A, Nuppenen-Puputti, M, Sohlberg, E, de Francesco, G, Perretti, G, Gibson, B. 2021. Sourdough cultures as reservoirs of maltose-negative yeasts for low-alcohol beer brewing. *Food Microbiol*, 94:103629 - doi:10.1016/j.fm.2020.103629.
- 4 Magalhães, F, Calton, A, Heiniö, R.L, Gibson, B. 2021. Frozen-dough baking potential of psychrotolerant *Saccharomyces* species and derived hybrids. *Food Microbiol*: in press - DOI: 10.1016/j.fm.2020.103640

Additional publications communicated by Kristoffer Krogerus <Kristoffer.Krogerus@vtt.fi>

- 5 Kuivanen J, Kannisto M, Mojzita D, Rischer H, Toivari M, Jäntti J. 2021. Engineering of *Saccharomyces cerevisiae* for anthranilate and methyl anthranilate production. *Microb Cell Factories* 20:34
<https://doi.org/10.1186/s12934-021-01532-3>
- 6 Ylinen A, Maaheimo H, Anghelescu-Hakala A, Penttilä M, Salusjärvi L, Toivari M. 2021. Production of D-lactic acid containing polyhydroxyalkanoate (PHA) polymers in yeast *Saccharomyces cerevisiae*. *J Indust Microbiol Biotechnol* kuab028 - <https://doi.org/10.1093/jimb/kuab028>

VIII Centro de Referencia en Levaduras y Tecnología Cervecera (CRELTEC). Instituto Andino Patagónico de Tecnologías Biológicas y Geoambientales (IPATEC) CONICET - UNComahue. Quintral 1250, Bariloche, Argentina. Communicated by Diego Libkind <www.ipatec.conicet.gob.ar>

Papers recently published, in press, or submitted.

- 1 Nizovoy P, Bellora N, Haridas S, Lipzen A, Daum C, Barry K, Grigoriev I, Libkind D, Connell L, Moline M. 2021. Unique genomic traits for cold adaptation in *Naganishia vishniacii*, a polyextremophile yeast isolated from Antarctica. *FEMS Yeast Res.* 21:foaa056 - doi: 10.1093/femsyr/foaa056.

- 2 Burini JA, Eizaguirre JI, Loviso C, Libkind D. 2021. Non-conventional yeasts as tools for innovation and differentiation in brewing. *Revista Argentina de Microbiología* - <https://doi.org/10.1016/j.ram.2021.01.003>. (In Spanish).

Constitutes a series of open source publications in Spanish, reviewing several brewing yeast aspects and meant to be available for Latinoamerican scientists and specially to advanced brewers. Previous papers included

reviews on the synthesis of flavor compounds such as fusel alcohols (<https://doi.org/10.1016/j.ram.2018.08.006>) and esters (doi: 10.1016/j.ram.2017.11.006).

- 3 Tiwari S, Baghela A, Libkind D. 2021. *Rhodotorula sampaioana* sp. nov., a novel carotenoid producing yeast of the order Sporidiobolales isolated from Argentina and India. *Antonie van Leeuwenhoek* In press.

Čadež N, Bellora N, Ulloa R, Tome M, Petković H, Groenewald M, Hittinger CT, Libkind D. 2021. *Hanseniaspora smithiae* sp. nov, a novel apiculate yeast species from Patagonian forests that lacks the typical genomic domestication signatures for fermentative environments. *Front Microbiol* - Submitted.

Book chapter.

- 4 Libkind D, Alvarez L. 2020. Levaduras en cerveza y panificados, aportes desde la Patagonia. En: *Alimentos Fermentados: microbiología, nutrición, salud y cultura*. (Eds). Ferrari, A, Vinderola G, Weill, R. Chapter 11, Editorial Instituto Danone. ISBN 978-987-25312-2-5. (In Spanish). Title: Yeast in brewing and bread, contributions from Patagonia.

IX Linking Landscape, Environment, Agriculture and Food Research Center (LEAF), Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, Lisboa, 1349-017, Portugal. Communicated by Manuel Malfeito-Ferreira <mmalfeito@isa.ulisboa.pt>

Recent publication.

- 1 Chandra M, Mota M, Silva AC, Malfeito-Ferreira M. 2020. Forest oak woodlands and fruit tree soils are reservoirs of wine-related yeast species. *Am J Enol Vitic* 71:191-197 - doi:10.5344/ajev.2020.19067

A large-scale sampling plan was performed over four years in three different vineyards to evaluate the occurrence of wine-related yeast species in the soils underneath both vines and forest oak and fruit trees close to the vineyards. Ascomycetous fermentative yeasts were present in 27% of 320 soil samples throughout the sampling years, with incidences that could not be related to sampling season. The greatest percentages of occurrence were found in soils under fig (76%), apple (73%), and oak (41 to 55%) trees. Soils were less contaminated under vines (6%), while these yeasts were not recovered from soil underneath chestnut trees. Other soils showed intermediate percentages of occurrence. A total of 139 fermentative ascomycetes were identified from 25 species. Ninety-six isolates came from 21 different non-*Saccharomyces* species and 43 isolates

from four *Saccharomyces* species. Soils underneath fruit trees had 11 different species. The most common isolates belonged to *Lachancea thermotolerans* and *Torulaspota delbrueckii*, while *Saccharomyces paradoxus* predominated in the soil underneath oak trees. *Saccharomyces cerevisiae* was found at low frequency (7% of total isolates) during all sampling years under fruit trees, but it was not recovered from vineyard or oak tree soils. The wine spoilage species *Zygosaccharomyces bailii* was recovered in only one sample of vineyard soil. For a given soil system, the species recovered varied strongly over the years, suggesting the existence of complex yeast communities. In particular, soils in the vicinity of vineyards were natural reservoirs for yeast species of enological interest.

The following article was recently published.

- 1 Melo WGP, Oliveira TB, Arcuri SL, Morais PB, Pagnocca FC. 2021. Yeasts in the nests of the leaf-cutter ant *Acromyrmex balzani* in a Savanna biome: exploitation of community and metabolic diversity. Antonie van Leeuwenhoek - <https://doi.org/10.1007/s10482-021-01555-1>.

The leaf-cutter ant *Acromyrmex balzani* is responsible for causing important losses in reforestation areas, crops, and pastures, and it is frequently found in the Brazilian savanna (Cerrado). So far, there is no information regarding the yeast communities that occur in their nests. Here, we evaluated the diversity, composition, and structure of yeast communities in both fungus gardens (FG) and external refuse dump (RD) of this ant species (Palmas, Tocantins, northern Brazil). A total of 720 yeasts were isolated, comprising 52 species distributed in 29 genera. The RDs have significantly richer and more diverse yeast communities than the fungus gardens, regardless of the

season and the level of preservation in the area. The isolates produced a wide range of carbon polymer-degrading enzymes and were able to assimilate carbon-sources present in plant materials. We observed a different proportion of enzyme-producers and carbon-assimilation found in external refuse dump and fungus gardens from preserved and disturbed areas, suggesting that this interaction may vary depending on the environmental conditions. *A. balzani* nests in the savanna biome are a hotspot of yeast species with ecological, clinical, and biotechnological implications.

The following are papers for 2020 and 2021 or in press.

- 1 Borovkova AN, Michailova YuV, Naumov GI. 2020. Molecular genetic characteristics of the *Saccharomyces* biological species. Microbiology (Moscow) 89(4):387–395.
- 2 Naumova ES, Borovkova AN, Shalamitskiy MYu, Naumov GI. 2021. Natural polymorphism of pectinase *PGU* genes in the *Saccharomyces* yeasts. Microbiology (Moscow) 90(3):349–360.

The distribution and properties of the pectinase-encoding *PGU* genes in different species of *Saccharomyces* yeasts was studied. Application of molecular karyotyping and Southern hybridization revealed that *S. arboricola*, *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, and *S. paradoxus* species had a single *PGU* gene located on chromosome X. The other three species had polymeric *PGU* genes of different chromosomal localization: *S. mikatae* and *S. jurei* (chromosomes X and VIII), *S. bayanus* (X, I, and XIV). This is the first report on the comparative analysis of the nucleotide and amino acid sequences of the *PGU* genes, carried out in all eight species of the genus *Saccharomyces*. Species-specificity of the *PGU* genes was revealed, as well as their intraspecific

polymorphism in *S. kudriavzevii* and *S. paradoxus*, associated with the geographical origin of the strains. The most divergent proteins were Pgu1a (*S. arboricola*) and Pgu1b, Pgu2b, Pgu3b (*S. bayanus*), for which the level of similarity to the Pgu proteins of other *Saccharomyces* species did not exceed 89%. The highest similarity (>95%) was noted for the Pgu proteins of *S. cerevisiae*, *S. paradoxus*, and *S. cariocanus*, as well as *S. mikatae* and *S. jurei*. Significant intraspecific polymorphism of endo-polygalacturonase secretion was observed in the studied *Saccharomyces* species, except for the species *S. bayanus*, all studied strains of which had a relatively high activity. The ability to secrete active endo-polygalacturonase is probably a specific feature of this species.

- 3 Lyutova LV, Naumov GI, Shnyreva AV, Naumova ES. 2021. Molecular polymorphism of β -galactosidase *LAC4* genes in dairy and natural strains of *Kluyveromyces* yeasts. *Molecular Biology (Moscow)* 55(1):66–74.

The ability to ferment lactose is a characteristic peculiarity of dairy *Kluyveromyces lactis* yeasts; the vast majority of other yeast species are not able to assimilate this disaccharide. Molecular polymorphism of *LAC4* genes encoding β -galactosidase controlling lactose fermentation is not well studied, and the published data concern only a single strain (*K. lactis* var. *lactis* NRRL Y-1140) isolated from cream in the United States. We studied β -galactosidase genes in lactose-fermenting *K. lactis* strains isolated from dairy products and natural sources in different regions of the world using molecular karyotyping, Southern hybridization, and sequencing. It was established that the ability to ferment lactose in *K. lactis* var. *lactis* dairy yeasts is controlled by at least three polymeric *LAC* loci with different chromosomal localization: *LAC1* (chromosome III), *LAC2* (II), and *LAC3* (IV). Most of the strains we studied had the *LAC2* locus. A comparative analysis of β -galactosidases of the *Kluyveromyces* genus

yeasts and these enzymes from other yeasts was conducted for the first time. Phylogenetic analysis detected significant differences between the *LAC4* proteins of yeasts of the *Kluyveromyces* genus (*K. lactis*, *K. marxianus*, *K. aestuarii*, *K. nonfermentans*, *K. wickerhamii*), *Scheffersomyces stipitis*, *Sugiyamaella lignohabitans*, and *Debaryomyces hansenii*. A correlation between β -galactosidase sequences and ecological origin (dairy products and natural sources) of *Kluyveromyces* strains was found. The group of dairy strains is heterogeneous and includes *K. lactis* var. *lactis* and *K. marxianus* yeasts (99.80–100% similarity), which indicates a common origin of their *LAC4* genes. Phylogenetic analysis of β -galactosidases indicates a close genetic relationship of dairy and hospital strains of *K. lactis* var. *lactis* and *K. marxianus*. Clinical isolates are able to ferment lactose and appear to originate from the dairy yeasts.

- 4 Naumova ES, Lee Ch-Fu, Naumov GI. 2021. Molecular genetic polymorphism of the yeast *Kluyveromyces dobzhanskii*. *Microbiology (Moscow)* 90(3): in press.

Based on strains of various ecological and geographical origin, we studied intraspecific polymorphism of the wild yeast *Kluyveromyces dobzhanskii*, the closest relative of the cultured dairy yeasts *K. lactis* and *K. marxianus*. Using microsatellite typing, phylogenetic analysis of the nucleotide sequences of the ITS1/ITS2 internal transcribed spacers and of the mitochondrial gene *COX2*, we found the species *K. dobzhanskii* to be of complex composition, consisting of at least three geographical populations: North

American (including the type culture CBS 2104), European, and Far Eastern. Strains of different geographic origin were characterized by unique nucleotide substitutions in the ITS1/ITS2 region and in the mitochondrial *COX2* gene. In European strains, a correlation was revealed between the (GTG)₅ profiles and the source of isolation. The strains isolated from insects in Spain had unique patterns.

XII University of Helsinki, Faculty of Biological and Environmental Sciences, Organismal and Evolutionary Biology Research Programme, and the Viikki Plant Science Centre, PL 65, Viikinkaari 1, 00014 Helsinki Finland. Communicated by Kirk Overmyer <kirk.overmyer@helsinki.fi>

The following paper has been published.

- 1 Kai Wang, Timo Sipilä, Kirk Overmyer. 2021. A novel *Arabidopsis* phyllosphere resident *Protomyces* species and a re-examination of genus *Protomyces* based on genome sequence data. *IMA Fungus* 12:8 <https://doi.org/10.1186/s43008-021-00054-2>

Protomyces is an understudied genus of yeast-like fungi currently defined as phytopathogens of only *Umbelliferae* and *Compositae*. Species relationships and boundaries remain controversial and molecular data are lacking. Of the 82 named *Protomyces*, we found few recent studies and six available cultures. We previously isolated *Protomyces* strains from wild *Arabidopsis thaliana*, a member of *Brassicaceae*, a family distant from accepted *Protomyces* hosts. We previously sequenced the genomes

of all available *Protomyces* species, and *P. arabidopsidicola* sp. nov. strain C29, from *Arabidopsis*. Phylogenomics suggests this new species occupied a unique position in the genus. Genomic, morphological, and physiological characteristics distinguished *P. arabidopsidicola* sp. nov. from other *Protomyces*. Nuclear gene phylogenetic marker analysis suggests *actin1* gene DNA sequences could be used with nuclear ribosomal DNA internal transcribed spacer sequences for rapid

identification of *Protomyces* species. Previous studies demonstrated *P. arabidopsidicola* sp. nov. could persist on the *Arabidopsis* phyllosphere and *Protomyces* sequences were discovered on *Arabidopsis* at multiple sites in different countries. We conclude that the strain C29

represents a novel *Protomyces* species and propose the name of *P. arabidopsidicola* sp. nov. Consequently, we propose that *Protomyces* is not strictly associated only with the previously recognized host plants.

XIII Fermentation Research and Yeast Selection, Fermentec, Av. Antônia Pazzinato Sturion, 1155 1 Piracicaba SP, Brasil 13420 640 - www.fermentec.com.br. Communicated by Silene Cristina de Lima Paulillo <silene@fermentec.com.br>

Progress Report.

1 Paulillo SCL, Souza CS, Lopes ML, Amorim Neto HB, Amorim HV. Customized yeast strains for ethanol production in Brazil.

The introduction of the electrophoretic karyotyping technique, starting in the 90s, was a milestone for the evolution of yeast monitoring and the selection of new strains for industrial processes of alcoholic fermentation in Brazil. This technique made it possible to identify which yeasts remain and dominate over other strains in industrial fermentations with recycling of cells. This method of yeast monitoring guided the selection process of new strains for ethanol production. Some examples are the strains PE-2 and CAT-1 that are the most used yeasts for ethanol production in Brazil. Both strains had their genomes completely sequenced. Strain PE2 presented a high genome plasticity, being able to accumulate large variations in its chromosomes. In the last years, molecular techniques have demonstrated that other industrial strains, FT858L and Fermel® have a close relationship with PE2 despite particular characteristics of industrial importance. We believe that FT858L and Fermel® may have originated from PE2.

Since 2008, Fermentec has been investing in the selection of customized yeasts for industrial fermentation processes. Customized yeasts are those that arise in the process and achieve dominance and persistence over other strains in fermentation tanks. These dominant strains are identified and monitored in industrial fermentations by molecular techniques based on polymorphisms of chromosomes and mitochondrial DNA. After isolation, these strains are evaluated in bench-scale fermentations that reproduce industrial conditions and the best ones are selected to be reintroduced in the same distillery where were originated. The plasticity of the genome, high-density of cells in a fermentation tank, successive recycling of cells throughout during a harvesting period of 8 months or more and the stressful conditions, to which these yeasts are

subjected, may generate new strains, more robust and adapted to each distillery.

The very industrial fermentation process selects the most robust and well-adapted strains (Process-driven selection). These strains have the highest rates of persistence and dominance, leading to a high rate of implantation. The use of customized yeasts has been an important step towards maintaining high industrial efficiencies, where fermentation becomes more stable. In the 2020/2021 season, 21 distilleries started their fermentation processes with customized yeast strains, which were responsible for the production of 3.46 billion liters of ethanol, representing 11.4% of Brazil's ethanol production. In the last nine years, the General Distillery Yield (GDY) of the Brazilian distilleries that used customized yeasts were higher (average of 90.5%) than those of the plants that used other yeast strains (average of 89.7%). This represents a total gain of 27.68 million liters of ethanol per year. For the beginning of the current sugar cane harvesting season (2021/2022), 28 Brazilian distilleries started the fermentation processes with their own customized yeast strain. In addition, there are distilleries that have two, three and even four customized yeasts to start the fermentation at the beginning of sugarcane harvesting season.

To select a customized yeast, the work must be continuous and, in some cases, require long-term research. The results achieved have been demonstrated by more stable fermentations, higher fermentation yields, lower residual sugars and faster fermentations in comparison to processes that do not have customized strains. These yeasts opened up a new opportunity to improve the results of large-scale fermentations for ethanol production in Brazil.

Please note that the affiliation of our collection has been changed to conform to a reorganization and renaming of our host university.

The following articles have been published since our last report.

- 1 Brysch-Herzberg M, Groenewald M, Dlačny D, Seidel M, Péter G. 2020. *Hyphopichia lachancei*, f.a., sp. nov., a yeast species from diverse origins. *Antonie van Leeuwenhoek* 113:773–778
doi: 10.1007/s10482-020-01387-5

Three strains originating from insect frass in South Africa, yellow foxglove in Hungary and soil in France, were characterised phenotypically and by sequencing of the D1/D2 domain of the large subunit and the ITS1-5.8S-ITS2 (ITS)-region of the rRNA gene. The strains have identical D1/D2 domain sequences and only one strain shows a 1 bp indel in a 9 bp homopolymer A/T repeat within the ITS region. Based on sequence analysis *Hyphopichia burtonii* is the closest related species. The investigated strains differ from the type strain of *H. burtonii* by 1.9% (9 substitutions and an indel) in the

D1/D2 domain and by 23 substitutions and 21–22 indels in the ITS-region. Since the sequence variability is very low among the three strains and the sequence divergence with the closely related *H. burtonii* exceeds the level generally encountered between species we propose the new species *Hyphopichia lachancei* f.a., sp. nov. to accommodate the three novel strains. From *H. burtonii* the new species can be distinguished phenotypically by its inability to ferment cellobiose and by the formation of endospores (Holotype: CBS 5999^T; Isotype: NCAIM Y.02228^T; MycoBank no.: MB833616).

- 2 Čadež N, Drumonde-Neves J, Sipiczki M, Dlačny D, Lima T, Pais C, Schuller D, Franco-Duarte R, Lachance MA, Péter G. 2020. *Starmerella vitis* f.a., sp. nov., a yeast species isolated from flowers and grapes. *Antonie van Leeuwenhoek* 113:1289–1298 - <https://doi.org/10.1007/s10482-020-01438-x>

A novel yeast species of *Starmerella vitis* f.a. sp. nov. is proposed to accommodate five strains isolated from flowers, grapes and an insect in the Azores, Canada, Hungary, Palau and Taiwan. As the strains were genetically distinct, we used parsimony network analysis based on ITS-D1/D2 sequences to delineate the species in a

statistically objective manner. According to sequence comparisons and phylogenetic analysis, the novel species is most closely related to *Starmerella lactis-condensi*. The two species cannot be distinguished by conventional physiological tests. The type strain of *Starmerella vitis* f.a., sp. nov. is CBS 16418^T; Mycobank number MB 835251.

- 3 Brysch-Herzberg M, Dlačny D, Seidel M, Péter G. 2021. *Cyberlindnera sylvatica* sp. nov., a yeast species isolated from forest habitats. *Int J Syst Evol Microbiol* 71 - doi: 10.1099/ijsem.0.004477

Five yeast strains isolated from forest habitats in Hungary and Germany were characterized phenotypically and by sequencing of the D1/D2 domain of the large subunit rRNA gene and the ITS1-5.8S-ITS2 (ITS) region of the rRNA gene. The strains have identical D1/D2 domain and ITS region sequences. By sequence comparisons, *Candida mycetangii* and *Candida maritima* were identified as the closest relatives among the currently recognized yeast species. The DNA sequences of the investigated strains differ by 1.2 % (six substitutions) in the D1/D2 domain and by 3.5 % (12 substitutions and eight indels) in the ITS region from the type strain of *C. mycetangii* (CBS 8675^T) while by 1.2 % (six substitutions and one indel) in the D1/D2 domain and by 7 % (32 substitutions and seven indels) in the ITS region

from the type strain of *C. maritima* (CBS 5107^T). Because the intraspecies heterogeneity seems to be very low and the distance to the most closely related species is above the commonly expected level for intraspecies variability *Cyberlindnera sylvatica* sp. nov. (holotype, CBS 16335^T; isotype, NCAIM Y.02233^T; MycoBank no., MB 835268) is proposed to accommodate the above-noted five yeast strains. Phenotypically the novel species can be distinguished from *C. mycetangii* and *C. maritima* by the formation of ascospores. *Cyberlindnera sylvatica* forms one or two hat-shaped ascospores per ascus on many different media as well as well-developed pseudohyphae and true hyphae. Additionally, we propose the transfer of three anamorphic members of the *Cyberlindnera americana* sub-clade to the genus *Cyberlindnera* as the

following new taxonomic combinations *Cyberlindnera maritima* f.a., comb. nov., *Cyberlindnera mycetangii* f.a.,

comb. nov. and *Cyberlindnera nakhonratchasimensis* f.a., comb. nov.

- 4 Čadež N, Dlačny D, Tome M, Péter G. 2021. *Novakomyces olei* sp. nov., the first member of a novel Taphrinomycotina lineage. *Microorganisms* 2021 9:301 - doi: 10.3390/microorganisms9020301

Taphrinomycotina is the smallest subphylum of the phylum Ascomycota. It is an assemblage of distantly related early diverging lineages of the phylum, comprising organisms with divergent morphology and ecology; however, phylogenomic analyses support its monophyly. In this study, we report the isolation of a yeast strain, which could not be assigned to any of the currently recognised five classes of Taphrinomycotina. The strain of the novel budding species was recovered from extra virgin olive oil and characterised phenotypically by standard methods. The ultrastructure of the cell wall was investigated by transmission electron microscopy. Comparisons of barcoding DNA sequences indicated that the investigated strain is not closely related to any known organism. Tentative phylogenetic placement was achieved by maximum likelihood analysis of the D1/D2 domain of the nuclear LSU rRNA gene. The genome of the investigated

strain was sequenced, assembled, and annotated. Phylogenomic analyses placed it next to the fission *Schizosaccharomyces* species. To accommodate the novel species, *Novakomyces olei*, a novel genus *Novakomyces*, a novel family Novakomycetaceae, a novel order Novakomycetales, and a novel class Novakomycetes is proposed as well. Functional analysis of genes missing in *N. olei* in comparison to *Schizosaccharomyces pombe* revealed that they are biased towards biosynthesis of complex organic molecules, regulation of mRNA, and the electron transport chain. Correlating the genome content and physiology among species of Taphrinomycotina revealed some discordance between pheno and genotype. *N. olei* produced ascospores in axenic culture preceded by conjugation between two cells. We confirmed that *N. olei* is a primary homothallic species lacking genes for different mating types.

XV Pathogenic Yeast Research Group, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, PO Box 339, Bloemfontein 9300, Republic of South Africa. Communicated by Carolina Pohl-Albertyn <PohlCH@ufs.ac.za>

The following papers were recently accepted for publication.

- 1 Mochochoko BM, Ezeokoli OT, Sebolai O, Albertyn J, Pohl CH. In Press. Role of the high-affinity reductive iron acquisition pathway of *Candida albicans* in prostaglandin E₂ production, virulence and interaction with *Pseudomonas aeruginosa*. *Med Mycol* DOI: 10.1093/mmy/myab015

Some components of the iron reductive pathway of *C. albicans* have been implicated in the production of immunomodulatory prostaglandin E₂ (PGE₂) and virulence. However, it is unknown if other components of the iron reductive pathway influence PGE₂. Here, we investigated the role of the iron reductive pathway of *Candida albicans* in biofilm formation, PGE₂ production and virulence in *Caenorhabditis elegans*. Additionally, as the co-occurrence of *C. albicans* and *Pseudomonas aeruginosa* in host tissues is frequent and involves competition for host-associated iron by both microorganisms, we examined the effects of co-incubation/co-infection with *P. aeruginosa*. Deletion of multicopper oxidase gene, *FET99*, and the iron permease genes, *FTH1* and *FTH2*, affected biofilm metabolic activity, and in the case of */fth2*, also biofilm morphology.

Deletion of *CCC1* (vacuolar iron transporter) and *CCC2* (P-type ATPase copper importer) also influenced biofilm morphology. In iron-competitive conditions, multicopper oxidases, including *Fet99*, are required for biofilm formation. Data also suggest that vacuolar iron transport is involved in biofilm formation. For PGE₂ production, deletion of *FET99*, *FTH1*, *FTH2*, *CCC1* and *CCC2* caused a significant reduction by monomicrobial biofilms, while */fth2* caused the highest reduction in polymicrobial biofilms. Furthermore, *URA3* positive strains of */fet99* and */fth2* demonstrated attenuated virulence in *C. elegans*, potentially due to the inability of mutants to form hyphae *in vivo*. Deductively, the role of the iron reductive pathway in PGE₂ synthesis is indirect; possibly due to their role in iron homeostasis.

- 2 Fourie R, Cason ED, Albertyn J, Pohl CH. In press. Transcriptional response of *Candida albicans* to *Pseudomonas aeruginosa* in a polymicrobial biofilm. Genes Genomes Genet DOI: 10.1093/g3journal/jkab042

Candida albicans is frequently co-isolated with the Gram-negative bacterium, *Pseudomonas aeruginosa*. *In vitro*, the interaction is complex, with both species influencing each other. Not only does the bacterium kill hyphal cells of *C. albicans* through physical interaction, it also affects *C. albicans* biofilm formation and morphogenesis, through various secreted factors and cell wall components. The present study sought to expand the current knowledge regarding the interaction between *C. albicans* and *P. aeruginosa*, using transcriptome analyses of early static biofilms. Under these conditions, a total of 2537 open reading frames (approximately 40% of the

C. albicans transcriptome) was differentially regulated in the presence of *P. aeruginosa*. Upon deeper analyses it became evident that the response of *C. albicans* towards *P. aeruginosa* was dominated by a response to hypoxia, and included those associated with stress as well as iron and zinc homeostasis. These conditions may also lead to the observed differential regulation of genes associated with cell membrane synthesis, morphology, biofilm formation and phenotypic switching. Thus, *C. albicans* in polymicrobial biofilms with *P. aeruginosa* have unique transcriptional profiles that may influence commensalism as well as pathogenesis.

XVI Environmental Microbiology, Dip. di BioScienze e Territorio (DiBT) , Università degli Studi del Molise, 86090 Pesche (IS), Italy. Communicated by Giancarlo Ranalli <ranalli@unimol.it>

Recent publication.

- 1 Ranalli G, Bosch-Roig P, Crudele S, Rampazzi L, Corti C, Zanardini E. 2021. Dry biocleaning of artwork: an innovative methodology for Cultural Heritage recovery? *Microbial Cell* 15:91-105.

An innovative methodology is proposed, based on applied bio-technology to the recovery of altered stonework: the "dry biocleaning", which envisages the use of dehydrated microbial cells without the use of free water or gel-based matrices. This methodology can be particularly useful for the recovery of highly-ornamented stoneworks, which cannot be treated using the conventional cleaning techniques. The experimental plan included initial laboratory tests on Carrara marble samples, inoculated with dehydrated *Saccharomyces cerevisiae* yeast cells, followed by on-site tests performed on "Quattro Fontane" (The Four Fountains), a travertine monumental

complex in Rome (Italy), on altered highly ornamented areas of about 1,000 cm². The mechanism is based on the spontaneous re-hydration process due to the environmental humidity and on the metabolic fermentative activity of the yeast cells. Evaluation by physical-chemical analyses, after 18 hours of the biocleaning, confirmed a better removal of salts and pollutants, compared to both nebulization treatment and control tests (without cells). The new proposed on-site dry biocleaning technique, adopting viable yeast cells, represents a promising method that can be further investigated and optimized for recovering specific altered Cultural Heritage stoneworks.

XVII University of the Free State, Department of Microbiology and Biochemistry, Bloemfontein, 9301, South Africa. Communicated by OM Sebolai <sebolaiom@ufs.ac.za>

Recent publication.

- 1 Ogundeji AO, Mjokane N, Folorunso OS, Pohl CH, Nyaga MM, Sebolai OM. 2021. The repurposing of acetylsalicylic acid as a photosensitizer to inactivate the growth of cryptococcal cells. *Pharmaceuticals* 14(5):404 - <https://doi.org/10.3390/ph14050404>

Photodynamic treatment (PDT) is often successful when used against aerobic microbes, given their natural susceptibility to oxidative damage. To this end, the current study aimed to explore the photodynamic action of acetylsalicylic acid (ASA; aspirin, which is commonly used to treat non-infectious ailments), when administered to respiring cryptococcal cells. The treatment of cryptococcal cells, i.e., exposure to 0.5 or 1 mM of ASA in

the presence of ultraviolet light (UVL) for 10 min, resulted in a significant ($p < 0.05$) reduction in the growth of tested cells when compared to non-treated (non-Rx) cells, i.e., no ASA and no UVL. The treated cells were also characterised by diseased mitochondria, which is crucial for the survival of respiring cells, as observed by a significant ($p < 0.05$) loss of mitochondrial membrane potential ($\Delta\Psi$) and significant ($p < 0.05$) accumulation of

reactive oxygen species (ROS) when compared to non-Rx cells. Moreover, the photolytic products of acetylsalicylic acid altered the ultrastructural appearance of treated cells as well as limited the expression levels of the capsular-associated gene, *CAP64*, when compared to non-Rx cells.

The results of the study highlight the potential use of ASA as a photosensitizer that is effective for controlling the growth of cryptococcal cells. Potentially, this treatment can also be used as an adjuvant, to complement and support the usage of current anti-microbial agents.

XVIII Laboratory of Applied Stress Microbiology, Division of Biological Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma Nara 630-0192, Japan - <https://bsw3.naist.jp/takagi/English.htm>. Communicated by Hiroshi Takagi <hiro@bs.naist.jp>

I was recently awarded the JSBBA Award from the Japan Society for Bioscience, Biotechnology, and Agrochemistry (JSBBA). A ceremony was held in Fukuoka on March 25, 2020, at which I presented a lecture entitled “Novel molecular mechanisms involved in stress tolerance and improved function of yeast”. This prize is awarded to members of with honorable research achievements in the field of Bioscience, Biotechnology, and Agrochemistry.

<https://bsw3.naist.jp/eng/research/index.php?id=2050>

Due to the COVID-19 pandemic, International Microorganism Day 2020 (IMD 2020) was held online this year.

<https://www.internationalmicroorganismday.org/>

I gave a 20 minute talk entitled "Enjoy exotic Japanese alcoholic beverage brewed with my YEAST" as the final speaker of this exciting event. A video recording of the conference is available:

<https://www.youtube.com/watch?v=Gy1GUAhOEUo>

In the video, The Chair, Dr. Isabel Sá Correia, introduced me at around 1:33:30 followed by my talk.

Recent publications.

1 Ohashi M, Nasuno R, Isogai S, Takagi H. 2020. High-level production of ornithine by expression of the feedback inhibition-insensitive N-acetyl glutamate kinase in the sake yeast *Saccharomyces cerevisiae*. *Metab Eng* 62:1-9.

<https://doi.org/10.1016/j.ymben.2020.08.005>

<https://bsw3.naist.jp/eng/research/index.php?id=2148>

https://eurekaalert.org/pub_releases/2020-08/nios-jst082720.php

2 Takagi H. 2021. Adventures in brewing exotic Japanese alcoholic beverages with “Amino Acid-rich Yeast”. *SIMB (Society for Industrial Microbiology and Biotechnology) News* 71:9-17

https://www.simbhq.org/docs/simbnews/JanMar2021_SIMBNEWS.pdf

XIX Yeast Research Group, Abertay University, Dundee, Scotland. Communicated by Graeme Walker <g.walker@abertay.ac.uk>

Recent publications.

1 Walker GM, Basso, TO. 2020. Mitigating stress in industrial yeasts. *Fungal Biol* 124:387-397
doi 10.1016/j.funbio.2019.10.010

The yeast, *Saccharomyces cerevisiae*, is the premier fungal cell factory exploited in industrial biotechnology. In particular, ethanol production by yeast fermentation represents the world’s foremost biotechnological process, with beverage and fuel ethanol contributing significantly to many countries economic and energy sustainability. During industrial fermentation processes, yeast cells are subjected

to several physical, chemical and biological stress factors that can detrimentally affect ethanol yields and overall production efficiency. These stresses include ethanol toxicity, osmotic stress, nutrient starvation, pH and temperature shock, as well as biotic stress due to contaminating microorganisms. Several cell physiological and genetic approaches to mitigate yeast stress during

industrial fermentations can be undertaken, and such approaches will be discussed with reference to stress mitigation in yeasts employed in Brazilian bioethanol processes. This article will highlight the importance of

furthering our understanding of key aspects of yeast stress physiology and the beneficial impact this can have more generally on enhancing industrial fungal bioprocesses.

- 2 Black K, Tziboula Clarke A, White PJ, Iannetta PPM, Walker G. 2020. Optimised processing of faba bean (*Vicia faba* L.) kernels as a brewing adjunct. *J Inst Brew* 127:13-20 - doi 10.1002/jib.632

Pulse (Fabaceae) grains, such as peas and beans, are derived from crops that are usually cultivated in the absence of mineral nitrogen fertiliser as these crops can obtain their nitrogen requirement naturally from the air via biological nitrogen fixation. Therefore, pulses present a significantly lower greenhouse gas (GHG) footprint than crops demanding nitrogen fertiliser, whilst also offering significant quantities of starch for the brewing and distilling industries. Mitigation of agriculture derived GHG emissions through utilisation of pulses can have a positive environmental impact. To this end, the potential of exploiting dry, dehulled faba bean (*Vicia faba* L.) kernel flour as an adjunct for beer production was evaluated. The

impact of different temperature regimes and commercial enzymes were assessed for their effect on wort: viscosity; run off rate; primary amino nitrogen content and fermentability. Faba beans demonstrated insufficient endogenous enzyme capacity for starch conversion and generated a viscous wort. However, using a stepped temperature mashing regime and exogenous enzyme additions, the faba bean wort was comparable in processability and fermentability to that of 100% malted barley wort. The faba based beer and co product qualities demonstrate the environmental, nutritional and commercial potential of pulses in brewing.

- 3 Daute M, Jack F, Baxter I, Harrison B, Grigor J, Walker G. 2021. Comparison of three approaches to assess the flavour characteristics of scotch whisky spirit. *App Sci* 11:1410 <https://doi.org/10.3390/app11041410>

This study compared the use of three sensory and analytical techniques: Quantitative Descriptive Analysis (QDA), Napping, and Gas Chromatography-Mass Spectrometry (GC-MS) for the assessment of flavour in nine unmaturing whisky spirits produced using different yeasts. Hierarchical Multiple Factor Analysis (HMFA) showed a similar pattern of sample discrimination (RV scores: 0.895–0.927) across the techniques: spirits were mostly separated by their Alcohol by Volume (ABV). Low ABV spirits tended to have heavier flavour characteristics (feinty, cereal, sour, oily, sulphury) than high ABV spirits, which were lighter in character (fruity, sweet, floral, solventy, soapy). QDA differentiated best between low

ABV spirits and GC-MS between high ABV spirits, with Napping having the lowest resolution. QDA was time-consuming but provided quantitative flavour profiles of each spirit that could be readily compared. Napping, although quicker, gave an overview of the flavour differences of the spirits, while GC-MS provided semi-quantitative ratios of 96 flavour compounds for differentiating between spirits. Ester, arenes and certain alcohols were found in higher concentrations in high ABV spirits and other alcohols and aldehydes in low ABV spirits. The most comprehensive insights on spirit flavour differences produced by different yeast strains are obtained through the application of a combination of approaches.

XX Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany – <http://www.dsmz.de>. Communicated by AM Yurkov <andrey.yurkov@dsmz.de>

Recent publications.

- 1 Yurkov A, Alves A, Bai FY, Boundy-Mills K, Buzzini P, Čadež N, Cardinali G, Casaregola S, Chaturvedi V, Collin V, Fell JW, Girard V, Groenewald M, Hagen F, Hittinger CT, Kachalkin AV, Kostrzewa M, Kouvelis V, Libkind D, Liu X, Maier T, Meyer W, Péter G, Piątek M, Robert V, Rosa CA, Sampaio JP, Sipiczki M, Stadler M, Sugita T, Sugiyama J, Takagi H, Takashima M, Turchetti B, Wang QM, Boekhout T. 2021. Nomenclatural issues concerning cultured yeasts and other fungi: why it is important to avoid unneeded name changes. *IMF Fungus* (in press).

The unambiguous application of fungal names is important to communicate scientific findings. Names are critical for (clinical) diagnostics, legal compliance, and regulatory controls, such as biosafety, food security, quarantine regulations, and industrial applications. Consequently, the stability of the taxonomic system and the traceability of nomenclatural changes is crucial for a broad range of users and taxonomists. The unambiguous application of names is assured by the preservation of nomenclatural history and the physical organisms representing a name. Fungi are extremely diverse in terms of ecology, lifestyle, and methods of study. Predominantly unicellular fungi known as yeasts are usually investigated as living cultures. Methods to characterize yeasts include physiological (growth) tests and experiments to induce a sexual morph; both methods require viable cultures. Thus, the preservation and availability of viable reference cultures are important, and cultures representing reference material

are cited in species descriptions. Historical surveys revealed drawbacks and inconsistencies between past practices and modern requirements as stated in the *International Code of Nomenclature for Algae, Fungi, and Plants* (ICNafp). Improper typification of yeasts is a common problem, resulting in a large number of invalid yeast species names. With this opinion letter, we address the problem that culturable microorganisms, notably some fungi and algae, require specific provisions under the ICNafp. We use yeasts as a prominent example of fungi known from cultures. But viable type material is important not only for yeasts, but also for other cultivable *Fungi* that are characterized by particular morphological structures (a specific type of spores), growth properties, and secondary metabolites. We summarize potential proposals which, in our opinion, will improve the stability of fungal names, in particular by protecting those names for which the reference material can be traced back to the original isolate.

- 2 Aime MC, Miller AN, Aoki T, Bensch K, Cai L, Crous PW, Hawksworth DL, Hyde KD, Kirk PM, Lücking R, May TW, Malosso E, Redhead SA, Rossman AY, Stadler M, Thines M, Yurkov AM, Zhang N, Schoch CL. 2021 How to publish a new fungal species, or name, version 3.0. *IMA fungus*. 12(1):1-5.

It is now a decade since The International Commission on the Taxonomy of Fungi (ICTF) produced an overview of requirements and best practices for describing a new fungal species. In the meantime the International Code of Nomenclature for algae, fungi, and plants (ICNafp) has changed from its former name (the International Code of Botanical Nomenclature) and introduced new formal requirements for valid publication of species scientific names, including the separation of provisions specific to Fungi and organisms treated as fungi in a new Chapter F. Equally transformative have been changes in the data collection, data dissemination, and analytical tools available to mycologists. This paper

provides an updated and expanded discussion of current publication requirements along with best practices for the description of new fungal species and publication of new names and for improving accessibility of their associated metadata that have developed over the last 10 years. Additionally, we provide: (1) model papers for different fungal groups and circumstances; (2) a checklist to simplify meeting (i) the requirements of the ICNafp to ensure the effective, valid and legitimate publication of names of new taxa, and (ii) minimally accepted standards for description; and, (3) templates for preparing standardized species descriptions.

- 3 Lücking R, Aime MC, Robbertse B, Miller AN, Aoki T, Ariyawansa HA, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, Jeewon R, Johnston PR, Kirk PM, Malosso E, May TW, Meyer W, Nilsson HR, Öpik M, Robert V, Stadler M, Thines M, Vu D, Yurkov AM, Zhang N, Schoch CL. 2021. Fungal taxonomy and sequence-based nomenclature. *Nature Microbiol* 6(5):540-8
DOI <https://doi.org/10.1186/s43008-021-00063-1>

The identification and proper naming of microfungi, in particular plant, animal and human pathogens, remains challenging. Molecular identification is becoming the default approach for many fungal groups, and environmental metabarcoding is contributing an increasing amount of sequence data documenting fungal diversity on a global scale. This includes lineages represented only by

sequence data. At present, these taxa cannot be formally described under the current nomenclature rules. By considering approaches used in bacterial taxonomy, we propose solutions for the nomenclature of taxa known only from sequences to facilitate consistent reporting and communication in the literature and public sequence repositories.

The following letter to IJSEM comments on four papers that surprisingly cited my 2016 paper on parapyly in (yeast) classification. The papers were on the topic of nanoparticles, and in each case, it was clear that the authors had no interest in parapyly. I find this highly amusing.

- 1 Lachance MA. 2021. On nanoparticles, parapyly, inventions, yeasts and diarrhea. Letter - Int J Syst Evol Microbiol 71(5) - <https://doi.org/10.1099/ijsem.0.004798>

Citations do not always guarantee that a paper aroused interest in the citing author(s).

Research articles.

- 2 de Vega C, Álvarez-Pérez S, Albaladejo R, Steenhuisen SL, Lachance MA, Johnson S, Herrera C. 2021. The role of plant-pollinator interactions in structuring nectar microbial communities. J Ecol (accepted May 2021).

1. Floral nectar harbours a diverse microbiome of yeasts and bacteria that depend predominantly on animal visitors for their dispersal. Since pollinators visit specific sets of flowers and carry their own unique microbiota, we hypothesize that plant species visited by the same set of pollinators may support non-random nectar microbial communities linked together by the type of pollinator. 2. Here we explore the importance of plant-pollinator interactions in the assembly of nectar microbiome and study the role of plant geographic location as a determinant of microbial community composition. We intensively sampled the nectar of 282 flowers of 48 plant species with beetles, birds, long-tongued and short-tongued insects as pollinators in wild populations in South Africa, one of the world's biodiversity hotspots, and using molecular techniques we identified nectar yeast and bacteria taxa. The analyses provided new insights into the richness, geographic structure and phylogenetic characterization of nectar microbiome, and compared patterns of composition of bacteria and yeast communities in relation to plant and pollinator guild. 3. Our results showed that plant-pollinator interactions played a crucial role in shaping nectar microbial communities. Plants visited by different

pollinator guilds supported significantly different yeast and bacterial communities. The pollinator guild also contributed to the maintenance of beta diversity and phylogenetic microbial segregation. The results revealed different patterns for yeast and bacteria; whereas plants visited by beetles supported the highest richness and phylogenetic diversity of yeasts, bacteria communities were significantly more diverse in plants visited by other insect groups. We found no clear microbial spatial segregation at different geographical scales for bacteria, and only the phylogenetic similarity of yeast composition was correlated significantly with geography. 4. *Synthesis*. Interactions of animal vector, plant host traits and microbe physiology contribute to microbial community assemblages in nectar. Our results suggest that plants visited by the same pollinator guild have a characteristic nectar microbiota signature that may transcends the geographic region they are in. Contrasted patterns for yeast and bacteria stress the need for future work aimed at better understanding the causes and consequences of the importance of plants and pollinators in shaping nectar microbial communities in nature.

- 3 Boekhout T, Aime MC, Begerow D, Gabaldón T, Heitman J, Kemler M, Khayhan K, Lachance MA, Louis EJ, Sun S, Vu D, Yurkov A. 2021. The evolving species concepts used for yeasts: from phenotypes and genomes to speciation networks. Fungal Biol (in press).

Here we review how evolving species concepts have been applied to understand yeast diversity. Initially, a phenotypic species concept was utilized taking into consideration morphological aspects of colonies and cells, and growth profiles. Later the biological species concept was added, which applied data from mating experiments. Biophysical measurements of DNA similarity between isolates were an early measure that became more broadly applied with the advent of sequencing technology, leading to a sequence-based species concept using comparisons of

parts of the ribosomal DNA. At present phylogenetic species concepts that employ sequence data of rDNA and other genes are universally applied in fungal taxonomy, including yeasts, because various studies revealed a relatively good correlation between the biological species concept and sequence divergence. The application of genome information is becoming increasingly common, and we strongly recommend the use of complete, rather than draft genomes to improve our understanding of species and their genome and genetic dynamics. Complete

genomes allow in-depth comparisons on the evolvability of genomes and, consequently, of the species to which they belong. Hybridization seems a relatively common phenomenon and has been observed in all major fungal lineages that contain yeasts. Note that hybrids may greatly differ in their post-hybridization development. Future in-

depth studies, initially using some model species or complexes may shift the traditional species concept as isolated clusters of genetically compatible isolates to a cohesive speciation network in which such clusters are interconnected by genetic processes, such as hybridization.

Remembering Lex Sheffers

I have the sad duty to inform you that Lex Scheffers died on May 12, at the age of 95.

Yeast research in Delft owes much to Lex. He performed foundational work on the regulation of alcoholic fermentation (last month, our group has submitted a paper about the Custers effect in which we cite Lex's 1966 Nature paper) and, when the Delft Microbiology group went through a prolonged rough patch in the 1970s after the departure of the then Chair, Lex played a major role in keeping the flame of yeast research burning. Lex's contributions to yeast science were in no way limited to a laboratory in Delft. For many years, he was an active member of the International Commission on Yeast, he organized several well-attended international symposia and, well into his retirement, Lex was the very active and equally successful founding Editor of FEMS Yeast Research.

I cannot claim a close scientific collaboration with Lex, who retired in the same month that I finished my PhD project. Lex, however, had his entirely own interpretation about retirement. Until we moved to a new laboratory in 2016, he remained a regular working visitor to the lab and I had the privilege to attend an number of conferences together with him. I cherish fond memories of an eminent scientist with a very strong sense of integrity, a beautifully dry sense of humour, a deep caring for the quality of academic writing and, until an advanced age, an exceptional drive to contribute to the international scientific community.

Churchill once said 'History will be kind to me, for I intend to write it'. Lex's integrity would have prevented him from manipulating the past to his advantage, but, six years ago, he did publish an eloquent account of his career in FEMS Yeast Research, which I recommend to those of you who want to spend a few moments to mark the passing of a great colleague: <https://doi.org/10.1093/femsyr/fov037>

Jack Pronk

Future Meetings

International Congress on Yeasts 15 — The Spirit of Yeast August 22-26 2021, Vienna University, Vienna, Austria

We are pleased to announce that ICY 15, originally planned for August 2020, will be held jointly with the 30th International Conference on Yeast Genetics and Molecular Biology (ICYGMB30), in the Heart of the City of Vienna, Austria, August 22 to 26, 2021. The conference is intended to attract all yeast researchers in all fields. For an overview of the program and other updates, please consult <http://icy15.boku.ac.at/>.

With kind regards,

Diethard Mattanovich and the “ICY15 meets ICYGMB30” organizing team



Every two years, the International Workshop on Brewing Yeasts (IWOBY) brings together yeast scientists and advanced brewers from all over the world seeking for the latest developments and innovations in brewing and other fermented beverages industries. An unforgettable event that allows you to enjoy the incredible patagonian natural surroundings together with the amazing local craft breweries and distilleries ecosystem. A set of unique social activities and amenities awaits you at the IWOBY 2021, as well as plenty of experimental beers brewed with new yeasts. IWOBY will provide you with a real taste of brewing science and innovation! The IWOBY is a NON PROFIT event organized by the Instituto Andino Patagónico de Tecnologías Biológicas y Geoambientales (IPATEC) from CONICET and Comahue's National University, and CRELTEC Foundation. The first conference took place in Bariloche city, Patagonia, Argentina in 2018. Due to our global context, the 2nd meeting had to be postponed in 2020 and finally will take place on 20th-21st November 2021. The event consists of two full days of plenary presentations of renown scientists from all over the world, as well as a variety of oral and e-poster presentations together with a commercial exhibition from our sponsors and a fantastic brewery and distillery tour. The format will be hybrid (on-line and face-to-face). Looking forward to meeting you at the IWOBY2021. More information in www.iwooby.com.ar/es,

D. Libkind

Brief News Item

Retirement: Prof Jeremy Thorner

I formally retired from active University service and am now (as of 1 July 2020) a Professor Emeritus. My laboratory is formally closing all its operations as of this summer (on 30 June 2021).

Jeremy Thorner, Professor Emeritus
Division of Biochemistry, Biophysics and Structural Biology
Department of Molecular and Cell Biology
University of California, Berkeley, USA

jthorner@berkeley.edu

Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

Official publication of the
International Council of Yeasts and Yeast-like Microorganisms

June 1971

Volume XX, Number 1

Editor

Herman J. Phaff, University of California, Davis, California 95616

Associate Editor

Anna Kocková-Kratochvilová, Slovak Academy of Sciences, Bratislava, Czechoslovakia

Associate Editor

Torsten O. Wikén, Laboratory for Microbiology, Technical University, Delft, Holland

Associate Editor

Richard Snow, Dept. of Genetics, University of California, Davis, California 95616

D. Yarrow of the Centraalbureau voor Schimmelcultures (Netherlands) communicated receipt of type strains of the new species *Candida lactose*, *Cryptococcus neoformans* v. *gatti*, *Hansenula malanga*, *Pichia ambrosia*, *Pichia meimii*, *Pichia spartinae*, and *Saccharomyces syneadendra*.

C. P. Kurtzman of the USDA Northern Utilization Research and Development Division in Peoria, Illinois shared abstracts of two recently published publications, one describing spoilage yeasts (especially *Saccharomyces bailii*) and bacteria in mayonnaise and salad dressings, and description of two new saturn-spored species, *Pichia mucosa* and *Pichia sargentensis*.

James A. Barnett of the Agricultural Research Council, Food Research Institute, England questioned whether identifying one yeast isolate of each colony morphology resulted in an accurate representation of species present, and also wanted to reduce the number of nutritional tests to identify species. Over a 3-year period, his laboratory identified over 1,500 yeasts from strawberries and other fruits. Publications resulting from this work included a revised panel of diagnostic tests, revised diagnostic keys, the description of *Torulopsis fragraria*, and the yeasts of strawberries. He reported working on a computerized diagnostic key superior to that in *The Yeasts: A Taxonomic Study* by J. Lodder (1970), based on physiological tests rather than ascospore formation. He requested yeast strains from the readers for his work on D-ribose utilization.

An abstract of a publication by **Shoji Goto** of Yamanishi University, Kofu, Japan detailed Himalayan yeasts including new species *Cryptococcus bhutanensis* and *Cr. himalayensis*.

Samuel P. Meyers of Louisiana State University and **D. G. Ahearn** of Georgia State University shared abstracts of two papers at the American Society for Microbiology meeting in Minneapolis, Minnesota on pulcherrimin-producing yeasts and the effect of crude oil on yeast populations, both in Louisiana marshland.

H. Klaushofer of the Lehrkanzel für Biochemische Technologie, Hochschule für Bodenkultur, Vienna, Austria published a paper on ascospore formation, and another on yeast systematics including a diagram of the distribute of species within different genera. The research group planned to present work on yeasts isolated from mead at the June 1971 International Specialized Symposium on Yeasts in Smolenice, CSSR.

Leslie R. Hedrick and **P. F. Dupont** of Illinois Institute of Technology measured DNA base composition of 49 strains of *Trichosporon*. Forty strains were retained in four clusters with a total of ten species of *Trichosporon*, and

others transferred to *Candida* or *Endomycopis*. A “numerical taxonomic analysis with the aid of a computer” was then performed using 81 characteristics of 25 strains, which resulted in 3 major branches that did not correlate well with the clusters based on DNA base composition.

A list of three papers published and five in press by **T. Nakase** of Ajinomoto Co., Inc., Kawasaki, Japan concerned the DNA base composition of *Pichia*, *Debaryomyces*, *Hansenula*, *Saccharomyces*, *Candida*, *Torulopsis*, *Cryptococcus*, and *Rhodotorula*.

The problem of the homonyms *Saccharomycopis* Schiønning and *Saccharomycopis* Guilliermond was addressed **J. P. van der Walt** of the South African Council for Scientific and Industrial Research, Pretoria, South Africa. He summarized the history of *S. capsularis* and *S. guttulata*, and proposed that *Endomycopis capsularis* be restored to *Saccharomycopis capsularis*, and proposed transfer of the second species to a new genus, *Cyniclomyces guttulata*. He also described new species of insect-associated yeasts *Pichia ambrosiae*, *Pichia cicatricosa*, *Hansenula dryadoides*, *Pichia xylophoci*, and *Saccharomycopis synnaedendra*.

M. C. Pignal, Université de Lyon, France published about the tannase enzymes from xylophagous yeasts from insect-infested wood and tanning liquor.

K. L. Malkin of the Animal Pathology Division, Canada Department of Agriculture, Québec, Canada studied cryptococcosis in domestic cats and dogs, and identified the causative agent as *Cryptococcus neoformans*

A paper in press by **Luis A. Roure**, Tropical Mycology Laboratory, University of Puerto Rico relates to an epidemic of fungal skin infections caused by *Candida albicans* and *Geotrichum candidum*, affecting over 100 of the 112 teenage boys at the Center of Studies and Work in rural Puerto Rico.

M. M. Shahin, postdoc at the Atomic Energy of Canada Limited, Ontario, Canada under Dr. A. Nasim, completed a thesis at the Institute of Biophysics, Free University of Berlin, Germany titled “Formation and regeneration of yeast protoplasts”.

P. Galzy, École Nationale Supérieure Agronomique de Montpellier, France was assembling manuscripts on agricultural microorganisms, genetic control of sporulation, metabolic changes during sporulation, and yeasts associated with Roquefort cheese.

John Gorman, University of Kentucky, studied three allele-specific missense suppressors of the his2-1 allele.

An abstract about gene-enzyme relationships in the fatty acid synthetase system of *Saccharomyces cerevisiae* was presented at the 7th Meeting of the European Biochemical Societies at Varna by **E. Schweizer**, Institut für Biochemie der Universität Würzburg, West Germany.

Ø. Strømnaes, University of Oslo, Norway developed the “ultrasonic-paraffin technique” to enrich ascospores from a mixture with diploid cells, using snail enzyme to digest the ascus wall; ultrasound treatment to selectively kill diploid cells; extraction with paraffin.

Several members of the yeast genetics group in the Department of Genetics at the University of California Berkeley shared summaries of their recent work. **E. Sena** and **D. Radin** developed techniques to enrich *S. cerevisiae* zygotes, to facilitate studies of zygote formation. **S. Henry** and **S. Fogel** studied fatty acid synthesis using mutants of *S. cerevisiae* that required saturated fatty acids for growth. **M. Bard** examined the sterol composition and genetics of nystatin resistant mutants of *S. cerevisiae*. **B. Wisnieski**, **R. James** and **A. Keith** correlated fatty acid specificity with membrane structure, function, and biosynthesis. The group used electron-spin resonance (ESR) spectroscopy with spin-labeled fatty acid analogues to study changes in the physical state of membranes. S. Fogel and collaborators developed a system to detect and recover *S. cerevisiae* mutants deficient in meiotic recombination, using a disomic a/alpha haploid strain.

N. A. Khan, City University of New York discovered clustering of maltose and sucrose genes in *Saccharomyces*.

F. K. Zimmermann Brooklyn College, New York compared the induction and inhibition of activity of *S. cerevisiae* maltose genes MAL1 and MAL3 by maltose and sucrose, and proposed that autoregulation explained the observed gene repression.

A. Maxwell, Stanford Research Institute, California determined that irradiation with light in wavelengths between 310 and 430 nm was lethal to *Rhodotorula glutinis*. Carotenoid pigments were not effective in this region.

E. Azoulay, Laboratoire de Chimie Bactérienne, Marseille, France studied alcohol and aldehyde dehydrogenases of *Candida tropicalis* cultivated on hydrocarbons.

R. V. Brunt, Bath University of Technology, England published discoveries about polysaccharide synthesis and glycolysis in *S. cerevisiae*, made using fluorinated carbohydrates. The structural and functional integrity of yeast mitochondria prepared by enzymatic vs. mechanical disintegration was also compared.

The results of a collaboration between Institut Pasteur(Paris) and Institut National de la Recherche Agronomique (Dijon) was described by **P. Brechot** and **P. Dupuy**, Station de Technologie, France. Oleanic acid was identified as the main constituent from the wax which covers grapes. Anaerobic cultures of *S. cerevisiae* both grew more strongly and fermented more sugar when oleanolic acid was added to the medium.

N. P. Elinov, Chemical-Pharmaceutical Institute, Leningrad, USSR examined the extracellular and cellular polysaccharides of 17 *Rhodotorula* species and *Aureobasidium pullulans*. C¹⁴-labeled polysaccharide complexes injected into white mice were absorbed on erythrocytes within 5 minutes, and remained for months.

J. R. Helbert, Miller Brewing Company, Milwaukee, Wisconsin presented a paper on spoilage of beer by *Dekkera intermedia* at the meeting of the American Society of Brewing Chemists in Montreal, Canada in May 1971.

Jessie Bodenhoff, Statens Serum Institut, Copenhagen, Denmark compared the sensitivity of 38 strains of *Candida*, *Torulopsis* and *Cryptococcus* to Bay b 5097, a new antimycotic developed by Bayer. Most *C. albicans*, and all *C. tropicalis* strains were only slight sensitive.

D. A. Lovett, Allied Breweries (Production) Limited, Burton-on-Trent, UK reported that colonies produced by a single strain of *S. carlsbergensis* grown on Difco WLN Agar varied significantly in growth, form and color; therefore this medium would not be useful to differentiate wild yeasts vs. *S. carlsbergensis*. The formation of hydrogen sulfide and sulfur dioxide was found to be effectively inhibited by methionine in *S. cerevisiae*, but much less so in *S. carlsbergensis*.

Meetings:

The Specialized Symposium on Yeast was scheduled for June 1971 at the Slovak Academy of Sciences, Smolenice Castle, Czechoslovakia. The program was listed in the newsletter, and included many familiar names.

The Specialized Symposium on Yeasts was scheduled for 1972 in Kyoto, Japan, in connection with the IVth International Fermentation Symposium.

Brief new items:

C. M. Clark, University of Illinois and Yeast Newsletter associate editor for many years, retired. Torsten D. Wiken, succeeded Clark as Associate Editor.

L. J. Wickerham and wife established a new home in Tucson, Arizona.

L. R. Batra, US Department of Agriculture, Beltsville, Maryland completed a monograph on mycelial hemiascomycetes.

S. Fogel and **R. Mortimer** received funding from the National Science Foundation to establish a yeast culture collection for the yeast genetics community.

K. Kodama provided a list of errors in Chapter 5, Sake Yeast, in *The Yeasts*, Vol. III (A. H. Rose and J. S. Harrison, eds.).

Editor **H. J. Phaff** notified readers that the subscription rate would increase from \$1.00 to \$1.50 per year, and could be paid by Unesco Coupons or International Postal Exchange Coupons in case of foreign currency restrictions.

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