

Yeast

A Newsletter for Persons Interested in Yeast

ISSN 0513-5222

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

JUNE 2023

Volume LXXII, Number I

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Communicated by WI Golubev <wig@ibpm.pushchino.ru>.**

Recent publication.

- 1 Golubev WI. 2022. Mycocinotyping of some unaffiliated *Candida* species. *Microbiology (Pleiades)* 91(5):831-833.

The studied type strains from the phylogenetically unaffiliated *Candida* species are insensitive to mycocins of ascosporogenous yeasts of seven genera. The data

obtained may be useful for assessing the taxonomic position of yeast strains within the large polyphyletic genus *Candida*.

II Laboratory of Genetics, Wisconsin Energy Institute, DOE Great Lakes Bioenergy Research Center, Center for Genomic Science Innovation, J. F. Crow Institute for the Study of Evolution, University of Wisconsin, Madison, WI 53726, USA. Communicated by Chris Todd Hittinger <cthittinger@wisc.edu>.

Recent publications.

- 1 Crandall JG, Fisher KJ, Sato TK, Hittinger CT. An adaptive interaction between cell type and metabolism drives ploidy evolution in a wild yeast. bioRxiv under - <https://doi.org/10.1101/2022.11.14.516370>.

Ploidy is an evolutionarily labile trait, and its variation across the tree of life has profound impacts on evolutionary trajectories and life histories. The immediate consequences and molecular causes of ploidy variation on organismal fitness are frequently less clear, although extreme mating type skews in some fungi hint at links between cell type and adaptive traits. Here we report an unusual recurrent ploidy reduction in replicate populations of the budding yeast *Saccharomyces eubayanus* experimentally evolved for improvement of a key metabolic trait, the ability to use maltose as a carbon source. We find that haploids have a substantial, but conditional, fitness advantage in the absence of other genetic variation. Using engineered genotypes that

decouple the effects of ploidy and cell type, we show that increased fitness is primarily due to the distinct transcriptional program deployed by haploid-like cell types, with a significant but smaller contribution from absolute ploidy. The link between cell-type specification and the carbon metabolism adaptation can be traced to the noncanonical regulation of a maltose transporter by a haploid-specific gene. This study provides novel mechanistic insight into the molecular basis of an environment-cell type fitness interaction and illustrates how selection on traits unexpectedly linked to ploidy states or cell types can drive karyotypic evolution in fungi.

- 2 Nalabothu RL, Fisher KJ, LaBella AL, Meyer TA, Opulente DA, Wolters JF, Rokas, A, Hittinger CT. 2023. Codon optimization improves the prediction of xylose metabolism from gene content in budding yeasts. *Mol Biol Evol* - epub <https://doi.org/10.1093/molbev/msad111>.

Xylose is the second most abundant monomeric sugar in plant biomass. Consequently, xylose catabolism is an ecologically important trait for saprotrophic organisms, as well as a fundamentally important trait for industries that hope to convert plant mass to renewable fuels and other bioproducts using microbial metabolism. Although common across fungi, xylose catabolism is rare within Saccharomycotina, the subphylum that contains most industrially relevant fermentative yeast species. The genomes of several yeasts unable to consume xylose have been previously reported to

contain the full set of genes in the *XYL* pathway, suggesting the absence of a gene-trait correlation for xylose metabolism. Here, we measured growth on xylose and systematically identified *XYL* pathway orthologs across the genomes of 332 budding yeast species. Although the *XYL* pathway coevolved with xylose metabolism, we found that pathway presence only predicted xylose catabolism about half of the time, demonstrating that a complete *XYL* pathway is necessary, but not sufficient, for xylose catabolism. We also found that *XYL1* copy number was positively

correlated, after phylogenetic correction, with xylose utilization. We then quantified codon usage bias of *XYL* genes and found that *XYL3* codon optimization was significantly higher, after phylogenetic correction, in species able to consume xylose. Finally, we showed that codon optimization of *XYL2* was positively correlated,

after phylogenetic correction, with growth rates in xylose medium. We conclude that gene content alone is a weak predictor of xylose metabolism and that using codon optimization enhances the prediction of xylose metabolism from yeast genome sequence data.

- 3 Parra M, Libkind D, Hittinger CT, Alvarez L, Bellora N. 2023. Assembly and comparative genome analysis of a Patagonian *Aureobasidium pullulans* isolate reveals unexpected intraspecific variation. Yeast - epub: <https://doi.org/10.1002/yea.3853>.

Aureobasidium pullulans is a yeast-like fungus with remarkable phenotypic plasticity widely studied for its importance for the pharmaceutical and food industries. So far, genomic studies with strains from all over the world suggest they constitute a genetically unstructured population, with no association by habitat. However, the mechanisms by which this genome supports so many phenotypic permutations are still poorly understood. Recent works have shown the importance of sequencing yeast genomes from extreme environments to increase the repertoire of phenotypic diversity of unconventional yeasts. In this study, we present the genomic draft of *A. pullulans* strain from a Patagonian yeast diversity

hotspot, re-evaluate its taxonomic classification based on taxonomic approaches, and annotate its genome with high-depth transcriptomic data. Our analysis suggests this isolate could be considered a novel variant at an early stage of the speciation process. The discovery of divergent strains in a genomically homogeneous group, such as *A. pullulans*, can be valuable in understanding the evolution of the species. The identification and characterization of new variants will not only allow finding unique traits of biotechnological importance, but also optimize the choice of strains whose phenotypes will be characterized, providing new elements to explore questions about plasticity and adaptation.

- 4 Schmitz JM, Wolters JF, Murray NH, Guerra RM, Bingman CA, Hittinger CT, Pagliarini DJ. 2023. Aim18p and Aim46p are CHI-domain-containing mitochondrial hemoproteins in *Saccharomyces cerevisiae*. J Biol Chem 299:102981- <https://doi.org/10.1015/j.jbc.223.102981>.

Chalcone isomerases (CHIs) have well-established roles in the biosynthesis of plant flavonoid metabolites. *Saccharomyces cerevisiae* possesses two predicted CHI-like proteins, Aim18p (encoded by YHR198C) and Aim46p (YHR199C), but it lacks other enzymes of the flavonoid pathway, suggesting that Aim18p and Aim46p employ the CHI fold for distinct purposes. Here, we demonstrate using proteinase K protection assays, sodium carbonate extractions, and crystallography that Aim18p and Aim46p reside on the mitochondrial inner membrane and adopt CHI folds, but they lack select active site residues and possess an extra fungal-specific

loop. Consistent with these differences, Aim18p and Aim46p lack CHI activity and also the fatty acid-binding capabilities of other CHI-like proteins, but instead bind heme. We further show that diverse fungal homologs also bind heme and that Aim18p and Aim46p possess structural homology to a bacterial hemoprotein. Collectively, our work reveals a distinct function and cellular localization for two CHI-like proteins, introduces a new variation of a hemoprotein fold, and suggests that ancestral CHI-like proteins were hemoproteins.

- 5 Peris D, Ubbelohde EJ, Kuang MC, Kominek J, Langdon QK, Adams M, Koshalek JA, Hulfachor AB, Opulente DA, Hall DJ, Hyma K, Fay JC, Leducq JB, Charron G, Landry CR, Libkind D, Gonçalves C, Gonçalves P, Sampaio JP, Wang QM, Bai FY, Wrobel RL, Hittinger CT. 2022. Macroevolutionary diversity of traits and genomes in the model yeast genus *Saccharomyces*. Nature Commun 14:690 - <https://doi.org/10.1101/2022.03.30.486421>.

Species is the fundamental unit to quantify biodiversity. In recent years, the model yeast *Saccharomyces cerevisiae* has seen an increased number of studies related to its geographical distribution, population structure, and phenotypic diversity. However, seven additional species from the same genus have been

less thoroughly studied, which has limited our understanding of the macroevolutionary events leading to the diversification of this genus over the last 20 million years. Here, we show the geographies, hosts, substrates, and phylogenetic relationships for approximately 1,800 *Saccharomyces* strains, covering

the complete genus with unprecedented breadth and depth. We generated and analyzed complete genome sequences of 163 strains and phenotyped 128 phylogenetically diverse strains. This dataset provides insights about genetic and phenotypic diversity within and between species and populations, quantifies

reticulation and incomplete lineage sorting, and demonstrates how gene flow and selection have affected traits, such as galactose metabolism. These findings elevate the genus *Saccharomyces* as a model to understand biodiversity and evolution in microbial eukaryotes.

- 6 Liu L, Ansari RU, Vang-Smith M, Hittinger CT, Sato TK. 2023. A role for ion homeostasis in yeast ionic liquid tolerance. *MicroPub Biol* 2023:10.17912/micropub.biology.000718 - <https://doi.org/10.17912/micropub.biology.000718>.

The model yeast *Saccharomyces cerevisiae* s being developed as a biocatalyst for the conversion of renewable lignocellulosic biomass into biofuels. The ionic liquid 1-ethyl-3-methylimidazolium chloride (EMIMCl) solubilizes lignocellulose for deconstruction into fermentable sugars, but it inhibits yeast fermentation. EMIMCl tolerance is mediated by the

efflux pump Sge1p and uncharacterized protein Ilt1p. Through genetic investigation, we found that disruption of ion homeostasis through mutations in genes encoding the Trk1p potassium transporter and its protein kinase regulators, Sat4p and Hal5p, causes EMIMCl sensitivity. These results suggest that maintenance of ion homeostasis is important for tolerance to EMIMCl.

- 7 Barros KO, Alvarenga FBM, Magni G, Souza GFL, Abegg MA, Palladino F, da Silva SS, Rodrigues RCLB, Sato TK, Hittinger CT, Rosa CA. 2023. The Brazilian Amazonian rainforest harbors a high diversity of yeasts associated with rotting wood, including many candidates for new yeast species. *Yeast* 40:84-101 - <https://doi.org/10.1002/yea.3837>.

This study investigated the diversity of yeast species associated with rotting wood in Brazilian Amazonian rainforests. A total of 569 yeast strains were isolated from rotting wood samples collected in three Amazonian areas (Universidade Federal do Amazonas-Universidade Federal do Amazonas [UFAM], Piquiá, and Carú) in the municipality of Itacoatiara, Amazon state. The samples were cultured in yeast nitrogen base (YNB)-d-xylose, YNB-xylose, and sugarcane bagasse and corncob hemicellulosic hydrolysates (undiluted and diluted 1:2 and 1:5). *Sugiyamaella* was the most prevalent genus identified in this work, followed by *Kazachstania*. The most frequently isolated yeast species were *Schwanniomyces polymorphus*, *Scheffersomyces amazonensis*, and *Wickerhamomyces* sp., respectively. The alpha

diversity analyses showed that the dryland forest of UFAM was the most diverse area, while the floodplain forest of Carú was the least. Additionally, the difference in diversity between UFAM and Carú was the highest among the comparisons. Thirty candidates for new yeast species were obtained, representing 36% of the species identified and totaling 101 isolates. Among them were species belonging to the clades *Spathaspora*, *Scheffersomyces*, and *Sugiyamaella*, which are recognized as genera with natural xylose-fermenting yeasts that are often studied for biotechnological and ecological purposes. The results of this work showed that rotting wood collected from the Amazonian rainforest is a tremendous source of diverse yeasts, including candidates for new species.

- 8 Li Y, Liu H, Steenwyk JL, LaBella AL, Harrison MC, Groenewald M, Zhou X, Shen XX, Zhao T, Hittinger CT, Rokas A. 2022. Contrasting modes of macro and microsynteny evolution in a eukaryotic subphylum. *Curr Biol* 32:5335-5343.e4 - <https://doi.org/10.1016/j.cub.2022.10.025>.

Examination of the changes in order and arrangement of homologous genes is key for understanding the mechanisms of genome evolution in eukaryotes. Previous comparisons between eukaryotic genomes have revealed considerable conservation across species that diverged hundreds of millions of years ago (e.g., vertebrates,^{1,2,3} bilaterian animals,^{4,5} and filamentous fungi⁶). However, understanding how genome organization evolves within and between eukaryotic major lineages remains underexplored. We analyzed

high-quality genomes of 120 representative budding yeast species (subphylum Saccharomycotina) spanning ~400 million years of eukaryotic evolution to examine how their genome organization evolved and to compare it with the evolution of animal and plant genome organization.⁷ We found that the decay of both macro-synteny (the conservation of homologous chromosomes) and microsynteny (the conservation of local gene content and order) was strongly associated with evolutionary divergence across budding yeast major clades. However,

although macrosynteny decayed very fast, within ~100 million years, the microsytenty of many genes—especially genes in metabolic clusters (e.g., in the *GAL* gene cluster⁸)—was much more deeply conserved both within major clades and across the subphylum. We further found that when genomes with similar evolutionary divergence times were compared, budding yeasts had lower macrosynteny conservation than

animals and filamentous fungi but higher conservation than angiosperms. In contrast, budding yeasts had levels of microsytenty conservation on par with mammals, whereas angiosperms exhibited very low conservation. Our results provide new insight into the tempo and mode of the evolution of gene and genome organization across an entire eukaryotic subphylum.

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The following are papers for 2023 or in press.

- 1 Borovkova AN, Shalamitskiy MYu, Naumova ES. 2023. Pectinolytic yeast *Saccharomyces paradoxus* is a new gene pool for winemaking. *Microbiology (Moscow)* 92(2):256–268.
- 2 Borovkova AN, Naumov GI, Shnyreva AV, Naumova ES. 2023. Genetically isolated population of *Saccharomyces bayanus* in New Zealand and Australia. *Russian Journal of Genetics*. 59(4):403–416 (in Russian).
- 3 Lyutova L.V, Naumova ES. 2023. Comparative analysis of lactose fermentation and its components, glucose and galactose, by inter-strain hybrids of dairy yeast *Kluyveromyces lactis*. *Biotekhnologiya (Moscow)* 39 (1): (in press).

Using the fermentation test and HPLC analysis, we studied the dynamics of lactose, glucose, and galactose fermentation and the formation of ethyl alcohol by dairy yeasts *Kluyveromyces marxianus*, *K. lactis* and inter-strain hybrids. Hybrids of *K. lactis* H1-2 (NRRL Y-1118

× NRRL Y-1140) and H3-1 (NRRL Y-1140 × VKM Y-1333), and *K. marxianus* VKM Y-1337 showed the highest fermentation activity. Inter-strain hybridization was shown to be a promising method for creating dairy strains of *Kluyveromyces* that actively ferment lactose.

- 4 Tellini N, De Chiara M, Mozzachiodi S, Tattini L, Vischioni C, Naumova ES, Warringer J., Bergström A, Liti G. 2023. Ancient and recent origins of shared polymorphisms in yeast. Preprint.

Shared polymorphisms between populations can be ascribed to ancestral variation or underlie more recent gene flows. These mechanisms occur at fundamentally different timescales but can be challenging to disentangle them. Here, we developed a computational framework to map shared polymorphisms in *Saccharomyces cerevisiae* and its closest relative *Saccharomyces paradoxus*, which diverged 4.0-5.8 million years ago. We mapped shared polymorphisms across 1,673 sequenced *S. cerevisiae* isolates with high resolution (mean distance 15.6 bp). We catalogued 11,863 introgression blocks with the majority being recent and clade-specific. The highly diverged wild Chinese lineages were depleted of introgressions, supporting, that secondary contacts occurred after the out-of-China event. However, these lineages retained an excess of individual ancestral polymorphisms derived from incomplete lineage sorting (ILS). We propose that

ILS polymorphisms in Chinese lineages, perhaps retained due to fewer or less dramatic bottlenecks during their demographic histories, dramatically contribute to inflating their genetic distance. In the non-Chinese lineages, we inferred introgression ancestry and revealed multiple pulses of introgressive hybridization at different timescales. We reconstructed major hybridization events and detected cases of overlapping introgression across distinct clades due to either shared histories or convergent events. We reconstruct an introgression block in otherwise isogenic backgrounds tagged with distinct fluorescent proteins. Competitive growth assay revealed that the recurrent *PADI-FDC1* gene-pair introgression potentiate stress response to multiple antifungal drugs and ferulic acid. Our study retraces past histories of divergence and contacts across *S. cerevisiae* and *S. paradoxus* populations and unveil their functional outcomes.

IV Laboratory of Yeast Systematics, Tokyo NODAI Research Institute (TNRI), Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502 Japan. Communicated by Masako Takashima <mt207623@nodai.ac.jp>.

Recent publication.

- 1 Aoki K, Yamamoto K, Ohkuma K, Sugita T, Tanaka N, Takashima M. 2023. Hyphal growth in *Trichosporon asahii* is accelerated by the addition of magnesium. *Microbiology Spectrum* (in press).

Fungal dimorphism involves two morphologies: a unicellular yeast cell and a multicellular hyphal form. Invasion of hyphae into human cells causes severe opportunistic infections. The transition between yeast and hyphal forms is associated with the virulence of fungi; however, the mechanism is poorly understood. Therefore, we aimed to identify factors that induce hyphal growth of *Trichosporon asahii*, a dimorphic basidiomycete that causes trichosporonosis. *T. asahii* showed poor growth and formed small cells containing large lipid droplets and fragmented mitochondria when cultivated for 16 h in a nutrient-deficient liquid medium. However, these phenotypes were suppressed via the addition of yeast nitrogen base. When *T. asahii* cells were cultivated in the presence of different compounds present in the yeast nitrogen base, we found that magnesium sulfate was a key factor for inducing cell

elongation, and its addition dramatically restored hyphal growth in *T. asahii*. In *T. asahii* hyphae, vacuoles were enlarged, the size of lipid droplets was decreased, and mitochondria were distributed throughout the cell cytoplasm and adjacent to the cell walls. Additionally, hyphal growth was disrupted due to treatment with an actin inhibitor. The actin inhibitor latrunculin A disrupted the mitochondrial distribution even in hyphal cells. Furthermore, magnesium sulfate treatment accelerated hyphal growth in *T. asahii* for 72 h when the cells were cultivated in a nutrient-deficient liquid medium. Collectively, our results suggest that an increase in magnesium levels triggers the transition from the yeast to hyphal form in *T. asahii*. These findings will support studies on the pathogenesis of fungi and aid in developing treatments.

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Recent publications.

- 1 Soares Rodrigues CI, den Ridder M, Pabst M, Gombert AK, Wahl SA. 2023. Comparative proteome analysis of different *Saccharomyces cerevisiae* strains during growth on sucrose and glucose. *Scientific Reports* 13(1):2126, 2023.
- 2 Eliodorio KP, Cunha GC, Lino FS, Sommer MO, Gombert AK, Giudici R, Basso TO. 2023. A fully defined synthetic medium mimicking sugar cane molasses. *bioRxiv* 2023.01. 27.525923

VI Department of Agriculture, Division of Grape and Wine Sciences, University of Naples Federico II, Viale Italia 60, 83100 Avellino, Italy. Communicated by Giuseppe Blaiotta <blaiotta@unina.it> <giuseppe.blaiotta@personalepec.unina.it>.

Recent publication.

- 1 Petruzzello E, Blaiotta G, Pittari E, Piombino E, Aponte M. 2023. Isolation and characterization of cryotolerant yeasts from Fiano di Avellino grapes fermented at low temperatures. *Foods* (MDPI) 12:526 - <https://doi.org/10.3390/foods12030526>.

A fermentation of Fiano di Avellino grape must was carried out at 9°C with the aim of selecting cryotolerant yeast strains and testing their fermentative performances and volatile production following molecular

characterization. A total of 20 yeast cultures were isolated at different fermentation stages. Based on molecular identification and characterization, *Metschnikowia* (*M.*) *pulcherrima*, *Hanseniaspora* (*H.*)

uvarum, *Staremerella (St.) bacillaris*, *Saccharomyces (S.) cerevisiae*, *S. kudriavzevii*, and *S. paradoxus* were found to be the yeast species dominating the fermentation. *S. paradoxus* has been rarely isolated in vineyards and never in the cellar environment. Moreover, in this study, *S. kudriavzevii* is detected for the first time in vine-wine environments. Both *S. kudriavzevii* and *S. paradoxus* co-occurred with

S. cerevisiae when grapes were micro-fermented at low temperatures. The growth kinetics of the three species were greatly affected by the fermentation temperature. As a consequence, Fiano wines obtained with *S. kudriavzevii* and *S. paradoxus* significantly differed from those made by *S. cerevisiae* in terms of chemical and volatile composition.

VII Department of Agricultural, Food and Environmental Sciences, Industrial Yeasts Collection DBVPG, University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. Communicated by Pietro Buzzini <pietro.buzzini@unipg.it>.

Recent publications.

- 1 Aiello D, Sannino C, Giannoni T, Fabbri G, Gelosia M, Nicolini A, Turchetti B, Cotana F, Buzzini P. 2021. Triacyl glycerols from yeast-catalysed batch and fed-batch bioconversion of hydrolysed lignocellulose from cardoon stalks. *Fermentation (MDPI)* 7:315.

The lipogenic ability of the yeast *Solicoccozyma terricola* DBVPG 5870 grown on hydrolyzed lignocellulose obtained from cardoon stalks was evaluated. Data on cell biomass, lipid production, and fatty acid profiles of triacylglycerols obtained in batch and fed-batch experiments were carried out at the laboratory scale in a 5L fermenter, and at two different temperatures (20 and 25 °C) were reported. The higher production of total intracellular lipids (13.81 g/L) was found in the fed-batch experiments carried out at 20 °C. *S. terricola* exhibited the ability to produce high amounts

of triacylglycerol (TAGs) with a characteristic fatty acids profile close to that of palm oil. The TAGs obtained from *S. terricola* grown on pre-treated lignocellulose could be proposed as a supplementary source of oleochemicals. Indeed, due to the rising prices of fossil fuels and because of the environmental-related issues linked to their employment, the use of TAGs produced by *S. terricola* grown on lignocellulose could represent a promising option as a supplementary oleochemical, especially for biodiesel production.

- 2 Mozzachiodi S, Bai FY, Baldrian P, Bell G, Boundy-Mills K, Buzzini P, Čadež N, Cubillos Rifo F, Dashko S, Dimitrov S, Fisher KJ, Gibson BR, Gouliamova D, Greig D, Heisteringer L, Hittinger CT, Jecmenica M, Koufopanou V, Landry CR, Mašínová TR, Naumova ES, Opulente D, Peña JJ, Petrovič U, Tsai IJ, Turchetti B, Villarreal P, Yurkov A, Liti G, Boynton P. 2022. Yeasts from temperate forests. *Yeast* 39:4-24.

Yeasts are ubiquitous in temperate forests. While this broad habitat is well-defined, the yeasts inhabiting it and their life cycles, niches, and contributions to ecosystem functioning are less understood. Yeasts are present on nearly all sampled substrates in temperate forests worldwide. They associate with soils, macroorganisms, and other habitats and no doubt contribute to broader ecosystem-wide processes. Researchers have gathered information leading to hypotheses about yeasts' niches and their life cycles based on physiological observations in the laboratory as well as genomic analyses, but the challenge remains to

test these hypotheses in the forests themselves. Here, we summarize the habitat and global patterns of yeast diversity, give some information on a handful of well-studied temperate forest yeast genera, discuss the various strategies to isolate forest yeasts, and explain temperate forest yeasts' contributions to biotechnology. We close with a summary of the many future directions and outstanding questions facing researchers in temperate forest yeast ecology. Yeasts present an exciting opportunity to better understand the hidden world of microbial ecology in this threatened and global habitat.

- 3 Sannino C, Cannone N, D'Alò F, Franzetti A, Gandolfi I, Pittino F, Turchetti B, Mezzasoma A, Zucconi L, Buzzini P, Guglielmin M, Onofri S. 2022. Fungal communities in European alpine soils are not affected by short-term *in-situ* simulated warming than bacterial communities. *Environ Microbiol* 24:4178-4192.

The impact of global warming on biological communities colonizing European alpine ecosystems was recently studied. Hexagonal open top chambers (OTCs) were used for simulating a short-term *in situ* warming (estimated around 1°C) in some alpine soils to predict the impact of ongoing climate change on resident microbial communities. Total microbial DNA was extracted from soils collected either inside or outside the OTCs over 3 years of study. Bacterial and fungal rRNA copies were quantified by qPCR. Metabarcoding sequencing of taxonomy target genes was performed (Illumina MiSeq) and processed by bioinformatic tools. Alpha- and beta-diversity were used to evaluate the

structure of bacterial and fungal communities. qPCR suggests that, although fluctuations have been observed between soils collected either inside and outside the OTCs, the simulated warming induced a significant ($p < 0.05$) shift only for bacterial abundance. Likewise, significant ($p < 0.05$) changes in bacterial community structure were detected in soils collected inside the OTCs, with a clear increase of oligotrophic taxa. On the contrary, fungal diversity of soils collected either inside and outside the OTCs did not exhibit significant ($p < 0.05$) differences, suggesting that the temperature increase in OTCs compared to ambient conditions was not sufficient to change fungal communities.

- 4 Turchetti B, Buzzini P, Baeza M. 2022. A genomic approach to analyze the cold adaptation of yeasts isolated from Italian Alps. *Frontiers Microbiol* 13:1026102.

Microorganisms including yeasts are responsible for mineralization of organic matter in cold regions, and their characterization is critical to elucidate the ecology of such environments on Earth. Strategies developed by yeasts to survive in cold environments have been increasingly studied in the last years and applied to different biotechnological applications, but their knowledge is still limited. Microbial adaptations to cold include the synthesis of cryoprotective compounds, as well as the presence of a high number of genes encoding the synthesis of proteins/enzymes characterized by a reduced proline content and highly flexible and large catalytic active sites. This study is a comparative genomic study on the adaptations of yeasts isolated from the Italian Alps, considering their growth kinetics. The optimal temperature for growth (OTG), growth rate (Gr), and draft genome sizes considerably varied (OTG, 10°C–20°C; Gr, 0.071–0.0726; genomes, 20.7–21.5 Mpb; % GC, 50.9–61.5). A direct relationship was observed between calculated protein flexibilities and OTG, but not for Gr. Putative genes encoding for cold

stress response were found, as well as high numbers of genes encoding for general, oxidative, and osmotic stresses. The cold response genes found in the studied yeasts play roles in cell membrane adaptation, compatible solute accumulation, RNA structure changes, and protein folding, i.e., dihydrolipoamide dehydrogenase, glycogen synthase, omega-6 fatty acid, stearoyl-CoA desaturase, ATP-dependent RNA helicase, and elongation of very-long-chain fatty acids. A redundancy for several putative genes was found, higher for P-loop containing nucleoside triphosphate hydrolase, alpha/beta hydrolase, armadillo repeat-containing proteins, and the major facilitator superfamily protein. Hundreds of thousands of small open reading frames (SmORFs) were found in all studied yeasts, especially in *Phenoliferia glacialis*. Gene clusters encoding for the synthesis of secondary metabolites such as terpene, non-ribosomal peptide, and type III polyketide were predicted in four, three, and two studied yeasts, respectively.

- 5 Coleine C, Delgado-Baquerizo M, Zerboni A, Turchetti B, Buzzini P, Franceschi P, Selbmann L. 2023. Rock traits drive complex microbial communities at the edge of life. *Astrobiology* 23(4):395-406.

Antarctic deserts are among the driest and coldest ecosystems of the planet; there, some microbes survive under these extreme conditions inside porous rocks, forming the so-called endolithic communities. Yet the contribution of distinct rock traits to support complex microbial assemblies remains poorly determined. Here, we combined an extensive Antarctic rock survey with rock microbiome sequencing and ecological networks and found that contrasting combinations of

microclimatic and rock traits such as thermal inertia, porosity, iron concentration, and quartz cement can help explain the multiple complex microbial assemblies found in Antarctic rocks. Our work highlights the pivotal role of rocky substrate heterogeneity in sustaining contrasting groups of microorganisms, which is essential to understand life at the edge on Earth and for the search for life on other rocky planets such as Mars.

- 6 Marozzi G, Benucci GMN, Turchetti B, Massaccesi L, Baciarelli Falini L, Bonito G, Buzzini P, Agnelli A, Donnini D, Albertini E. 2023. Fungal and bacterial diversity in the *Tuber magnatum* 1 ecosystem and microbiome. *Microbial Ecol* 85:508–521.

Fungi belonging to the genus *Tuber* produce edible ascocarps known as truffles. *Tuber magnatum* Picco may be the most appreciated truffle species given its peculiar aroma. While its life cycle is not yet fully elucidated, some studies demonstrated an active role of microorganisms. The main goal of this study was to determine how the *T. magnatum* microbiome varies across space and time. To address this, we characterized microbial communities associated with *T. magnatum* through high-throughput amplicon sequencing of internal transcribed spacer (ITS) and 16S rDNAs in three productive natural sites in Italy across 2 years. At each site, four truffles were sampled as well as the soil underneath and at 40, 100, and 200 cm from the harvesting points, to assess for microbial variation between substrates, years, and sites. A statistically significant site-related effect on microbial communities

was identified, whereas only the prokaryotic community was significantly affected by the distance of soil from the truffle. Significant differences between sampling years were also found, demonstrating a possible relation among rainfall precipitation and *Firmicutes* and Actinobacteria. Thirty-six bacterial OTUs in truffles and 11 bacterial OTUs in soils beneath truffles were identified as indicator taxa. As shown for other truffle species, the dominance of *Bradyrhizobium*, *Rhizobium*, and *Ensifer* spp. within the truffle fruiting body suggests an evolutionary adaptation of this microorganism to the genus *Tuber*. The present work offers novel and relevant insights into the microbial ecology of *T. magnatum* ecosystems and fruiting bodies. The function and role of these bacteria in the truffle microbiome and life cycle need further investigation.

- 7 Guglielmin M, Azzaro M, Buzzini P, Battistel D, Roman M, Ponti S, Turchetti B, Sannino C, Borruso L, Papale M, Lo Giudice A. 2023. A possible unique ecosystem in the endoglacial hypersaline brines in Antarctica. *Sci Rep* 13:177.

Here, we present the results related to a new unique terrestrial ecosystem found in an englacial hypersaline brine found in Northern Victoria Land (Antarctica). Both the geochemistry and microbial (prokaryotic and fungal) diversity revealed a unicity with respect to all the other known Antarctic brines and suggested a probable ancient origin mainly due a progressive cryo-concentration of seawater. The prokaryotic community presented some peculiarities, such as the occurrence of sequences of Patescibacteria (which can thrive in nutrient-limited

water environments) or few Spirochaeta, and the presence of archaeal sequences of Methanomicrobia closely related to *Methanoculleus*, a methanogen commonly detected in marine and estuarine environments. The high percentage (35%) of unassigned fungal taxa suggested the presence of a high degree of undiscovered diversity within a structured fungal community (including both yeast and filamentous life forms) and reinforce the hypothesis of a high degree of biological uniqueness of the habitat under study.

- 8 Sannino C, Borruso L, Mezzasoma A, Turchetti B, Ponti S, Buzzini P, Mimmo T, Guglielmin M. 2023. The unusual dominance of the yeast genus *Glaciozyma* in the deeper layer in an Antarctic permafrost core (Adélie Cove, Northern Victoria Land) is driven by elemental composition. *J Fungi (MDPI)* 9:435.

Rock glaciers are relatively common in Antarctic permafrost areas and could be considered postglacial cryogenic landforms. Although the extensive presence of rock glaciers, their chemical–physical and biotic composition remain scarce. Chemical–physical parameters and fungal community (by sequencing the ITS2 rDNA, Illumina MiSeq) parameters of a permafrost core were studied. The permafrost core, reaching a depth of 6.10 m, was divided into five units based on ice content. The five units (U1–U5) of the permafrost core exhibited several significant ($p < 0.05$) differences in terms of chemical and physical characteristics, and significant ($p < 0.05$) higher values of Ca, K, Li, Mg,

Mn, S, and Sr were found in U5. Yeasts dominated on filamentous fungi in all the units of the permafrost core; additionally, Ascomycota was the prevalent phylum among filamentous forms, while Basidiomycota was the dominant phylum among yeasts. Surprisingly, in U5 the amplicon sequence variants (ASVs) assigned to the yeast genus *Glaciozyma* represented about two-thirds of the total reads. This result may be considered extremely rare in Antarctic yeast diversity, especially in permafrost habitats. Based on of the chemical–physical composition of the units, the dominance of *Glaciozyma* in the deepest unit was correlated with the elemental composition of the core.

- 9 Troiano E, Larini I, Binati RL, Gatto V, Torriani S, Buzzini P, Turchetti, Salvetti E, Felis G. 2023. Finding a correct species assignment for a *Metschnikowia* strain: insights from the genome sequencing of strain DBT012. FEMS Yeast Res 23:foad024.

Metschnikowia pulcherrima is an important yeast species that is attracting increased interest thanks to its biotechnological potential, especially in agri-food applications. Phylogenetically related species of the so-called 'pulcherrima clade' were first described and then reclassified in one single species, which makes the identification an intriguing issue. Starting from the whole-genome sequencing of the pro-technological strain *Metschnikowia* sp. DBT012, this study applied comparative genomics to calculate similarity with the *M. pulcherrima* clade publicly available genomes with the aim to verify if novel single-copy putative phylogenetic markers could be selected, in comparison with the commonly used primary and secondary barcodes.

The genome-based bioinformatic analysis allowed the identification of 85 consensus single-copy orthologs, which were reduced to three after split decomposition analysis. However, wet-lab amplification of these three genes in non-sequenced type strains revealed the presence of multiple copies, which made them unsuitable as phylogenetic markers. Finally, average nucleotide identity (ANI) was calculated between strain DBT012 and available genome sequences of the *M. pulcherrima* clade, although the genome dataset is still rather limited. Presence of multiple copies of phylogenetic markers as well as ANI values were compatible with the recent reclassification of the clade, allowing the identification of strain DBT012 as *M. pulcherrima*.

- 10 Barili S, Bernetti A, Montegiove N, Calzoni E, Cesaretti A, Pinchuk I, Pezzolla D, Turchetti B, Buzzini P, Emiliani C, Gigliotti G. 2023. Impact of PVC microplastics on soil chemical and microbiological parameters. Environ Res 229:115891.

Microplastics (MPs) are emerging pollutants whose occurrence is a global problem in natural ecosystems including soil. Among MPs, polyvinyl chloride (PVC) is a well-known polymer with remarkable resistance to degradation, and because its recalcitrant nature serious environmental concerns are created during manufacturing and waste disposal. The effect of PVC (0.021% w/w) on chemical and microbial parameters of an agricultural soil was tested by a microcosm experiment at different incubation times (from 3 to 360 days). Among chemical parameters, soil CO₂ emission, fluorescein diacetate (FDA) activity, total organic C (TOC), total N, water extractable organic C (WEOC), water extractable N (WEN) and SUVA₂₅₄ were considered, while the structure of soil microbial communities was studied at different taxonomic levels (phylum and genus) by sequencing bacterial 16S and fungal ITS2 rDNA (Illumina MiSeq). Although some

fluctuations were found, chemical and microbiological parameters exhibited some significant trends. Significant ($p < 0.05$) variations of soil CO₂ emission, FDA hydrolysis, TOC, WEOC and WEN were found in PVC-treated soils over different incubation times. Considering the structure of soil microbial communities, the presence of PVC significantly ($p < 0.05$) affected the abundances of specific bacterial and fungal taxa: Candidatus Saccharibacteria, Proteobacteria, Actinobacteria, Acidobacteria and Bacteroides among bacteria, and Basidiomycota, Mortierellomycota and Ascomycota among fungi. After one year of experiment, a reduction of the number and the dimensions of PVC was detected supposing a possible role of micro-organisms on PVC degradation. The abundance of both bacterial and fungal taxa at phylum and genus level was also affected by PVC, suggesting that the impact of this polymer could be taxa-dependent.

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The following papers have been recently published.

- 1 Santos ARO, Souza GFL, Barros KO, Alvarenga FBM, Lopes MR, Souza LMD, Rosa LH, Góes-Neto A, Morais PB, Lachance MA, Rosa CA. 2023. *Spathaspora brunopereirae* sp. nov. and *Spathaspora domphillipsii* sp. nov., two d-xylose-fermenting ascosporogenous yeasts from Amazonian Forest biomes. Int J Syst Evol Microbiol 73(3) - <https://doi.org/10.1099/ijsem.0.005752>.

Four isolates of *Spathaspora* species were recovered from rotting wood collected in two Brazilian Amazonian biomes. The isolates produced unconjugated allantoid asci with a single elongated ascospore with curved ends. Sequence analysis of the ITS-5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that the isolates represent two different novel *Spathaspora* species, phylogenetically related to *Sp. boniae*. Two isolates were obtained from rotting wood collected in two different sites of the Amazonian forest in the state of Pará. The name *Spathaspora brunopereirae* sp. nov. is proposed to accommodate these

isolates. The holotype of *Spathaspora brunopereirae* sp. nov. is CBS 16119^T (Mycobank MB846672). The other two isolates were obtained from a region of transition between the Amazonian forest and the Cerrado ecosystem in the state of Tocantins. The name *Spathaspora domphillipsii* sp. nov. is proposed for this novel species. The holotype of *Spathaspora domphillipsii* sp. nov. is CBS 14229^T (Mycobank MB846697). Both species are able to convert d-xylose into ethanol and xylitol, a trait with biotechnological applications.

- 2 Barros KO, Alvarenga FBM, Magni G, Souza GFL, Abegg MA, Palladino F, da Silva SS, Rodrigues RCLB, Sato TK, Hittinger CT, Rosa CA. 2023. The Brazilian Amazonian rainforest harbors a high diversity of yeasts associated with rotting wood, including many candidates for new yeast species. *Yeast* 40(2):84-101 - <https://doi.org/10.1002/yea.3837>.

This study investigated the diversity of yeast species associated with rotting wood in Brazilian Amazonian rainforests. A total of 569 yeast strains were isolated from rotting wood samples collected in three Amazonian areas (Universidade Federal do Amazonas-Universidade Federal do Amazonas [UFAM], Piquiá, and Carú) in the municipality of Itacoatiara, Amazon state. The samples were cultured in yeast nitrogen base (YNB)-d-xylose, YNB-xylan, and sugarcane bagasse and corn cob hemicellulosic hydrolysates (undiluted and diluted 1:2 and 1:5). *Sugiyamaella* was the most prevalent genus identified in this work, followed by *Kazachstania*. The most frequently isolated yeast species were *Schwanniomyces polymorphus*, *Scheffersomyces amazonensis*, and *Wickerhamomyces* sp., respectively.

The alpha diversity analyses showed that the dryland forest of UFAM was the most diverse area, while the floodplain forest of Carú was the least. Additionally, the difference in diversity between UFAM and Carú was the highest among the comparisons. Thirty candidates for new yeast species were obtained, representing 36% of the species identified and totaling 101 isolates. Among them were species belonging to the clades *Spathaspora*, *Scheffersomyces*, and *Sugiyamaella*, which are recognized as genera with natural xylose-fermenting yeasts that are often studied for biotechnological and ecological purposes. The results of this work showed that rotting wood collected from the Amazonian rainforest is a tremendous source of diverse yeasts, including candidates for new species.

- 3 Palladino F, Rodrigues RCLB, da Silva SP, Rosa CA. 2023. Strategy to reduce acetic acid in sugarcane bagasse hemicellulose hydrolysate concomitantly with xylitol production by the promising yeast *Cyberlindnera xylosilytica* in a bioreactor. *Biotechnol Lett* 45(2):263-272 - <https://doi.org/10.1007/s10529-022-03337-9>.

The yeast *Cyberlindnera xylosilytica* UFMG-CM-Y309 has been identified as a promising new xylitol producer from sugarcane bagasse hemicellulosic hydrolysate (SCHH). However, SCHH pretreatment process generates byproducts, which are toxic to cell metabolism, including furans, phenolic compounds, and carboxylic acids, such as acetic acid, typically released at high concentrations. This research aims to reduce acetic acid in sugarcane hemicellulose hydrolysate concomitantly with xylitol production by yeast strain *Cy. xylosilytica* UFMG-CM-Y309 in a bioreactor by strategically evaluating the influence of volumetric oxygen transfer coefficient (k_La) (21 and 35 h⁻¹). Experiments were conducted on a bench bioreactor (2 L volumetric capacity) at different initial k_La values (21

and 35 h⁻¹). SCHH medium was supplemented with rice bran extract (10 g L⁻¹) and yeast extract (1 g L⁻¹). *Cy. xylosilytica* showed high xylitol production performance (19.56 g L⁻¹), xylitol yield (0.56 g g⁻¹) and, maximum xylitol-specific production rate ($\mu_{p_{max}}$ 0.20 g_{xylitol}·g⁻¹·h⁻¹) at k_La value of 21 h⁻¹, concomitantly slowing the rate of acetic acid consumption. A faster acetic acid consumption (100%) by *Cy. xylosilytica* was observed at k_La of 35 h⁻¹, concomitantly with an increase in maximum cellular growth (14.60 g L⁻¹) and reduction in maximum xylitol production (14.56 g L⁻¹ and $Y_{p/s}$ 0.34 g g⁻¹). This study contributes to pioneering research regarding this yeast performance in bioreactors, emphasizing culture medium detoxification and xylitol production.

- 4 Souza GFL, Barros KO, Alvarenga FBM, Santos ARO, Fonseca CRV, Abegg MA, Lachance MA, Rosa CA 2023. *Sugiyamaella bielyi* f. a., sp. nov. and *Sugiyamaella amazoniana* f.a., sp. nov., two yeast species isolated from passalid beetles and rotting wood in Amazonia. Int J Syst Evol Microbiol 73 - <https://doi.org/10.1099/ijsem.0.005839>.

Sixteen yeast isolates representing two novel species of the genus *Sugiyamaella* were obtained from passalid beetles, their galleries and rotting wood collected in three sites of Amazonian Forest in Brazil. Sequence analyses of the ITS-5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that the first species, described here as *Sugiyamaella amazoniana* f. a., sp. nov. (holotype CBS 18112, MycoBank 847461) is phylogenetically related to *S. bonitensis* with these species differing by 37 nucleotide substitutions and six gaps in D1/D2 sequences. *S. amazoniana* is represented by nine isolates obtained

from the guts of the passalid beetles *Popilius marginatus*, *Veturius magdalenae*, *Veturius sinuosus* and *Spasalus aquinoi*, a beetle gallery and rotting wood. The second species, *Sugiyamaella bielyi* f. a., sp. nov. (holotype CBS 18148, MycoBank 847463), is most phylogenetically related to several undescribed *Sugiyamaella* species. *S. bielyi* is described based on seven isolates obtained from the guts of *V. magdalenae* and *V. sinuosus*, a beetle gallery and rotting wood. Both species appear to be associated with passalid beetles and their ecological niches in Amazonian biome.

- 5 de Souza LMD, Ogaki MB, Teixeira EAA, de Menezes GCA, Convey P, Rosa CA, Rosa LH. 2022. Communities of culturable freshwater fungi present in Antarctic lakes and detection of their low-temperature-active enzymes. Braz J Microbiol 24 - <https://doi.org/10.1007/s42770-022-00834-x>.

We evaluated the diversity and enzymatic activities of culturable fungi recovered from cotton baits submerged for 2 years in Hennequin Lake, King George Island, and from benthic biofilms in Kroner Lake, Deception Island, South Shetland Islands, maritime Antarctica. A total of 154 fungal isolates were obtained, representing in rank abundance the phyla Ascomycota, Basidiomycota and Mortierellomycota. *Thelebolus globosus*, *Goffeauzyma* sp., *Pseudogymnoascus verrucosus* and *Metschnikowia australis* were the most abundant taxa. The fungal community obtained from the biofilm was more diverse and richer than that recovered from the cotton baits. However, diversity indices suggested that the lakes may harbour further fungal diversity. The capabilities of all cultured fungi to produce the extracellular enzymes cellulase, protease, lipase, agarase, carrageenase, invertase, amylase,

esterase, pectinase, inulinase and gelatinase at low temperature were evaluated. All enzymes were detected, but the most widely produced were protease and pectinase. The best enzymatic indices were obtained from *Holtermanniella wattica* (for invertase, esterase), *Goffeauzyma* sp. (amylase), *Metschnikowia australis* (protease), *Mrakia blollopis* (cellulase, pectinase), *Pseudogymnoascus verrucosus* (agarase, carrageenase) and *Leucosporidium fragarium* (inulinase). The detection of multiple enzymes reinforces the ecological role of fungi in nutrient cycling in Antarctic lakes, making nutrients available to the complex aquatic food web. Furthermore, such low-temperature-active enzymes may find application in different biotechnological processes, such as in the textile, pharmaceutical, food, detergent and paper industries, as well as environmental application in pollutant bioremediation processes.

- 6 Campos VJ, Ribeiro LE, Albuini FM, de Castro AG, Fontes PP, da Silveira WB, Rosa CA, Fietto LG 2022. Physiological comparisons among *Spathaspora passalidarum*, *Spathaspora arborariae*, and *Scheffersomyces stipitis* reveal the bottlenecks for their use in the production of second-generation ethanol. Braz J Microbiol 53(2):977-990 - <https://doi.org/10.1007/s42770-022-00693-6>.

The microbial conversion of pentoses to ethanol is one of the major drawbacks that limits the complete use of lignocellulosic sugars. In this study, we compared the yeast species *Spathaspora arborariae*, *Spathaspora passalidarum*, and *Scheffersomyces stipitis* regarding their potential use for xylose fermentation. Herein, we evaluated the effects of xylose concentration, presence of glucose, and temperature on ethanol production. The

inhibitory effects of furfural, hydroxymethylfurfural (HMF), acetic acid, and ethanol were also determined. The highest ethanol yield (0.44 g/g) and productivity (1.02 g/L.h) were obtained using *Sp. passalidarum* grown in 100 g/L xylose at 32 °C. The rate of xylose consumption was reduced in the presence of glucose for the species tested. Hydroxymethylfurfural did not inhibit the growth of yeasts, whereas furfural extended their lag

phase. Acetic acid inhibited the growth and fermentation of all yeasts. Furthermore, we showed that these xylose-fermenting yeasts do not produce ethanol concentrations greater than 4% (v/v), probably due to the inhibitory effects of ethanol on yeast physiology. Our data confirm that among the studied yeasts, *Sp. passalidarum* is the most promising for xylose fermentation, and the low tolerance to ethanol is an important aspect to be

improved to increase its performance for second-generation (2G) ethanol production. Our molecular data showed that this yeast failed to induce the expression of some classical genes involved in ethanol tolerance. These findings suggest that *Sp. passalidarum* may have not activated a proper response to the stress, impacting its ability to overcome the negative effects of ethanol on the cells.

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The following 4 papers have been published.

- 1 Dygas D, Kregiel D, Berłowska J. 2023. Sugar beet pulp as a biorefinery substrate for designing feed. *Molecules* (MDPI), 28(5):2064 - <https://doi.org/10.3390/molecules28052064>.

An example of the implementation of the principles of the circular economy is the use of sugar beet pulp as animal feed. Here, we investigate the possible use of yeast strains to enrich waste biomass in single-cell protein (SCP). The strains were evaluated for yeast growth (pour plate method), protein increment (Kjeldahl method), assimilation of free amino nitrogen (FAN), and reduction of crude fiber content. All the tested strains were able to grow on hydrolyzed sugar beet pulp-based medium. The greatest increases in protein content were observed for *Candida utilis* LOCK0021 and

Saccharomyces cerevisiae Ethanol Red ($\Delta N = 2.33\%$) on fresh sugar beet pulp, and for *Scheffersomyces stipitis* NCYC1541 ($\Delta N = 3.04\%$) on dried sugar beet pulp. All the strains assimilated FAN from the culture medium. The largest reductions in the crude fiber content of the biomass were recorded for *Saccharomyces cerevisiae* Ethanol Red ($\Delta = 10.89\%$) on fresh sugar beet pulp and *Candida utilis* LOCK0021 ($\Delta = 15.05\%$) on dried sugar beet pulp. The results show that sugar beet pulp provides an excellent matrix for SCP and feed production.

- 2 Steglińska A, Sulyok M, Janas R, Grzesik M, Liszkowska W, Kregiel, D, Gutarowska B. 2023. Metabolite formation by fungal pathogens of potatoes (*Solanum tuberosum* L.) in the presence of bioprotective agents. *International Journal of Environmental Research and Public Health* (MDPI) 20(6) - <https://doi.org/10.3390/ijerph20065221>.

The potato is a crop of global importance for the food industry. This is why effective protection against pathogens is so important. Fungi as potato pathogens are responsible for plant diseases and a significant reduction in yields, as well as for the formation of mycotoxins. This study focuses on the effect of three natural biocides, yeast *Metschnikowia pulcherrima*, lactic acid bacteria *Lactiplantibacillus plantarum*, and aqueous garlic extract, on the improvement of the physiology of planted potato tubers and the reduction in mycotoxin formation. The secondary metabolites produced by the fungal pathogens of genera *Fusarium*, *Alternaria*, *Colletotrichum*, *Rhizoctonia*, and *Phoma* in the presence of these biocontrol agents were compared to profiles

obtained from contaminated potatoes. Analysis of liquid chromatography coupled with tandem mass spectrometry data showed the presence of 68 secondary metabolites, including the mycotoxins: alternariol, alternariol methyl ether, altertoxin-I, aurofusarin, beauvericin, diacetoxyscirpenol, enniatin B, and sterigmatocystin. The studies showed that the applied biocontrol agents had a positive effect on the physiological parameters of potatoes (including root growth, stem growth, gas exchange, and chlorophyll content index) and on the reduction in the production of mycotoxins and other secondary metabolites by *Fusarium*, *Alternaria*, and *Phoma*.

- 3 Liszkowska W, Motyl I, Pielech-Przybylska K, Dziugan P, Berłowska, J. 2023. Plant biomass as a source of low-temperature yeasts. *BioResources* 18(1):599-612 - <https://doi.org/10.15376/biores.18.1.599-612>.

More than 40 yeast strains were isolated from various types of plant biomass and then evaluated for potential applications in biotechnological processes conducted at low temperature. Adaptation to low temperature was tested by passaging the isolates at decreasing temperatures, from 30 to 15 °C. Only the strains that were able to adapt to the final temperature and reached the stationary growth phase relatively quickly were submitted to further experimentation. These included eight environmental yeast isolates from four types of materials of plant origin: wheat, rye, and

cucumber, containing glucose, fructose, sucrose, and starch; yeast-fermentable sugars; red beetroot, containing large amounts of glucose and fructose; and fruits (grapes and apples) containing glucose, fructose, and sucrose. The strains were identified and then subjected to a series of experiments to assess their suitability for use in low-temperature biotechnological industrial processes incorporating microbial biomass. The growth dynamics and assimilation profiles of the yeast strains were investigated, as well as their ability to produce volatile compounds.

- 4 Steglinska A, Koltuniak A, Berłowska J, Czyzowska A, Szulc J, Cieciora-Wloch W, Okrasa M, Kregiel D, Gutarowska B. 2022. *Metschnikowia pulcherrima* as a biocontrol agent against potato (*Solanum tuberosum*) pathogens. *Agronomy* (MDPI) 12(10):2546 - <https://doi.org/10.3390/agronomy12102546>.

An increasing trend in protecting plants against phytopathogens is the replacement of chemical pesticides with environmentally acceptable biopreparations. This article focuses on the possible use of yeast *Metschnikowia pulcherrima* as a biocontrol agent against potato pathogens. The scope included an assessment of the antimicrobial activity of 10 *M. pulcherrima* isolates against 10 phytopathogens: *Fusarium oxysporum*, *Fusarium sambucinum*, *Rhizoctonia solani*, *Alternaria solani*, *Alternaria tenuissima*, *Alternaria alternata*, *Colletotrichum coccodes*, *Phoma exigua*, *Pectobacterium carotovorum*, and *Streptomyces scabiei*, by the agar-well diffusion method. Pulcherrimin formation, enzymatic profiles detected by the API ZYM system, and metabolite

formation evaluated by HPLC analysis were conducted for the most active *M. pulcherrima* isolates. Leucine arylamidase, valine arylamidase, α - and β -glucosidase, and esterases were the most noteworthy in the pattern of activity. In turn, ethanol, glycerol, and organic acids (acetic, succinic, lactic acids) were determined in the largest quantities. The isolate *M. pulcherrima* TK1 was selected and cultured on supplemented acid whey. An in situ experiment was carried out on the seed potatoes, which showed a 30%–100% reduction in nine phytopathogens; only *P. carotovorum* was insensitive to yeast treatment. Therefore, *M. pulcherrima* TK1 was proposed as the potential biological solution for seed potato protection against phytopathogens.

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Recent publications.

- 1 Krogerus K, Rettberg N, Gibson B. 2023. Increased volatile thiol release during beer fermentation using constructed interspecies yeast hybrids. *Eur Food Res Technol* 249:55-69 - <https://doi.org/10.1007/s00217-022-04132-6>
- 2 Kakko N, Rantasalo A, Koponen T, Vidgren V, Kannisto M, Maiorova N, Nygren H, Mojzita D, Penttilä M, Jouhten P. 2023. Inducible synthetic growth regulation using the ClpXP proteasome enhances cis,cis-muconic acid and glycolic acid yields in *Saccharomyces cerevisiae*. *ACS Synth Biol* - In Press. <https://doi.org/10.1021/acssynbio.2c00467>.
- 3 de Ruijter J, Aisala H, Jokinen I, Krogerus K, Rischer H, Toivari M. 2023. Production and sensory analysis of grape favoured beer by co fermentation of an industrial and a genetically modified laboratory yeast strain. *Eur Food Res Technol* - In Press - <https://doi.org/10.1007/s00217-023-04274-1>.
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Recent Publications.

- 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(1):foaa056 - <https://doi.org/10.1093/femsyr/foaa056>.
- 2 Burini JA, Eizaguirre JI, Loviso C, Libkind D. 2021. Levaduras no convencionales como herramientas de innovación y diferenciación en la producción de cerveza. Revista Argentina de Microbiología - <https://doi.org/10.1016/j.ram.2021.01.003>.
- 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. 114(8):1237-1244 - <https://doi.org/10.1007/s10482-021-01597-5>.
- 3 Yurkov A, Alves A, Bai F-Y, Boundy-Mills K, Buzzini P, Čadež N, Cardinali G, Casaregola S, Chaturvedi V, Collin V, Fell J, Girard V, Groenewald M, Hagen F, Hittinger CT, Kachalkin A.V, Kostrzewa M, Kouvelis V, Libkind D, Liu X, Maier T, Meyer W, Péter G, Piątek M, Robert V, Rosa CA, Sampaio J.P, Sipiczki M, Stadler X, Sugita T, Sugiyama J, Takagi H, Takashima M, Turchetti B, Wang Q-M, Boekhout T. 2021. Nomenclatural issues concerning cultured yeasts and other fungi: why it is important to avoid unneeded name changes. IMA Fungus 12, 18 - <https://doi.org/10.1186/s43008-021-00067-x>
- 4 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. Frontiers in Microbiology 12:679894 - <https://doi.org/10.3389/fmicb.2021.679894>.
- 5 Burini JA, Eizaguirre JI, Loviso C, Libkind D. 2022. Selection of *S. eubayanus* strains from Patagonia (Argentina) with brewing potential and performance in the craft beer industry. European Food Research & Technology - <https://doi.org/10.1007/s00217-021-03897-6>.
- 6 Methner Y, Dancker P, Maier R, Latorre M, Hutzler M, Zarnkow M.: Steinhaus M, Libkind D, Frank S, Jacob F. 2022. Influence of varying fermentation parameters of the yeast strain *Cyberlindnera saturnus* on the concentrations of selected flavor components in non-alcoholic beer focusing on (E)- β -dDamascenone. Foods (MDPI). 11:1038 - <https://doi.org/10.3390/foods11071038>
- 7 Latorre M, Bruzone M.C, de García V, Libkind D. 2022. Contaminantes microbianos en cervezas artesanales embotelladas de la Patagonia Andina Argentina. Revista Argentina de Microbiología. RAM-D-21-00065. In press - <https://doi.org/10.1016/j.ram.2022.05.006>.
- 8 Trochine A, Bellora N, Nizovoy P, Duran R, Greif G, de García V, Batthyany C, Robello C, Libkind D. 2022. Genomic and proteomic analysis of *Tausonia pullulans* reveals a key role for a GH15 glucoamylase in starch hydrolysis. Appl Microbiol Biotechnol - <https://doi.org/10.1007/s00253-022-12025-7>.
- 9 Molinet J, Eizaguirre JI, Quintrel P, Bellora N, Villarroel CA, Villarreal P, Benavides-Parra J, Nespolo RF, Libkind D, Cubillos FA. 2022. Natural Variation for diauxic shift between Patagonian *Saccharomyces eubayanus* strains. Msystems - <https://doi.org/doi:10.1128/msystems.00640-22>.

- 10 Peris D, Ubbelohde EJ, Kuang MK, Kominek J, Langdon QK, Adams M, Koshalek JA, Hulfachor AB, Opulente DA, Hall DJ, Hyma K, Fay J, Leducq J-B, Charron G, Landry C, Libkind D, Gonçalves C, Gonçalves P, Sampaio JP, Wang QM, Bai F-Y, Wrobel RL, Hittinger CT. 2023. Population and macroevolutionary diversity of traits and genomes in the model yeast genus *Saccharomyces*. *Nature Communications*. Feb 8;14(1):690 - <https://doi.org/10.1038/s41467-023-36139-2>.
- 11 Sepúlveda D, Campusano S, Moliné M, Barahona S, Baeza M, Alcaíno J, Colabella F, Urzúa B, Libkind D, Cifuentes V. 2023. Unraveling the molecular basis of mycosporine biosynthesis in fungi. *International Journal of Molecular Sciences (MDPI)*. 24:5930 - <https://doi.org/10.3390/ijms24065930>.

Book chapter.

- 12 Flores M, Bruzone MC; Origone A, Burini J, Rodriguez ME, Lopes CA, Libkind D. 2022. Yeasts in the beverage industry: Patagonia Gets Wild. (Eds). Alves Junior SL, Treichel H, Basso TO, Stambuk BU. *Yeasts: from Nature to Bioprocesses. Mycology: Current and Future Developments. Volume 2. Chapter 10*. Bentham Science Publishers. Singapore. ISSN: 2452-0772; ISBN: 978-981-5051-06-3.

XII Lallemand Inc., Montréal, QC H1W 2N8, Canada. Communicated by Ildar Nisamedtinov <inisamedtinov@lallemand.com>.

Recent publication.

- 1 Arju G, Berg HY, Lints T, Nisamedtinov I. 2022. Methodology for analysis of peptide consumption by yeast during fermentation of enzymatic protein hydrolysate supplemented synthetic medium using UPLC-IMS-HRMS. *Fermentation (MDPI)* 8:145 - <https://doi.org/10.3390/fermentation8040145>.

Several studies have shown the ability of yeast to consume peptides as a nitrogen source in single-peptide containing media. However, a suitable and cost-effective methodology to study the utilization of peptides by yeast and other microorganisms in a complex peptide mixture has yet to be put forward. This article addresses this issue by presenting a screening methodology for tracking the consumption of peptides by yeast during alcoholic fermentation. As a peptide source, the methodology makes use of an in-house prepared peptide-mapped bovine serum albumin (BSA) proteolytic digest, which was applied to a synthetic grape must. The peptide uptake was analyzed using high-throughput ultra-high-pressure liquid chromatography coupled to data-independent acquisition-based ion mobility separation-enabled high-resolution mass spectrometry (UPLC-DIAIMS-HRMS) analysis. The relative changes of

abundance of 123 di- to hexapeptides were monitored and reported during fermentations with three commercial wine strains, demonstrating different uptake kinetics for individual peptides. Using the same peptide-mapped BSA hydrolysate, the applicability of an untargeted workflow was additionally assessed for peptide profiling in unelucidated matrixes. The comparison of the results from peptide mapping and untargeted analysis experiments highlighted the ability of untargeted analysis to consistently identify small molecular weight peptides on the length and amino acid composition. The proposed method, in combination with other analytical techniques, such as gene or protein expression analysis, can be a useful tool for different metabolic studies related to the consumption of complex nitrogen sources by yeast or other microorganisms.

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New papers.

- 1 Peris D, Ubbelohde EJ, Kuang MC, Kominek J, Langdon QK, Adams M, Koshalek JA, Hulfachor AB, Opulente DA, Hall DJ, Hyma K, Fay JC, Leducq JB, Charron G, Landry CR, Libkind D, Gonçalves C, Gonçalves P, Sampaio JP, Wang QM, Bai FY, Wrobel RL, Hittinger CT. 2023. Macroevolutionary diversity of traits and genomes in the model yeast genus *Saccharomyces*. Nature Communications 14:690.

Species is the fundamental unit to quantify biodiversity. In recent years, the model yeast *Saccharomyces cerevisiae* has seen an increased number of studies related to its geographical distribution, population structure, and phenotypic diversity. However, seven additional species from the same genus have been less thoroughly studied, which has limited our understanding of the macroevolutionary events leading to the diversification of this genus over the last 20 million years. Here, we show the geographies, hosts, substrates, and phylogenetic relationships for approximately 1,800 *Saccharomyces* strains, covering

the complete genus with unprecedented breadth and depth. We generated and analyzed complete genome sequences of 163 strains and phenotyped 128 phylogenetically diverse strains. This dataset provides insights about genetic and phenotypic diversity within and between species and populations, quantifies reticulation and incomplete lineage sorting, and demonstrates how gene flow and selection have affected traits, such as galactose metabolism. These findings elevate the genus *Saccharomyces* as a model to understand biodiversity and evolution in microbial eukaryotes.

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Recent publications.

- 1 Das S, Tamang JP. 2023. Fermentation dynamics of naturally fermented palm beverages of West Bengal and Jharkhand in India. Fermentation (MDPI) 9:301 - <https://doi.org/10.3390/fermentation9030301>.

The term ‘*toddy*’ represents a group of different varieties of mild-alcoholic palm beverages of coastal and mainland India, produced from the fresh saps of various palm trees through uncontrolled natural fermentation. In this study, we analysed the successional changes of microbial abundances and various physico-chemical parameters during natural fermentation (0 h to 48 h) of *taal toddy*, prepared from Palmyra palm and *khejur toddy* prepared from date palm (of West Bengal and Jharkhand in India). Microorganisms from different successional levels were isolated and grouped using repetitive element sequence-based PCR (rep-PCR) technique and identified by the sequencing of 16S rRNA gene and D1-D2 region of 26S rRNA gene for bacteria and yeasts, respectively. *Enterococcus faecalis*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei* and yeast *Saccharomyces cerevisiae* were identified during natural fermentation of *toddy*. During

fermentation, average pH decreased from 6.30±0.5 to 3.15±0.03, average titratable acidity increased from 1.32±0.29 g/100 mL to 2.29±2.2 g/100 mL, ethanol increased from 1.92%±0.70 to 6.30%±0.17, methanol increased from 0.12%±0.03 to 0.43%±0.06, average sugar content decreased from 29.51%±5.30 to 17.85±6.71%, total ester content increased from 15.79±11.81 g/L to 29.17±10.07 g/L. and total protein content enhanced from 8.03±2.58 mg/L to 30.33±9.54 mg/L. Bio-active potential of *toddy* was also analysed and observed an increase in total phenolics (0 h t 48 h) from 86.66±20.81 mg/L to 223.33±33.33 mg/L, total flavonoids from 93.33±75.7 mg/L to 253.33±51.67 mg/L along with the subsequent increase in antioxidant activity (DPPH assay) from 19.82%±18.01 to 33.91%±17.03 indicating *toddy* as a functional low-alcoholic drink.

- 2 Tamang JP, Lama S. 2023. Diversity of yeasts in Indian fermented foods and alcoholic beverages. FEMS Yeast Research 21:1-7 - <https://doi.org/10.1093/femsyr/foad011>.

Ethnic Indian people have been domesticating beneficial microorganisms (bacteria, yeasts and moulds) by their wisdom of ethno-microbiological knowledge for production of flavoured and socio-culturally preferred fermented foods and alcoholic beverages for more than 8000 years. The purpose of this review is to collate the available literatures of diversity of *Saccharomyces* and non-*Saccharomyces* species associated with Indian fermented foods and alcoholic beverages. A colossal diversity of enzyme- and alcohol-producing yeasts under phylum *Ascomycota* has been reported from Indian fermented foods and alcoholic beverages. The

distributions of yeast species show 13.5% of *Saccharomyces cerevisiae* and 86.5% of some non-*Saccharomyces* spp. in Indian fermented foods and alcoholic beverages, based on the reported literatures available till date. There is a research gap on prospect of yeasts research in India. Hence, we suggest that validation of traditional knowledge of domestication of functional yeasts needs to be studied to develop the functional genomics platforms for *Saccharomyces* and non-*Saccharomyces* spp. in Indian fermented foods and alcoholic beverages.

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Communicated by M.A. Lachance <lachance@uwo.ca>.

I am pleased to announce that the transfer of my yeast culture collection (UWOPS) to the Phaff Yeast Culture Collection in Davis California is now complete. Of the six and a half thousand cultures accumulated over 41 years, approximately five thousand were shipped, in relatively small batches and at cryogenic temperatures, across North America, an interesting process to say the least. I continue to preserve the remaining cultures that could not be transferred due to legal constraints. I am

immensely grateful to Kyria Boundy-Mills, who not only assured the long-term survival of the cultures in Davis, California, but also secured funding from the National Science Foundation (USA) to cover expenses associated with the cryogenic shipments as well as the re-identification and cataloguing of the isolates. I am also indebted to our respective institutions for their cooperation in swiftly putting in place the required agreements.

The following papers have been accepted since the last issue. Please see abstracts under Dr. Rosa's communication.

- 1 Santos ARO, Gisele F. L. Souza GFL, Barros KO, Alvarenga FBM, Lopes MR, Souza LMD, Rosa LH, Góes-Neto A, Morais PB, Lachance MA, Rosa CA. 2022. *Spathaspora brunopereirae* sp. nov. and *Spathaspora domphillipsii* sp. nov., two D-xylose-fermenting ascosporegenous yeasts from Amazonian Forest biomes. Int J Syst Evol Microbiol 73(3): 005752 - <https://doi.org/10.1099/ijsem.0.005752>.
- 2 Souza GFL, Barros KO, Alvarenga FBM, Santos ARO, Fonseca CRV, Abegg MA, Lachance MA, Rosa CA. 2023. *Sugiyamaella bielyi* f. a., sp. nov., and *Sugiyamaella amazoniana* f. a., sp. nov., two yeast species isolated from passalid beetles and rotting wood in Amazonia. Int J Syst Evol Microbiol 73(4):005839 - <https://doi.org/10.1099/ijsem.0.005839>.

The following is a "retrospective", prepared with editorial help from Terry Cooper, for which I am grateful. A must longer version of an account of my work with yeasts, with an emphasis on the discovery or new species, is in preparation, with no fixed target date.

- 3 Lachance MA. 2023. Travels with *Metschnikowia*. FEMS Yeast Research - <https://doi.org/10.1093/femsyr/foad025>.

For over four decades, I have explored hotspots of biodiversity in search of yeasts that could increase our understanding of the meaning of species as the concept applies to yeasts. This led to the discovery, description, and characterization of many *Metschnikowia* and other species. What published species descriptions do not report is the context of their discoveries, the people and

the places involved. This is an abridged account of some of the paths I followed in making these yeast discoveries and some of the wonderful people that have made them possible. Selected memories about education, serendipity, boots, dress trousers, pantyhose, t-shirts, hugs, magic, friendships, symbioses, beetles, morning glories, missing nuclei, love, and loss.

Oral presentation.

- 4 Lachance MA. 2013. Yeast biogeography, a personal account. Seminar. Department of Experimental and Functional Morphology, Institute of Botany, The Czech Academy of Sciences, Třeboň, Czech Republic.

GMVV - Italian Group on Vine and Wine Microbiology

Coordinated by Prof. Patrizia Romano

Faculty of Economy, Universitas Mercatorum, Piazza Mattei, 10, 00186 Rome, Italy

The following are papers on wine yeast topics from GMVV partners for 2022.

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Communicated by Professor Patrizia Romano <pot2930@gmail.com>.

- 1 Romano P, Siesto G, Capece A, Pietrafesa R, Lanciotti R, Patrignani F, Granchi L, Galli V, Bevilacqua A, Campaniello D, Spano G, Caridi A, Poiana M, Foschino R, Vigenini I, Blaiotta G, Corich V, Giacomini A, Cardinali G, Corte A, Toffanin A, Agnolucci M, Comitini F, Ciani M, Mannazzu I, Budroni M, Englezos V, Rantsiou K, Iacumin L, Comi G, Capozzi V, Grieco F, Tufariello M. 2022. Validation of a standard protocol to assess the fermentative and chemical properties of *Saccharomyces cerevisiae* wine strains. *Frontiers in Microbiology*, 13:830277 - <https://doi.org/10.3389/fmicb.2022.830277>.

This paper reports on a common experiment performed by 17 Research Units of the Italian Group of Microbiology of Vine and Wine (GMVV), which belongs to the Scientific Society SIMTREA, with the aim to validate a protocol for the characterization of wine strains of *Saccharomyces cerevisiae*. For this purpose, two commercial *S. cerevisiae* strains (EC 1118 and AWRI796) were used to carry out inter-laboratory-scale comparative fermentations using both synthetic medium and grape musts and applying the same protocol to obtain reproducible, replicable, and statistically valid results. Ethanol yield, production of acetic acid, glycerol, higher alcohols, and other volatile compounds were assessed. Moreover, the Fourier transform infrared spectroscopy was also applied to define the metabolomic fingerprint of yeast cells from each experimental trial. Data were standardized as unit of compounds or yield per gram of sugar (glucose and fructose) consumed throughout fermentation, and analyzed through

parametric and non-parametric tests, and multivariate approaches (cluster analysis, two-way joining, and principal component analysis). The results of experiments carried out by using synthetic must showed that it was possible to gain comparable results from three different laboratories by using the same strains. Then, the use of the standardized protocol on different grape musts allowed pointing out the goodness and the reproducibility of the method; it showed the main traits of the two yeast strains and allowed reducing variability amongst independent batches (biological replicates) to acceptable levels. In conclusion, the findings of this collaborative study contributed to the validation of a protocol in a specific synthetic medium and in grape must and showed how data should be treated to gain reproducible and robust results, which could allow direct comparison of the experimental data obtained during the characterization of wine yeasts carried out by different research laboratories.

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- 1 Tedesco F, Siesto G, Pietrafesa R, Romano P, Salvia R, Scieuzo C, Falabella P, Capece A. 2022. Chemical methods for microbiological control of winemaking: an overview of current and future applications. *Beverages* (MDPI) 8(3):58 - <https://doi.org/10.3390/beverages8030058>.

Preservation technologies for winemaking have relied mainly on the addition of sulfur dioxide (SO₂), in

consequence of the large spectrum of action of this compound, linked to the control of undesirable

microorganisms and the prevention of oxidative phenomena. However, its potential negative effects on consumer health have addressed the interest of the international research on alternative treatments to substitute or minimize the SO₂ content in grape must and wine. This review is aimed at analyzing chemical methods, both traditional and innovative, useful for the microbiological stabilization of wine. After a preliminary description of the antimicrobial and technological properties of SO₂, the additive traditionally used during wine production, the effects of the addition (in must and wine) of other compounds officially permitted in winemaking, such as sorbic acid, dimethyl dicarbonate (DMDC), lysozyme and chitosan, are discussed and evaluated. Furthermore, other substances showing antimicrobial properties, for which the use for

wine microbiological stabilization is not yet permitted in EU, are investigated. Even if these treatments exhibit a good efficacy, a single compound able to completely replace SO₂ is not currently available, but a combination of different procedures might be useful to reduce the sulfite content in wine. Among the strategies proposed, particular interest is directed towards the use of insect-based chitosan as a reliable alternative to SO₂, mainly due to its low environmental impact. The production of wines containing low sulfite levels by using pro-environmental practices can meet both the consumers' expectations, who are even more interested in the healthy traits of foods, and wine-producers' needs, who are interested in the use of sustainable practices to promote the profile of their brand.

- 2 Capece A, Pietrafesa A, Pietrafesa R, Garrigós V, Tedesco F, Romano P, Matallana M, Siesto G, Aranda A. 2022. Impact of *Starmerella bacillaris* and *Zygosaccharomyces bailii* on ethanol reduction and *Saccharomyces cerevisiae* metabolism during mixed wine fermentations. Food Research International 159:111649 - <https://doi.org/10.1016/j.foodres.2022.111649>.

The bulk of grape juice fermentation is carried out by the yeast *Saccharomyces cerevisiae*, but *non-Saccharomyces* yeasts can modulate many sensorial aspects of the final products in ways not well understood. In this study, some of such non-conventional yeasts were screened as mixed starter cultures in a defined growth medium in both simultaneous and sequential inoculations. One strain of *Starmerella bacillaris* and another of *Zygosaccharomyces bailii* were chosen by their distinct phenotypic footprint and their ability to reduce ethanol levels at the end of fermentation. *S. bacillaris* losses viability strongly at the end of mixed fermentations, while *Z. bailii* remains viable. *S. cerevisiae* viability was unchanged by the

presence of the other yeasts. Physiological characterization of both strains indicates that *S. bacillaris* behavior is overall more different from *S. cerevisiae* than *Z. bailii*. In addition, *S. cerevisiae* transcriptome changes to a bigger degree in the presence of *S. bacillaris* in comparison to mixed fermentation with *Z. bailii*. *S. bacillaris* induces the translation machinery and repress vesicular transport. Both non-*Saccharomyces* yeasts induce *S. cerevisiae* glycolytic genes, and that may be related to ethanol lowering, but some aspects of carbon-related mechanisms are specific for each strain. *Z. bailii* presence increases the stress-related polysaccharides trehalose and glycogen, while *S. bacillaris* induces gluconeogenesis genes.

- 3 Siesto G, Pietrafesa R, Infantino V, Thanh C, Pappalardo I, Romano P, Capece A. 2022. In vitro study of probiotic, antioxidant and anti-inflammatory activities among indigenous *Saccharomyces cerevisiae* strains. Foods (MDPI) 11(9):1342 - <https://doi.org/10.3390/foods11091342>.

Nowadays, the interest toward products containing probiotics is growing due to their potential health benefits to the host and the research is focusing on search of new probiotic microorganisms. The present work was focused on the characterization of indigenous *Saccharomyces cerevisiae* strains, isolated from different food matrixes, with the goal to select strains with probiotic or health-beneficial potential. A preliminary screening performed on fifty *S. cerevisiae* indigenous strains, in comparison to a commercial probiotic strain, allowed to individuate the most suitable ones for

potential probiotic aptitude. Fourteen selected strains were tested for survival ability in the gastrointestinal tract and finally, the strains characterized for the most important probiotic features were analyzed for health-beneficial traits, such as the content of glucan, antioxidant and potential anti-inflammatory activities. Three strains, 4LBI-3, LL-1, TA4-10, showing better attributes compared to the commercial probiotic *S. cerevisiae* var. *boulardii* strain, were characterized by interesting health-beneficial traits, such as high content of glucan, high antioxidant and potential anti-

inflammatory activities. Our results suggest that some of the tested *S. cerevisiae* strains have potential as probiotics and candidate for different applications, such

as dietary supplements, and starter for the production of functional foods or as probiotic to be used therapeutically.

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- 1 Paradiso VM, Sanarica L, Zara I, Pisarra C, Gambacorta G, Natrella G, Cardinale M. 2022. Cultivar-dependent effects of non-*Saccharomyces* yeast starter on the oenological properties of wines produced from two autochthonous grape cultivars in Southern Italy. *Foods* (MDPI) 11(21):3373 - <https://doi.org/10.3390/foods11213373>.

Must fermentation with non-*Saccharomyces* yeast starter is a possible approach to limit the negative effects of climate change, leading to desirable effects such as an increase in total acidity and/or aroma improvement. The aim of this study was to evaluate the effects of the use of a non-*Saccharomyces* starter (*Lachancea thermotolerans*) on the chemical and sensory properties of wines obtained by the the fermentation of two autochthonous Apulian grape cultivars, namely Bombino nero and Minutolo, as compared to the traditional *Saccharomyces cerevisiae* driven fermentation. Bombino and Minutolo wines fermented with either *Lachancea thermotolerans* or *Saccharomyces cerevisiae* were characterized for their oenological parameters, volatile profiles, and sensory properties. Both chemical and

sensory properties were affected by the yeast starter. Inoculation of *L. thermotolerans* increased sensory complexity, with different floral and sweet-like attributes for both cultivars. Bombino nero, a neutral cultivar, showed a clear effect on wine composition, with both an increase in lactic acid and a change in the volatile profile. On the contrary, the impact of *L. thermotolerans* was partially masked in Minutolo due to the strong primary aroma background of this highly terpenic cultivar. In this work, we evidenced a notable cultivar × yeast interaction, showing how generalizations of the effects of non-*Saccharomyces* yeasts on vinification are difficult to achieve, as they show a cultivar-specific outcome.

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- 1 Capozzi V, Tufariello M, Berbegal C, Fragasso M, De Simone N, Spano G, Russo P, Venerito P, Bozzo F, Grieco F. 2022. Microbial resources and sparkling wine differentiation: state of the arts. *Fermentation* (MDPI) 8(6):275 - <https://doi.org/10.3390/fermentation8060275>.

Consumers' increasing interest in sparkling wine has enhanced the global market's demand. The pro-technological yeasts strains selected for the formulation of microbial starter cultures are a fundamental parameter for exalting the quality and safety of the final product. The management of the employed microbial resource is nowadays highly requested by the stakeholders since the increasing economic importance of this enological sector. Here, we report an overview of the production processes of sparkling wine and the main characterisation criteria to select *Saccharomyces* and

non-*Saccharomyces* strains appropriate for the preparation of commercial starter cultures dedicated to the primary and, in particular, the secondary fermentation of sparkling wines. We also focused on the possible uses of selected indigenous strains to improve the unique traits of sparkling wines from particular productive areas. In summary, the sparkling wine industry will get an important advantage from the management of autochthonous microbial resources associated with vineyard/wine microbial diversity.

- 2 Tufariello M, Palombi L, Rizzuti A, Musio B, Capozzi V, Gallo V, Mastroilli P, Grieco F. 2022. Volatile and chemical profiles of Bombino sparkling wines produced with autochthonous yeast strains. *Food Control* 145:109462 - <https://doi.org/10.1016/j.foodcont.2022.109462>.

This study investigated the effects of four autochthonous yeast strains and one commercial strain of *Saccharomyces cerevisiae* on the volatile and chemical profiles of rosé and sparkling wines (Bombino cultivar). HPLC-HRMS, GC-MS, and odorant series analyses were performed on sparkling wine produced on an industrial scale. Statistical elaboration of GC-MS and HPLC-HRMS data suggested that the adoption of autochthonous yeast strains significantly influenced the composition of sparkling wines in terms of volatile and

non-volatile compounds. the GC-MS and HPLC-HRMS data to determine a pool of compounds for discrimination of winemaking processes using native versus commercial yeast strains. The results indicated a significant strain-specific effect of the autochthonous yeast strains on the aroma and metabolome of regional sparkling wines compared to the commercial strain. This study may provide stakeholders with a powerful tool suitable to bring innovation and market differentiation.

- 3 De Gioia, M, Russo, P, De Simone, N, Grieco, F, Spano, G, Capozzi, V, Fragasso, M. 2022. Interactions among relevant non-*Saccharomyces*, *Saccharomyces*, and lactic acid bacteria species of the wine microbial consortium: towards advances in antagonistic phenomena and biocontrol potential. Applied Sciences (MDPI) 12:12760 - <https://doi.org/10.3390/app122412760>.

The topic of microbial interactions is of notable relevance in oenology, being connected with their impact on microbial biodiversity and wine quality. The interactions among different couples of microorganisms, in particular, yeasts and lactic acid bacteria representative of the must/wine microbial consortium, have been tested in this study. This interaction's screening has been implemented by means of plate assays, using culture medium, grape juice and wine agar as substrates. Different antagonistic phenomena have been detected, belonging to the following interaction categories: yeast-yeast, yeast-bacteria, bacteria-yeast and bacteria-bacteria. In general, the inhibitory activity has been observed onto all the three media agar used as substrates, resulting in more frequent on culture medium, followed by grape juice and, finally, wine.

demonstrating the reciprocal interactions between non-*Saccharomyces* yeasts (NSY) and malolactic bacteria. The findings shed a new light on the co-inoculation of the yeast starter culture with malolactic bacteria, as well as the biocontrol potential of Lactic Acid Bacteria (LAB) strains. Highlighted microbial interactions are relevant for the management of alcoholic fermentation, malolactic fermentation and the development of distinctive aroma profile, control of spoilage yeasts and the selection of tailored mixed starter cultures. In addition, the plate assay method could be a fast, cheap and suitable method to exclude negative interactions among *Saccharomyces* spp., NSY, and malolactic bacteria during trials from regional spontaneous fermentations with the aim to select tailored mixed starter cultures.

Specifically, the work is one of the first reports

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- 1 Guzzon R, Franciosi E, Toffanin A. 2022. Investigation by high-throughput sequencing methods of microbiota dynamics in spontaneous fermentation of Abruzzo (South Italy) wines. Agronomy (MDPI) 12(12):3104 - <https://doi.org/10.3390/agronomy12123104>.

Spontaneous wine fermentation is a meaningful topic that cannot be disregarded among winemakers and consumers due to the peculiarity of the organoleptic profile that it confers to the wine. Nevertheless, in this process the activity of indigenous microorganisms might be a threat. We studied the evolution of the spontaneous fermentation process in a traditional Italian winery in order to understand the origin of spoilage microorganisms, and to characterize the peculiarity of the microbiota associated with spontaneous

fermentation. Six Trebbiano and Montepulciano wine production chains were monitored by plate counts made by OIV methods and by Illumina MiSeq technique. Despite some compositional deficiencies, all grape musts were characterized by a highly concentrated microbial population. Non-*Saccharomyces* yeasts revealed an unexpected tolerance to ethanol, which has contributed to the evolution of alcoholic fermentation. Lactic bacteria were detectable from the very first steps of the winemaking process, with a prevalence of *Leuconostoc*

spp. which is nowadays, rarely isolated in wine. The combination between culture-dependent and high-throughput sequencing (HTS) approaches allowed to estimate microbial diversity and growth dynamics in wine fermentations of different grape varieties and under

different treatments; these results could be used by winemakers as a starting point to drive a more mindful, accurate and, controlled fermentation process and to set up the most suitable environmental conditions to enhance wine singularities.

- 2 Guzzon R, Nardin T, Larcher R. 2022. The controversial relationship between chitosan and the microorganisms involved in the production of fermented beverages. *European Food Research and Technology* 248:751–765 - <https://doi.org/10.1007/s00217-021-03919-3>.

Chitosan is a promising antimicrobial agent available in the beverage industry, because it ensures the control of a wide range of spoilage microorganisms. As chitosan does not alter the characteristics of fermented beverages, it is nowadays widely employed in the wine sector. In this work, an exhaustive chemical characterization of 12 commercial chitosans was performed in accordance with the OIV methods. These analyses made it possible to confirm or determine the animal or fungal origin of the 12 samples. Furthermore, ionic chromatography coupled with an amperometric detector (IC-PAD) confirmed peculiar polysaccharide profiles for fungal and animal-derived chitosans. The antimicrobial activity of chitosans was evaluated against the microorganisms involved in beverage fermentation or capable spoil wine, beer and cider. Chitosans were

tested in static and stirred conditions, in a synthetic medium that reproduces fermented beverage conditions, to discriminate against the physical settling of cells and their specific antimicrobial activity. Moreover, the activity of the soluble portion of chitosan was checked by inoculating microorganisms in the media after chitosans removal. The results highlighted the different sensitivity of microorganisms to chitosans, allowing selective control of spoilage agents. However, the yeast and bacteria involved in fermentation were damaged by chitosan, and the synthetic media treated with this molecule showed a less fermentative aptitude. These results suggest that chitosan is a promising tool in fermented beverage production, but an in-depth study of the biochemical interaction between chitosan and food microorganisms is necessary.

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- 1 Ferremi Leali N, Binati RL, Martelli F, Gatto V, Luzzini G, Salini A, Slaghenaufi D, Fusco S, Ugliano M, Torriani S, Salvetti E. 2022. Reconstruction of simplified microbial consortia to modulate sensory quality of Kombucha tea. *Foods (MDPI)* 11:3045 - <https://doi.org/10.3390/foods11193045>.

Kombucha is a fermented tea with a long history of production and consumption. It has been gaining popularity thanks to its refreshing taste and assumed beneficial properties. The microbial community responsible for tea fermentation—acetic acid bacteria (AAB), yeasts, and lactic acid bacteria (LAB)—is mainly found embedded in an extracellular cellulosic matrix located at the liquid–air interphase. To optimize the production process and investigate the contribution of individual strains, a collection of 26 unique strains was established from an artisanal-scale kombucha production; it included 13 AAB, 12 yeasts, and one LAB. Among these, distinctive strains, namely *Novacetimonas hansenii* T7SS-4G1, *Brettanomyces bruxellensis* T7SB-5W6, and *Zygosaccharomyces*

parabailii T7SS-4W1, were used in mono- and co-culture fermentations. The monocultures highlighted important species-specific differences in the metabolism of sugars and organic acids, while binary co-cultures demonstrated the roles played by bacteria and yeasts in the production of cellulose and typical volatile acidity. Aroma complexity and sensory perception were comparable between reconstructed (with the three strains) and native microbial consortia. This study provided a broad picture of the strains' metabolic signatures, facilitating the standardization of kombucha production in order to obtain a product with desired characteristics by modulating strains presence or abundance.

- 2 Binati RL, Larini I, Salvetti E, Torriani S. 2022. Glutathione production by non-*Saccharomyces* yeasts and its impact on winemaking: a review. *Food Research International*, 156:111333 - <https://doi.org/10.1016/j.foodres.2022.111333>.

Glutathione (GSH) is a non-protein thiol naturally present in grape berries and produced by yeasts during fermentation. It has a strong antioxidant activity; thus, the addition of pure GSH during winemaking is recommended to limit the oxidative phenomena of wine, preserving sensory characteristics and stability, ultimately promoting a healthier product by reducing the need for SO₂ addition. A promising alternative approach considers the use of yeast starter cultures high producers of this compound in situ, during the fermentation process, in substitution of external GSH addition. Recent research showed that multistarter fermentations with non-*Saccharomyces* yeasts produce even higher concentrations of GSH compared to single *Saccharomyces cerevisiae*. Accumulation of GSH in yeast cells

is also considered valuable during the growth and dehydration of biomass for starter production, aiding strains to overcome the stressful conditions of industrial process. Moreover, a current trend in oenology is the use during fermentation of inactivated dry yeasts preparations as a source of nutrients, and many of them contain GSH-enriched cells. The aim of this review was to assess the significance of GSH production for the exploitation of wine-related non-*Saccharomyces* yeasts, both in starter biomass production and during fermentations, which were until now studied in detail for *S. cerevisiae*. This compendium highlights an interesting new feature of non-conventional yeasts and upgrade the strategy of multistarter fermentation as a valuable tool to positively modulate wine composition.

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- 1 Tofalo R, Perpetuini G, Rossetti AP, Gaggiotti S, Piva A, Olivastri L, Cichelli A, Compagnone D, Arfelli G. 2022. Impact of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts to improve traditional sparkling wines production. *Food Microbiology* 108:104097 - <https://doi.org/10.1016/j.fm.2022.104097>.

In this study the effect of a co-inoculum of *S. cerevisiae* (F6789) with *Torulaspora delbrueckii* (TB1) or *Starmerella bacillaris* (SB48) on the oenological and aroma characteristics of sparkling wines obtained with the Champenoise method was investigated. The autolytic outcome and the sensory profile of sparkling wines were also evaluated. The secondary fermentations were completed by all mixed and single starter cultures with the only exception of those guided by *Starm. bacillaris*. Sparkling wines produced with *S. cerevisiae* F6789 + *Starm. bacillaris* SB48 showed the highest amounts of glycerol (6.51 g/L). The best autolytic potential was observed in sparkling wines produced with *S. cerevisiae* + *Starm. bacillaris* (81.98 mg leucin/L) and *S. cerevisiae* + *T. delbrueckii* (79.03 mg leucin/L). The lowest value was observed for sparkling wines obtained with *S. cerevisiae* F6789 (53.96 mg leucin/L). Sparkling wines

showed different aroma and sensory profiles. Esters were mainly present in sparkling wines obtained with *S. cerevisiae* F6789 (88.09 mg/L) followed by those obtained with *S. cerevisiae* + *T. delbrueckii* (87.20 mg/L), *S. cerevisiae* + *Starm. bacillaris* (81.93 mg/L). The content of esters decreased over time, and that might be related to the adsorption on lees and chemical hydrolysis. The highest concentrations of higher alcohols were found in sparkling wines produced with *S. cerevisiae* + *T. delbrueckii* (27.50 mg/L). Sparkling wines obtained with *S. cerevisiae* + *Starm. bacillaris* were well differentiated from the others due to their high score for the descriptor for spicy, bread crust, freshness and floral. Tailored strains with different autolytic potential might represent an interesting strategy to improve traditional sparkling wine production and favour their differentiation.

- 2 Perpetuini, G, Rossetti, A.P, Battistelli, N, Zulli, C, Cichelli, A, Arfelli, G, Tofalo, R. 2022. Impact of vineyard management on grape fungal community and Montepulciano d'Abruzzo wine quality. Food Research International, 158:111577 - <https://doi.org/10.1016/j.foodres.2022.111577>.

In this study the effect of farming practices on the diversity of grape fungal community of *Vitis vinifera* L. cultivar Montepulciano and the microbial metabolic activity was investigated. Small-scale vinifications were performed and wines were characterized. Agronomic management system affected the fungal community composition. *Hanseniaspora*, *Areobasidium* and *Botrytis* genera represented 30%, 20% and 10% of the total reads in all samples. *A. pullulans*, and *Cladosporium clado-sporioides* mainly occurred on organic and biodynamic grapes. *Saccharomyces* and *Pseudopithomyces* genera were present only on organic or biodynamic grapes, respectively. The agronomic managements also influenced the potential functionality of microbial community. In fact, the metabolic function was increased in organic and biodynamic grapes. Polymers

were used only by organic and biodynamic microbial communities, which also showed the highest values were observed for the oenological parameters analyzed, with the only exception of the content of sugars which were higher on conventional grapes. The fermentation profiles showed that conventional wines had a lower residual sugars content, and a higher amount of alcohol. The differences observed in the volatile composition of the wines were both quantitative and qualitative. Conventional wines showed a lower content of esters, and a higher concentration of alcohols than organic and biodynamic ones. Biodynamic wines were characterized by the highest content of organic acids. The obtained results revealed that farming practices shape the fungal community influencing wine traits linking the wine with the viti-vinicultural area of origin.

- 3 De Bellis D, Di Stefano A, Simeone P, Catitti G, Vespa S, Patruno A, Marchisio M, Mari E, Granchi L, Viti C, Chiacchiaretta P, Cichelli A, Tofalo R, Lanuti P. 2022. Rapid detection of *Brettanomyces bruxellensis* in wine by polychromatic flow cytometry. International Journal of Molecular Science (MDPI) 23:15091 - <https://doi.org/10.3390/ijms232315091>.

Brettanomyces bruxellensis is found in several fermented matrices and produces relevant alterations to the wine quality. The methods usually used to identify *B. bruxellensis* contamination are based on conventional microbiological techniques that require long procedures (15 days), causing the yeast to spread in the meantime. Recently, a flow cytometry kit for the rapid detection (1–2 h) of *B. bruxellensis* in wine has been developed. The feasibility of the method was assessed in a synthetic medium as well as in wine samples by detecting *B. bruxellensis* in the presence of other yeast species (*Saccharomyces cerevisiae* and *Pichia* spp.) and at the concentrations that produce natural contaminations (up

to 10⁵ cells/mL), as well as at lower concentrations (10³–10² cells/mL). Wine samples naturally contaminated by *B. bruxellensis* or inoculated with four different strains of *B. bruxellensis* species together with *Saccharomyces cerevisiae* and *Pichia* spp., were analyzed by flow cytometry. Plate counts were carried out in parallel to flow cytometry. We provide evidence that flow cytometry allows the rapid detection of *B. bruxellensis* in simple and complex mixtures. Therefore, this technique has great potential for the detection of *B. bruxellensis* and could allow preventive actions to reduce wine spoilage.

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- 1 De Bellis D, Di Stefano A, Simeone P, Catitti G, Vespa S, Patruno A, Marchisio M, Mari E, Granchi L, Viti C, Chiacchiaretta P, Cichelli A, Tofalo R, Lanuti P. 2022. Rapid detection of *Brettanomyces bruxellensis* in wine by polychromatic flow cytometry. International Journal of Molecular Sciences (MDPI) 23:15091 - <https://doi.org/10.3390/ijms232315091>.

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- 1 Siesto, G, Corbo, M.R, Pietrafesa, R, Sinigaglia, M, Romano, P, Bevilacqua, A. 2022. Screening of *Saccharomyces* and Non-*Saccharomyces* wine yeasts for their decarboxylase activity of amino acids. Foods (MDPI) 11:3587 - <https://doi.org/10.3390/foods11223587>.

The type and quantity of precursor amino acids present in grape must that are used by wine yeasts affect the organoleptic and health properties of wine. The aim of this work was to conduct a preliminary screening among *Saccharomyces* and non-*Saccharomyces* indigenous strains, which were previously isolated from different Italian regional grape varieties. This was performed in order to evaluate their decarboxylase activity on certain important amino acids—such as arginine, proline, serine, and tyrosine—that are present in grape must. In particular, a qualitative test on 122 wine yeasts was performed on a decarboxylase medium using arginine, proline, serine, and tyrosine as precursor amino acids. Our results showed a considerable

variability among the microbial species tested for this parameter. Indeed, *Saccharomyces cerevisiae* strains exhibited a high decarboxylase capability of the four amino acids tested; moreover, only 10% of the total (i.e., a total of 81) did not show this trait. A high recovery of decarboxylation ability for at least one amino acid was also found for *Zygosaccharomyces bailii* and *Hanseniaspora* spp. These findings can, therefore, promote the inclusion of decarboxylase activity as an additional characteristic in a wine yeast selection program in order to choose starter cultures that possess desirable technological traits; moreover, this also can contribute to the safeguarding of consumer health.

- 2 Petruzzi, L, Campaniello, D, Corbo, M.R, Speranza, B, Altieri, C, Sinigaglia, M, Bevilacqua, A. 2022. Wine Microbiology and Predictive Microbiology: A Short Overview on Application, and Perspectives. Microorganisms (MDPI) 10:421 - <https://doi.org/10.3390/microorganisms10020421>.

Predictive microbiology (PM) is an essential element in food microbiology; its aims are the determination of the responses of a given microorganism combining mathematical models with experimental data under certain environmental conditions, and the simulation a priori of the growth/inactivation of a population based on the known traits of a food matrix. Today, a great variety of models exist to describe the behaviour of several pathogenic and spoilage microorganisms in foods. In winemaking, many mathematical models have been used for monitoring

yeast growth in alcoholic fermentation as well as to predict the risk of contamination of grapes and grape products by mycotoxin producing fungi over the last years, but the potentialities of PM in wine microbiology are underestimated. Thus, the goals of this review are to show some applications and perspectives in the following fields: (1) kinetics of alcoholic and malolactic fermentation; (2) models and approaches for yeasts and bacteria growth/inactivation; (3) toxin production and removal.

- 3 Bevilacqua A, Petruzzi L, Arevalo-Villena M, Kandyli P, Nisiotou A. 2022. Editorial: Wine Microbiology: Current Trends and Approaches. *Frontiers in Microbiology* 13:873980 - <https://doi.org/10.3389/fmicb.2022.873980>.

GMVV-X Department of Agricultural, Forestry and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy. Communicated by Professor Luca Cocolin <lucasimone.cocolin@unito.it>.

- 1 Englezos V, Di Gianvito P, Peyer L, Giacosa S, Río Segade S, Edwards N, Rolle L, Rantsiou K, Cocolin L. 2022. Bioprotective effect of *Pichia kluyveri* and *Lactiplantibacillus plantarum* in winemaking conditions. *American Journal of Enology and Viticulture* 73(4):293–306 - <https://doi.org/10.5344/ajev.2022.22008>.

Consumer demand for wines free of or with reduced levels of preservatives provide new challenges for innovation in winemaking. The addition of microorganisms as bioprotective agents to avoid or reduce the sulphur dioxide (SO₂) addition during winemaking is a possible intervention strategy that could be of interest to winemakers. To this regard, a strain of *Pichia kluyveri* and one of *Lactiplantibacillus plantarum* were inoculated in a Nebbiolo red grape must, previously inoculated with a mix of microorganisms to mimic a grape must environment. The synergistic effect of the above-mentioned strains with no, low (1 g/hL) or moderate (3 g/hL) addition of total SO₂ was explored in two vintages (2019 and 2020). Wine fermentations were monitored for microbiological (yeasts, lactic and acetic,

acid bacteria populations) and physicochemical attributes (standard chemical parameters, chromatic characteristics, phenolic and aroma compounds). Microbiological analyses showed a decrease of undesired microorganisms, namely *Acetobacter aceti* and *Hanseniaspora uvarum*, independently from the strain used, while this decrease was faster when starter cultures were combined with SO₂. Chemical analyses revealed a decrease of acetic acid and ethyl acetate in the bio-protected wines. The bioprotective ability of the starter cultures was confirmed in both vintages. This study reveals new knowledge about the use of *P. kluyveri* and *L. plantarum* as bioprotective agents in winemaking and could assist to reduce the use of SO₂ in the first steps of wine production.

- 2 Englezos V, Jolly NP, Di Gianvito P, Rantsiou K, Cocolin L. 2022. Microbial interactions in winemaking: Ecological aspects and effect on wine quality. *Trends in Food Science and Technology* 127:99–113 - <https://doi.org/10.1016/j.tifs.2022.06.015>.

Background: Wine microbiota is a dense and diverse ecosystem that is directly involved in the production and synthesis of many metabolites of oenological interest thereby directly affecting wine composition. The biodiversity and successional evolution of yeast and lactic acid bacteria (LAB) species and strains within species during alcoholic (AF) and malolactic fermentation (MLF) is greatly influenced by the complexity of the wine environment. Consequently, the successful prediction of wine characteristics is limited. Scope and approach: The use of starter cultures has allowed better control of the fermentation process and the production of wines with desired characteristics. Mixed culture fermentations with selected non-*Saccharomyces* and *Saccharomyces* yeasts has regained

attention in recent years due to their potential to modulate a wide range of metabolites of oenological interest. In this context, interactions among yeast species and LAB throughout the AF and MLF are known to influence the main enological parameters and aromatic profile of the wines. Studies have been conducted to uncover the nature of these interactions, with the aim to better control the AF and MLF. Key findings and conclusions: This review provides an overview of microorganism interactions during the different steps of the winemaking process. This gives wine producers the ability to control and fine-tune microorganism population dynamics and therefore the fermentation process and finally wine quality.

- 3 Gianvito PD, Englezos V, Rantsiou K, Cocolin L. 2022. Bioprotection strategies in winemaking. *International Journal of Food Microbiology* 364:109532 - <https://doi.org/10.1016/j.ijfoodmicro.2022.109532>.

Worldwide the interest for biological control of food spoilage microorganisms has significantly increased over the last decade. Wine makes no exception to this trend, as consumer demands for wines free of preservatives that are considered negative for human health, increase. Biological control during wine fermentation aims at producing high quality wines, while minimizing, or even eliminating, the use of chemical additives. Its success lies in the inoculation of microorganisms to prevent, inhibit or kill undesired

microbes, therefore maintaining wine spoilage at the lowest level. The food industry already makes use of this practice, with dedicated commercial microbes already on the market. In winemaking, there are commercial microbes currently under investigation, particularly with the aim to reduce or replace the use of sulphur dioxide. In this review, the potential of wine yeasts and lactic acid bacteria as bioprotection agents and their mechanisms of action during wine fermentation are presented.

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- 1 Canonico L, Agarbati A, Comitini F, Ciani M. 2022. Assessment of spontaneous fermentation and non-*Saccharomyces* sequential fermentation in Verdicchio wine at winery scale. *Beverages* (MDPI) 8(3):49 - <https://doi.org/10.3390/beverages8030049>.

The use of non-*Saccharomyces* yeasts in sequential fermentation is a suitable biotechnological process to provide specific oenological characteristics and to increase the complexity of wines. In this work, selected strains of *Lachancea thermotolerans* and *Starmerella bombicola* were used in sequential fermentations with *Saccharomyces cerevisiae* and compared with spontaneous and pure *S. cerevisiae* fermentation trials in Verdicchio grape juice. *Torulasporea delbrueckii* together with the other two non-*Saccharomyces* strains (*L. thermotolerans*, *S. bombicola*) in multi-sequential fermentations was also evaluated. Wines, obtained under winery vinification conditions, were evaluated for their analytical and sensorial profile. The results indicated that

each fermentation gave peculiar analytical and aromatic features of the final wine. *L. thermotolerans* trials are characterized by an increase of total acidity, higher alcohols and monoterpenes as well as citric and herbal notes. *S. bombicola* trials showed a general significantly high concentration of phenylethyl acetate and hexyl acetate and a softness sensation while multi-sequential fermentations showed a balanced profile. Spontaneous fermentation was characterized by the production of acetate esters (ethyl acetate and isoamyl acetate), citrus and herbal notes, and tannicity. The overall results indicate that multi-starter fermentations could be a promising tool tailored to the desired features of different Verdicchio wine styles.

- 2 Agarbati A, Canonico L, Comitini F, Ciani M. 2022. Ecological distribution and oenological characterization of native *Saccharomyces cerevisiae* in an organic winery. *Fermentation* (MDPI) 8(5):224 - <https://doi.org/10.3390/fermentation8050224>.

The relation between regional yeast biota and the organoleptic characteristics of wines has attracted growing attention among winemakers. In this work, the dynamics of a native *Saccharomyces cerevisiae* population was investigated in an organic winery. In this regard, the occurrence and the persistence of native *S. cerevisiae* fermentation of two nonconsecutive vintages. From a total of 98 strains, nine different *S. cerevisiae* biotypes were identified that were distributed through the whole winemaking process, and five of them persisted in both vintages. The results of the oenological characterization of the dominant biotypes (I and II) show

a fermentation behavior comparable to that exhibited by three common commercial starter strains, exhibiting specific aromatic profiles. Biotype I was characterized by some fruity aroma compounds, such as isoamyl acetate and ethyl octanoate, while biotype II was differentiated by ethyl hexanoate, nerol, and β -damascenone production also in relation to the fermentation temperature. These results indicate that the specificity of these resident strains should be used as starter cultures to obtain wines with distinctive aromatic profiles.

- 3 Agarbati A, Canonico L, Pecci T, Romanazzi G, Ciani M, Comitini F. 2022. Biocontrol of non *Saccharomyces* yeasts in vineyard against the gray mold disease agent *Botrytis cinerea*. *Microorganisms* (MDPI) 10(2):200 - 10.3390/microorganisms10020200.

Background: *Botrytis cinerea* (*B. cinerea*) is responsible for grape infection and damage to the winemaking and table grape sectors. Although *anti-Botrytis* chemicals are available, they are considered unsustainable for resistance phenomenon and adverse effects on the environment and human health. Research is focused on developing alternative approaches, such as exploiting biological control agents (BCAs). In this context, 19 yeasts of the genera *Cryptococcus*, *Aureobasidium*, *Metschnikowia*, *Kluyveromyces* and *Wickerhamomyces* were tested as antimicrobial agents against *B. cinerea* development. Methods: A combination of in vitro tests based on dual-culture methods, volatile organic compound production assay,

laboratory tests on grape berries (punctured and sprayed with yeasts) and field experiments based on yeast treatments on grapes in vineyards allowed the selection of two potential BCAs. Results: *M. pulcherrima* DiSVA 269 and *A. pullulans* DiSVA 211 exhibited the best ability to contain the development of *B. cinerea*, showing the severity, the decay and the McKinney index lower than a commercial biological formulation consisting of a mixture of two different *A. pullulans* strains, which were used as positive controls. Conclusions: The results indicated that the selected strains were effective BCA candidates to counteract *B. cinerea* in the field, applying them in the partial or total replacement of conventional treatments.

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- 1 Moreira LDPD, Nadai C, da Silva Duarte V, Brearley-Smith EJ, Marangon M, Vincenzi S, Giacomini A, Corich, V. 2022. *Starmerella bacillaris* strains used in sequential alcoholic fermentation with *Saccharomyces cerevisiae* improves protein stability in white wines. *Fermentation* (MDPI) 8(6):252 - <https://doi.org/10.3390/fermentation8060252>.

Haze can appear in white wines as a result of the denaturation and subsequent aggregation of grape pathogenesis-related (PR) proteins. Yeast cell-wall polysaccharides, particularly mannoproteins, represent a promising strategy to reduce the incidence of this phenomenon. The aim of this study was to evaluate the effects of 13 *Starmerella bacillaris* strains, in sequential fermentation with *Saccharomyces cerevisiae*, on wine protein stability of three white wines (Sauvignon blanc, Pinot grigio, and Manzoni bianco). The resulting wines were characterized in terms of their chemical composition, content of PR proteins and polysaccharides, and heat stability. In addition, the mannoprotein fraction was purified from six wines, five produced with *S. bacillaris* and one with *S. cerevisiae* EC1118 used as

control. Generally, wines produced with *S. bacillaris* strains were more heat-stable, despite generally containing higher amounts of PR proteins. The increased heat stability of *Starmerella* wines was attributed to the stabilizing effect resulting from their higher concentrations of both total polysaccharides and mannoprotein fractions. In particular, for the most heat unstable wine (Manzoni bianco), the low MW mannoprotein fraction resulted to be the most involved in wine stability. The ability to produce wines with different heat stability was demonstrated to be strain-dependent and was more evident in the most unstable wines. By reducing fining waste, the use of *S. bacillaris* as an enological starter can be proposed as a new tool to manage wine protein stability for a more sustainable winemaking.

- 1 Francesca N, Gaglio R, Matraxia M, Naselli V, Prestianni R, Settanni L, Badalamenti N, Columba P, Bruno M, Maggio A, Alfonzo A, Moschetti G. 2022. Technological screening and application of *Saccharomyces cerevisiae* strains isolated from fermented honey by-products for the sensory improvement of *Spiritu re fascitrari*, a typical Sicilian distilled beverage. Food Microbiology 104:103968 - <https://doi.org/10.1016/j.fm.2021.103968>.

“Spiritu re fascitrari” is a Sicilian alcoholic beverage obtained through distillation of a decoction of spontaneously fermented honey by-products (FHP). The production process often leads to sensorial defects due to the unstable alcoholic fermentation. The objective of this work was to select *Saccharomyces cerevisiae* strains from FHP to be used as starter in decoction fermentation. Based on chemical, microbiological and technological data, from a total of 91 strains three *S. cerevisiae* were selected for further testing to produce FHP at laboratory scale level. After FHP distillation, the analysis of volatile organic compounds showed a complex mixture of

sensory active molecules, mainly alcohols and aldehydes. Among the alcohols, 3-methyl-1-butanol, 2-methyl-1-butanol, phenylethyl alcohol, hexadecanol and octadecanol were found at the highest concentrations. Among the carboxylic acids, acetic acid was mainly detected in the spontaneously fermented samples. FHP fermented with the three selected strains were not characterized by the presence of off-odors or off-flavours. The results obtained in this work demonstrate that the selected *S. cerevisiae* strains are promising starters to stabilize the production of distilled alcoholic beverages produced from honey by-products.

- 1 Romano P, Braschi G, Siesto G, Patrignani F, Lanciotti R. 2022. Role of yeasts on the sensory component of wines. Foods (MDPI) 11(13):1921 - <https://doi.org/10.3390/foods11131921>.

The aromatic complexity of a wine is mainly influenced by the interaction between grapes and fermentation agents. This interaction is very complex and affected by numerous factors, such as cultivars, degree of grape ripeness, climate, mashing techniques, must chemical-physical characteristics, yeasts used in the fermentation process and their interactions with the grape endogenous microbiota, process parameters (including new non-thermal technologies), malolactic fermentation (when desired), and phenomena occurring during aging. However, the role of yeasts in the formation of aroma compounds has been universally recognized. In fact, yeasts (as starters or naturally occurring microbiota) can contribute both with the formation of compounds deriving from the primary

metabolism, with the synthesis of specific metabolites, and with the modification of molecules present in the must. Among secondary metabolites, key roles are recognized for esters, higher alcohols, volatile phenols, sulfur molecules, and carbonyl compounds. Moreover, some specific enzymatic activities of yeasts, linked above all to non-*Saccharomyces* species, can contribute to increasing the sensory profile of the wine thanks to the release of volatile terpenes or other molecules. Therefore, this review will highlight the main aroma compounds produced by *Saccharomyces cerevisiae* and other yeasts of oenological interest in relation to process conditions, new non-thermal technologies, and microbial interactions.

- 2 Patrignani F, Siesto G, Gottardi D, Vigentini I, Toffanin A, Englezos V, Blaiotta G, Grieco F, Lanciotti R, Speranza B, Bevilacqua A, Romano P. 2022. Impact of two commercial *S. cerevisiae* strains on the aroma profiles of different regional musts. Beverages (MDPI) 8(4):59 - <https://doi.org/10.3390/beverages8040059>.

The present research is aimed at investigating the potential of two commercial *Saccharomyces cerevisiae* strains (EC1118 and AWRI796) to generate wine-

specific volatile molecule fingerprinting in relation to the initial must applied. To eliminate the effects of all the process variables and obtain more reliable results,

comparative fermentations on interlaboratory scale of five different regional red grape musts were carried out by five different research units (RUs). For this purpose, the two *S. cerevisiae* strains were inoculated separately at the same level and under the same operating conditions. The wines were analyzed by means of SPME-GC/MS. Quali-quantitative multivariate approaches (two-way joining, MANOVA and PCA) were used to explain the contribution of strain, must, and their interaction to the final wine volatile fingerprinting. Our results showed that the five wines analyzed for

volatile compounds, although characterized by a specific aromatic profile, were mainly affected by the grape used, in interaction with the inoculated *Saccharomyces* strain. In particular, the AWRI796 strain generally exerted a greater influence on the aromatic component resulting in a higher level of alcohols and esters. This study highlighted that the variable strain could have a different weight, with some musts experiencing a different trend depending on the strain (i.e., Negroamaro or Magliocco musts).

Forthcoming Meetings

37th International Specialized Symposium on Yeasts - ISSY37
Adelaide, Australia 27 Nov–1 Dec 2023



Registration is open for the 37th International Specialized Symposium on Yeasts, to be held in Adelaide, Australia. Earlybird registration fees will be available till August 28th 2023. Please consult <https://issy2023.com.au/>. Abstracts will be accepted till June 30th 2023. Submissions are sought along the following suggested themes. These themes are broad and inclusive and aim to assist with directing your submission:

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New Yeast and New Properties
Application of Yeast to Agriculture
Yeast in Food & Beverages
Yeast in Medicine

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The Yeast Bioeconomy
Yeast Cell Factories
Designer Yeast – Systems/Synthetic Biology
Design – The Technology-Biology Interface

The speakers selected so far can be seen at <https://issy2023.com.au/speakers/>. For further information, contact: conference@aomevents.com

16th International Congress on Yeasts Cape Town, South Africa September 29th to October 3rd 2024

The 16th International Congress on Yeasts will be held in Cape Town, South Africa, September 29th to October 3rd 2024. As more information becomes available, it will be posted on the conference website: www.icy2024.com. The congress will be managed by Eastern Sun Events <yeasts@easternsun.co.za>.



International Workshop on Brewing Yeasts November 2024 in Bariloche, Patagonia, Argentina

The International Workshop on Brewing Yeasts planned for November 2023 in Bariloche, Patagonia, Argentina, was postponed to November 2024. Stay tuned for updates! <https://iwoby.com.ar>

Diego Libkind

Brief News Items

INCT Yeasts: Biodiversity, Preservation and Biotechnological Innovation

A research network involving several universities in different regions of Brazil obtained a 5-years grant (starting January 2023) from the Federal Government for a big project entitled "INCT Yeasts: Biodiversity, preservation and biotechnological innovation". The aims of the project are: to characterize and describe the biodiversity of yeasts in Brazilian ecosystems considered of high diversity, preserving microorganisms in ex-situ collections and making isolates available for taxonomic, genetic and physiological studies, complemented by omics, as well as for developing new biotechnological processes and products (bioinnovation). The

Brazilian universities involved are located in the following states: Rio Grande do Sul and Santa Catarina (South), São Paulo and Minas Gerais (Southeast), Tocantins and Amazonas (North), Bahia and Alagoas (Northeast). Some international collaborators are also involved, namely Chris Hittinger, José Paulo Sampaio, Marc-André Lachance and Johan Thevelein, as well as some companies. The project is coordinated by Prof. Carlos Rosa (Federal University of Minas Gerais) and Prof. Andreas K. Gombert (University of Campinas). A website and other resources of information are under development and we hope to announce them to the community soon.

A.K. Gombert <gombert@unicamp.br>

Wikipedia, Fungi in Art, and VisibleFungi

What have Santa Claus, a tapestry wall, Pointillism, a mythical beverage in Norse mythology, a Mexican tortilla, a musical theatre on WWI, and Björk's new album *Fossora* have in

common? You might already know: Fungi.

Fungi have influenced the arts for millenia and across cultures. Now it is possible to explore the enormous infiltration of [Fungi in art](#) on a new

Wikipedia page. Virtually all fungal forms (aside from mildew perhaps) and all art forms are covered. Yeasts too, of course, have influenced the arts, and not only indirectly through the [Mead of Poetry](#) and other such dissoluteness. Even a veteran mycologist and art aficionado might find some surprising gems. Personally, while researching I particularly enjoyed discovering the poem of Margaret Atwood *Mushrooms* (1981) and the mesmerizing short movie about rotting and decay *Wrought* (2022; freely available on [vimeo](#)).

While I hope it will make an interesting read, I truly appreciate any feedback on how to improve the page or what sources to add. I am particularly interested in stereotype-bending examples, like fungi entering literature with a more 'positive' trope (*Amanita muscaria* as murder weapon, really? again?), the representation of fungal forms other than mushrooms in the graphic arts, or the utilization of mushrooms to create (contemporary) art. But everything is welcome!

Corrado Nai <corrado.nai@gmail.com>

You can do so per email at visiblefungi@gmail.com, using the hashtags #FungiInArt on social media, which I will regularly check, or by editing the page directly of course. This is part of the [#VisibleFungi initiative](#). If you feel being part of this informal group and consider collaborating, or just feel like reaching out per email, I appreciate connecting virtually.

Further readings:

- 1 (oldish): The Beauty and The Morbid: Fungi as inspiration in contemporary arts
<https://fungalbiolbiotech.biomedcentral.com/articles/10.1186/s40694-016-0028-4>
- 2 #VisibleFungi Initiative:
<https://blogs.biomedcentral.com/on-biology/2022/09/14/most-fungi-are-invisible-even-on-wikipedia/>
- 3 Wikipedia page:
https://en.wikipedia.org/wiki/Fungi_in_art

Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

Official Publication of the
International Commission on Yeasts and Yeast-like Microorganisms
of the International Association of Microbiological Societies (IAMS)

June 1973 Volume XXII, Number 1

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

Associate Editors

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| <p>Anna Kocková-Kratochvilová Slovak Academy of Sciences Bratislava, Czechoslovakia</p> <p>Richard Snow Dept. of Genetics, Univ. of California Davis, Calif. 95616</p> | <p style="text-align: right;"><i>National Women's University: Kita -</i> Susumu Nagai; Joto-Ku Fuenicht <i>Koya -</i> Kitadori 4-1; Shiei-jutaku 1-3 <i>NARA 630, Osaka 536, Japan</i> <i>Nishimachi</i></p> <p>Torsten O. Wikén Lab. for Microbiology Techn. University, Delft, Holland</p> |
|--|--|

D. Yarrow, Centraal Bureau voor Schimmelcultures, reported deposit of type strains in the CBS collection: *Candida iberica*, *Candida subtropicalis*, *Deparyomyces formicarius*, *Kazachstania viticola*, *Saccharomycopsis crataegensis*, and *Schisaccharomyces sloofiae*.

C. P. Kurtzman, Northern Regional Research Laboratory, US Department of Agriculture shared abstracts of papers being submitted for publication: "Formation of extracellular 3-D-hydroxypalmitic acid by

Saccharomyces mananga comb. nov.”, “Formation of extracellular C₁₄-C₁₈ 2-D-hydroxy fatty acids by species of *Saccharomyces*,” “Scanning electron microscopy of ascospores of *Schwanniomyces*,” and “Growth of *Hansenula holstii* on cadavers”.

J. W. Fell, University of Miami published a paper titled, “Marine basidiomycetous yeasts (*Rhodospidium* spp. n) with tetrapolar and multiple allelic bipolar mating systems”.

A. Kocková-Kratichvolová, Slovak Academy of Sciences, CSSR published a paper on the relationships within the genus *Rhodotorula*, testing 99 strains by means of 60 morphological, physiological, biochemical and serological properties, and GC content. A second publication proposed automation of yeast diagnostics through numerical taxonomy because the current process was “tedious”. In a third publication, they used ten ploidal strains of *Saccharomyces uvarum* from haploid through hexaploidy to learn that cell volume was proportional with ploidy, and that industrial brewing yeasts were more likely to be triploid or tetraploid. In the fourth paper, after testing 63 characters of 121 *Torulopsis* strains, linkage cluster analysis grouped the strains into nine phenons which correlated with GC content analysis. They described new species *Sorulopsis kruisii*, *T. schatavii*, *Candida pseudolipolytica*, and *Prototheca hydrocarbonea*.

V. K. Jain, Institut für Biologie, Frankfurt/Main, West Germany published two papers on repair of X-ray damage in living cells.

B. A. Siddiqi of University of Oslo, Norway shared results on radiation studies with monosomics and disomics of yeast.

Norman E. Gentner, Atomic Energy of Canada Limited, Ontario, Canada summarized recently completed research projects on repair in *Schizosaccharomyces pombe* after post-irradiation lethal enhancement by caffeine, and time course of recovery from both UV- and gamma-irradiation.

Takashi Ito, University of Tokyo, Japan presented two papers at the 15th Annual Meeting of the Japan Radiation Society, Kanazawa, Japan on mechanisms of photosensitizing action of Acridine Orange in yeast. A manuscript on photodynamic actions on synchronous cultures of *Saccharomyces cerevisiae* was submitted for publication.

Sayaka Nakai published a paper on induction and repair of gene conversion in UV-sensitive mutants of *S. cerevisiae*.

A.M. A. ten Berge, University of Utrecht, The Netherlands published seven papers on regulation of maltose fermentation by MAL₆ in *Saccharomyces carlsbergensis*, including four papers that were part of the Ph.D. thesis of A.M.A. ten Berge.

Norman R. Eaton, City University of New York, USA published work on genes involved in disaccharide fermentation, and genetic and biochemical evidence of sucrose fermentation by maltase.

F. K. Zimmermann, Fachbereich Biologie Technische Hochschule, Darmstadt, Germany summarized work on the six unlinked SUC genes involved in sucrose fermentation in *S. cerevisiae*, and developed a diploid strain, D5, for visual demonstration of mitotic crossing over.

Eckhart Schweizer, Institut für Biochemie, Universität Würzburg, Germany summarized their work on pantothenate free mutants of the *Saccharomyces cerevisiae* fatty acid synthase complex.

Reed Wickner, Yeshiva University, Bronx, New York USA related work on mutants of *S. cerevisiae* that incorporate dTMP into DNA *in vivo*.

H. de Robichon-Szulmajster, Laboratoire d'Enzymologie, Gif-Sur-Yvette, France had one manuscript in press and one submitted related to biosynthesis of methionine in *Saccharomyces cerevisiae*, using strains with

mutations in loci *eth2*, *eth3* and *eth10*. Mutant *eth2-2* had a 20-fold higher methionine pool than the wild-type strain due to overproduction.

H. Heslot, Institut National Agronomique, Paris, France had two papers in press, one on conjugation, sporulation and meiotic segregation in *Candida lipolytica* and one on regulation of biosynthesis of purine nucleotides in *Schizosaccharomyces pombe*.

M. W. Miller, University of California Davis, USA shared abstracts of two published papers on electron micrography of bud formation in *Metschnikowia krissii*, and of asci and ascospores of *Metschnikowia kamienski*.

Harlyn O. Halvorson, Brandeis University, Massachusetts, USA observed sporulation and germination in “genotypically petite” cultures of *S. cerevisiae* that lacked detectable mitochondrial DNA. They also listed three publications, on ascospore formation in yeast, β -glucosidase mutants of *Saccharomyces lactis*, and mutants of *S. lactis* controlling both β -glucosidase and β -galactosidase activities.

A. Sommer, University of California Davis, USA completed his Ph.D. dissertation on spheroplast membrane of *Saccharomyces carlsbergensis* under the guidance of M. J. Lewis.

H. J. Phaff, University of California Davis, USA presented a summary of the Ph. D. dissertation of **Graham H. Fleet** on the association of β -glucanases of *Schizosaccharomyces pombe*, *S. versatilis*, *S. malidevorans* and *S. octosporus* selected for their high levels of α -1,3-linked glucan in its cell walls. Dr. Fleet planned a 2-year postdoc in the laboratory of David Manners, Heriot-Watt University, Edinburgh, Scotland.

J. M. Bastide, Université de Montpellier, France shared the abstract of the Ph. D. dissertation of Pédro Travé. Effectiveness of a reducing agent (thiols or sodium sulphide) and glucanolytic enzymes to produce protoplast were used to group 46 species of *Candida* into two clades. These clades correlated with ability to hydrolyse urea and utilize glucose anaerobically, indicating that cell wall structure may be an important taxonomic criterium.

J. R. Villanueva, Universidad de Salamanca, Spain summarized the Doctoral Thesis of J. M. Sierra on the enzymatic characterization of the biosynthesis of mannan and glucan in the cell wall of *S. cerevisiae*.

K. Beran, Czechoslovak Academy of Sciences, Prague, CSSR published work on the chitin-glucan complex in *S. cerevisiae* through IR and X-ray observations, and localization of the complex in the encircling region of the bud scar; hypertrophic two-stage continuous cultivation of *Candida utilis*; production of native protein from yeasts; and automated sampling during continuous cultivation.

J. O. Lampen, Rutgers University, New Jersey, USA was on sabbatical in Mill Hill, England. Recent publications included work on structure of external enzymes of *Saccharomyces*, control of synthesis of exoenzymes, effects of antibiotics including cycloheximide and lomofungin on enzyme secretion and RNA synthesis, and biosynthesis of mannan.

N. P. Elinov, Leningrad Chemical-Pharmaceutical Institute, USSR announced that **A. K. Matveyeva** completed their post-graduate thesis on the extracellular polysaccharide production by *Pullularia (Aureobasidium) pullulans* strain N 8. Dr. Elinov was appointed Rector of the same institute.

Byron F. Johnson, National Research Council of Canada published work on the role of mitochondria in sex-directed flocculation and ultrastructural changes during ascospore formation in *Schizosaccharomyces pombe*.

E. Azoulay, Laboratoire de Structure et Fonction des Biomembranes, Marseille, France published work on the intracellular localization of some enzymes and cytochromes, and alkane oxidation, of *Candida tropicalis* grown on alkanes.

Edward Spoerl, Department of the Army, Virginia, USA published a paper on the effects of sugar transport by competitive sugars, cycloheximide, and added nitrogen sources in *S. cerevisiae*.

H. Suomalainen, State Alcohol Monopoly, Helsinki, Finland published a book chapter and presented results at a conference in Ireland on heat formation during anaerobic and aerobic growth of *Saccharomyces cerevisiae*, and respiratory enzymes in oxygen and glucose limited continuous cultivations.

S. P. Meyers, Louisiana State University, USA published papers on microbial degradation of oil pollutants. A news letter entitled Oil Biodegradation Newsletter was made available on request.

V. Kostov, Bulgarian Academy of Sciences compared the paraffin-oxidizing properties of 20 strains, several species, within the genus *Candida*, when grown on malt agar, or on agar or in a liquid emulsion of either Rumanian (Ploesti) kerosene or a mixture of Dolni Dabnik and Romashkinski (USSR) kerosene. Paraffin-oxidizing properties were preserved just as well in yeasts maintained on media with or without kerosene.

J. T. Rice, Miller Brewing Company, Milwaukee, Wisconsin, USA presented a paper at the American Society of Brewing Chemists meeting on the kinetics of diacetyl formation and assimilation during fermentation.

A. Fiechter, Swiss Federal Institut of Technology, Zürich, Switzerland listed several publications on automatic determination of glucose, ethanol, amino nitrogen and ammonia; kinetics of yeast growth on hydrocarbons; automated assays of adenylate, citrate, pyruvate and glucose-6-phosphate; and *S. pombe* malate dehydrogenase.

Yataro Nunokawa, Research Institute of Brewing, Tokyo, Japan summarized published work on the physicochemical characteristics of non-foaming sake yeasts.

E. Minarik, Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia listed publications in press. Rare species in grapes and musts were isolated and identified as *Kluveromyces veronae*, *Hansenula subpelliculosa*, *Kloeckera cortices* and *Trigonopsis variabilis*. Sulphate and sulphite formation and reduction by wine yeasts was proposed to have taxonomic value.

D. M. Levine, Massachusetts Institute of Technology, USA completed a M. S. thesis on methanol utilization by a thermotolerant strain of *Hansenula polymorpha*.

R. V. Chudyk, Horticultural Research Institute of Ontario, Canada described yeast research at the institute, including its wine unit, and located in the Niagara Peninsula of Ontario where over 90% of Canada's grapes and wines were produced. The laboratory maintained a culture collection of over 250 industrial yeasts. Recent studies included comparison of media for airborne winery yeasts, microflora of Ontario wineries, microbiology of bottled wines, and yeast populations in vineyards.

J. Lodder moved from Delft to Bennekom, The Netherlands.

N. J. W. Kreger-van Rij published "Electron microscopy of septa in ascomycetous yeasts" in Antonie van Leeuwenhoek.

Yeast researchers in Japan gathered in Kyoto in May 1973 to plan a joint symposium, coordinate communication among the 9 groups, and agreed that Dr. Nagai would act as the Japanese editor of the Yeast News Letter.

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