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

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

Editorial

Three Obituaries

I have the sad duty to report the loss of three colleagues.

Dr. Jack D. Rogers (1937-2021) of Washington State University, Pullman, Washington, an expert of Xylariaceous fungi, contributed to the discovery and characterization of nematodophilous yeast species. Jack was a long-time Yeast Newsletter reader, and I had the opportunity to experience his compassionate nature first-hand.

Dr. Byron F. Johnson (1928-2023), of the National Research Council of Canada and later of Carleton University in Ottawa, was a major contributor to the biology of *Schizosaccharomyces pombe*, a frequent participant in conferences of the International Commission on Yeasts, and a strong supporter of the Yeast Newsletter.

Dr. Enrico Cabib (1925-2023) had a long career as a researcher at the National Institutes of Health, in Bethesda, Maryland. He is well known for his contributions to our understanding of the biochemistry of the yeast cell wall.

Obituaries appear in this issue.

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

M.A. Lachance

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

Recent publication.

1 Golubev W.I., 2023. Activity of *Wickerhamomyces anomalus* mycocins against ascomycetous yeasts. Adv Med Mycology 25:188. (In Russian).

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 Opulente DA, Leavitt LaBella A, Harrison MC, Wolters JF, Liu C, Li Y, Kominek J, Steenwyk JL, Stoneman HR, VanDenAvond J, Miller CR, Langdon QK, Silva M, Gonçalves C, Ubbelohde EJ, Li Y, Buh KV, Jarzyna M, Haase MAB, Rosa CA, Čadež N, Libkind D, DeVirgilio JH, Beth Hulfachor A, Kurtzman CP, Sampaio JP, Gonçalves P, Zhou X, Shen XX, Groenewald M, Rokas A, Hittinger CT. Genomic and ecological factors shaping specialism and generalism across an entire subphylum. bioRxiv - https://doi.org/10.1101/2023.06.19.545611

Organisms exhibit extensive variation in ecological niche breadth, from very narrow (specialists) to very broad (generalists). Paradigms proposed to explain this variation either invoke trade-offs between performance efficiency and breadth or underlying intrinsic or extrinsic factors. We assembled genomic (1,154 yeast strains from 1,049 species), metabolic (quantitative measures of growth of 843 species in 24 conditions), and ecological (environmental ontology of 1,088 species) data from nearly all known species of the ancient fungal subphylum Saccharomycotina to examine niche breadth evolution. We found large interspecific differences in carbon breadth stem from intrinsic differences in genes encoding specific metabolic pathways but no evidence of trade-offs and a limited role of extrinsic ecological factors. These comprehensive data argue that intrinsic factors driving microbial niche breadth variation.

2 Harrison MC, Ubbelohde EJ, LaBella AL, Opulente DA, Wolters JF, Zhou X, Shen XX, Groenewald M, Hittinger CT, Rokas A. Machine learning illuminates how diet influences the evolution of yeast galactose metabolism. bioRxiv - https://doi.org/10.1101/2023.07.20.549758

How genomic differences contribute to phenotypic differences across species is a major question in biology. The recently characterized genomes, isolation environments, and qualitative patterns of growth on 122 sources and conditions of 1,154 strains from 1,049 fungal species (nearly all known) in the subphylum Saccharomycotina provide a powerful, yet complex, dataset for addressing this question. In recent years, machine learning has been successfully used in diverse analyses of biological big data. Using a random forest classification algorithm trained on these genomic, metabolic, and/or environmental data, we predicted growth on several carbon sources and conditions with high accuracy from presence/absence patterns of genes and of growth in other conditions. Known structural genes involved in assimilation of these sources were important features contributing to prediction accuracy, whereas isolation environmental data were poor predictors. By further examining growth on galactose, we found that it can be predicted with high accuracy from either genomic (92.6%) or growth data in 120 other conditions (83.3%) but not from isolation environment data (65.7%). When we combined genomic and growth data, we noted that prediction accuracy was even higher (93.4%) and that, after the <u>*GAL*</u>actose utilization genes, the most important feature for predicting growth on galactose was growth on galactitol. These data raised the hypothesis that several species in two orders, Serinales and Pichiales (containing *Candida auris* and the genus *Ogataea*, respectively), have an alternative galactose utilization pathway because they lack the *GAL* genes. Growth and biochemical assays of several of these species confirmed that they utilize galactose through an oxidoreductive D-galactose pathway, rather than the canonical *GAL* pathway. We conclude that machine learning is a powerful tool for investigating the evolution of the yeast genotype-phenotype map and that it can help uncover novel biology, even in well-studied traits.

3 David KT, Harrison MC, Opulente DA, LaBella AL, Wolters JF, Zhou X, Shen XX, Groenewald M, Pennell M, Hittinger CT, Rokas A. Saccharomycotina yeasts defy longstanding macroecological patterns. bioRxiv - https://doi.org/10.1101/2023.08.29.555417

The Saccharomycotina yeasts ("yeasts" hereafter) are a fungal clade of scientific, economic, and medical significance. Yeasts are highly ecologically diverse, found across a broad range of environments in every biome and continent on earth; however, little is known about what rules govern the macroecology of yeast species and their range limits in the wild. Here, we trained machine learning models on 12,221 occurrence records and 96 environmental variables to infer global distribution maps for 186 yeast species (~15% of described species from 75% of orders) and to test environmental drivers of yeast biogeography and macroecology. We found that predicted yeast diversity hotspots occur in mixed montane forests in temperate climates. Diversity in vegetation type and topography were some of the greatest predictors of yeast species richness, suggesting that microhabitats and environmental clines are key to yeast diversification. We further found that range limits in yeasts are significantly influenced by carbon niche breadth and range overlap with other yeast species, with carbon specialists and species in high diversity environments exhibiting reduced geographic ranges. Finally, yeasts contravene many longstanding macroecological principles, including the latitudinal diversity gradient, temperature-dependent species richness, and latitude-dependent range size (Rapoport's rule). These results unveil how the environment governs the global diversity and distribution of species in the yeast subphylum. These high-resolution models of yeast species distributions will facilitate the prediction of economically relevant and emerging pathogenic species under current and future climate scenarios.

4 Gonçalves C, Harrison MC, Steenwyk JL, Opulente DA, LaBella AL, Wolters JF, Zhou X, Shen XX, Groenewald M, Hittinger CT, Rokas A. Diverse signatures of convergent evolution in cacti-associated yeasts. bioRxiv - https://doi.org/10.1101/2023.09.14.557833

Many distantly related organisms have convergently evolved traits and lifestyles that enable them to live in similar ecological environments. However, the extent of phenotypic convergence evolving through the same or distinct genetic trajectories remains an open question. Here, we leverage a comprehensive dataset of genomic and phenotypic data from 1,049 yeast species in the subphylum Saccharomycotina (Kingdom Fungi, Phylum Ascomycota) to explore signatures of convergent evolution in cactophilic yeasts, ecological specialists associated with cacti. We inferred that the ecological association of yeasts with cacti arose independently ~17 times. Using machine-learning, we further found that cactophily can be predicted with 76% accuracy from functional genomic and phenotypic data. The most informative feature for predicting cactophily was thermotolerance, which is likely associated with duplication and altered evolutionary rates of genes impacting the cell envelope in several cactophilic lineages. We also identified horizontal gene transfer and duplication events of plant cell wall-degrading enzymes in distantly related cactophilic clades, suggesting that putatively adaptive traits evolved through disparate molecular mechanisms. Remarkably, multiple cactophilic lineages and their close relatives are emerging human opportunistic pathogens, suggesting that the cactophilic lifestyle—and perhaps more generally lifestyles favoring thermotolerance—may preadapt yeasts to cause human disease. This work underscores the potential of a multifaceted approach involving high throughput genomic and phenotypic data to shed light onto ecological adaptation and highlights how convergent evolution to wild environments could facilitate the transition to human pathogenicity.

5 Wolters JF, LaBella AL, Opulente DA, Rokas A, Hittinger CT. Mitochondrial genome diversity across the subphylum Saccharomycotina. Front Microbiol epub - https://www.frontiersin.org/articles/10.3389/fmicb.2023.1268944/

Eukaryotic life depends on the functional elements encoded by both the nuclear genome and organellar genomes, such as those contained within the mitochondria. The content, size, and structure of the mitochondrial genome varies across organisms with potentially large implications for phenotypic variance and resulting evolutionary trajectories. Among yeasts in the subphylum Saccharomycotina, extensive differences have been observed in various species relative to the model yeast *Saccharomyces cerevisiae*, but mitochondrial genome sampling across many groups has been scarce, even as hundreds of nuclear genomes have become available. By extracting mitochondrial assemblies from existing short-read genome sequence datasets, we have greatly expanded both the number of available genomes and the coverage across sparsely sampled clades. Comparison of 353 yeast mitochondrial genomes revealed that, while size and GC-content were fairly consistent across species, those in the genera *Metschnikowia* and *Saccharomyces* trended larger, while several species in the order Saccharomycetales, which includes S. cerevisiae, exhibited lower GC-content. Extreme examples for both size and GC-content were scattered throughout the subphylum. All mitochondrial genomes shared a core set of protein-coding genes for Complexes III, IV, and V, but they varied in the presence or absence of mitochondrially-encoded canonical Complex I genes. We traced the loss of Complex I genes to a major event in the ancestor of the orders Saccharomycetales and Saccharomycodales, but we also observed several independent losses in the orders Phaffomycetales, Pichiales, and Dipodascales. In contrast to prior hypotheses based on smaller-scale datasets, comparison of evolutionary rates in protein-coding genes showed no bias towards elevated rates among aerobically fermenting (Crabtree/Warburg-positive) yeasts. Mitochondrial introns were widely distributed, but they were highly enriched in some groups. The majority of mitochondrial introns were poorly conserved within groups, but several were shared within groups, between groups, and even across taxonomic orders, which is consistent with horizontal gene transfer, likely involving homing endonucleases acting as selfish elements. As the number of available fungal nuclear genomes continues to expand, the methods described here to retrieve mitochondrial genome sequences from these datasets will prove invaluable to ensuring that studies of fungal mitochondrial genomes keep pace with their nuclear counterparts.

6 Opulente DA, Langdon QK, Jarzyna M, Buh KV, Haase MAB, Groenewald M, Hittinger CT. Taxogenomic analysis of a novel yeast species isolated from soil, *Pichia galeolata* sp. nov. Yeast epub - https://doi.org/10.1002/yea.3905

A novel budding yeast species was isolated from a soil sample collected in the United States of America. Phylogenetic analyses of multiple loci and phylogenomic analyses conclusively placed the species within the genus *Pichia*. Strain yHMH446 falls within a clade that includes *Pichia norvegensis*, *Pichia pseudocactophila*, *Candida inconspicua*, and *Pichia cactophila*. Whole genome sequence data were analyzed for the presence of genes known to be important for carbon and nitrogen metabolism, and the phenotypic data from the novel species were compared to all *Pichia* species with publicly available genomes. Across the genus, including the novel species candidate, we found that the inability to use many carbon and nitrogen sources correlated with the absence of metabolic genes. Based on these results, *Pichia galeolata* sp. nov. is proposed to accommodate yHMH446^T (=NRRL Y-64187 = CBS 16864). This study shows how integrated taxogenomic analysis can add mechanistic insight to species descriptions.

7 Crandall JG, Fisher KJ, Sato TK. Hittinger CT. 2023. Ploidy evolution in a wild yeast is linked to an interaction between cell type and metabolism. PLoS Biol 21:e3001909 - https://doi.org/10.1371/journal.pbio.3001909

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.

8 Santos ARO, Barros KO, Batista TM, Souza GFL, Alvarenga FBM, Abegg MA, Sato TK, Hittinger CT, Lachance MA, Rosa CA. 2023. *Saccharomycopsis praedatoria* sp. nov., a predacious yeast isolated from soil and rotten wood in an Amazonian rainforest biome. Int J Syst Evol Microbiol 73:006125 - https://doi.org/10.1099/ijsem.0.006125

Three yeast isolates were obtained from soil and rotting wood samples collected in an Amazonian rainforest biome in Brazil. Comparison of the intergenic spacer 5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that the isolates represent a novel species of the genus *Saccharomycopsis*. A tree inferred from the D1/D2 sequences placed the novel species near a subclade containing *Saccharomycopsis lassenensis*, *Saccharomycopsis fermentans*, *Saccharomycopsis javanensis*, *Saccharomycopsis babjevae*, *Saccharomycopsis schoenii* and *Saccharomycopsis oosterbeekiorum*, but with low bootstrap support. In terms of sequence divergence, the novel species had the highest identity in the D1/D2 domains with *Saccharomycopsis capsularis*, from which it differed by 36 substitutions. In contrast, a phylogenomic analysis based on 1061 single-copy orthologs for a smaller set of *Saccharomycopsis* species whose whole genome sequences are available indicated that the novel species represented by strain UFMG-CM-Y6991 is phylogenetically closer to *Saccharomycopsis fodiens* and *Saccharomycopsis* sp. TF2021a (=*Saccharomycopsis phalluae*). The novel yeast is homothallic and produces asci with one spheroidal ascospore with an equatorial or subequatorial ledge. The name *Saccharomycopsis praedatoria* sp. nov. is proposed to accommodate the novel species. The holotype of *Saccharomycopsis praedatoria* is CBS 16589^{T}. The MycoBank number is MB849369. *S. praedatoria* was able to kill cells of *Saccharomyces cerevisiae* by means of penetration with infection pegs, a trait common to most species of *Saccharomycopsis*.

9 de Lima AEP, Wrobel RL, Paul B, Anthony LC, Sato TK, Zhang Y, Hittinger CT, Maravelias CT. 2023. High yield co-production of isobutanol and ethanol from switchgrass: experiments, and process synthesis and analysis. Sustain Energy Fuels. 7:3266-75 - https://doi.org/10.1039/D2SE01741E

Biofuels from sustainable feedstocks are a promising option for carbon-neutral bioenergy, where isobutanol has been receiving attention due to its advantageous physical and chemical properties. In this work, the production of isobutanol from carbohydrates in ammonia fiber expansion-pretreated switchgrass hydrolysate is investigated. We engineer a yeast strain by hybridizing an industrial starch isobutanologen with a strain that can tolerate the stresses of lignocellulosic hydrolysates. This strategy increases isobutanol production through ethanol co-production, which enables improved yeast growth and higher metabolic flux under these stressful conditions, likely due to the presence of at least some pyruvate decarboxylase. Furthermore, we develop a process for the recovery of isobutanol and ethanol from the broth and perform technoeconomic analysis of the switchgrass-to-alcohol biorefinery based on experiments. The yeast consumes all available glucose, but no xylose, available in the hydrolysate and co-produces isobutanol and ethanol at 23.7% and 61.8% theoretical yields, respectively. An estimated baseline minimum selling price of $11.41 per GGE for isobutanol and ethanol is determined and sensitivity analysis identified the key parameters affecting the economic feasibility of the process. Specifically, hydrolysis enzyme loading, the sugar concentration in hydrolysate, and potential fermentation technological advances, such as xylose conversion to alcohols, were shown to have the greatest economic impact.

10 Groenewald M, Hittinger CT, Bensch K, Opulente DA, Shen XX, Li Y, Liu C, LaBella AL, Zhou X, Limtong S, Jindamorakot S, Gonçalves P, Robert V, Wolfe KH, Rosa CA, Boekhout T, Ĉadež N, Péter G, Sampaio JP, Lachance MA, Yurkov AM, Daniel HM, Takashima M, Boundy-Mills K, Libkind D, Aoki K, Sugita T, Rokas A. 2023. A genome-informed higher rank classification of the biotechnologically important fungal subphylum Saccharomycotina. Stud Mycol 105:1-22 - https://doi.org/10.3114/sim.2023.105.01

The subphylum *Saccharomycotina* is a lineage in the fungal phylum *Ascomycota* that exhibits levels of genomic diversity similar to those of plants and animals. The *Saccharomycotina* consist of more than 1 200 known species currently divided into 16 families, one order, and one class. Species in this subphylum are ecologically and metabolically diverse and include important opportunistic human pathogens, as well as species important in biotechnological applications. Many traits of biotechnological interest are found in closely related species and often restricted to single phylogenetic clades. However, the biotechnological potential of most yeast species remains unexplored. Although the subphylum *Saccharomycotina* has much higher rates of genome sequence evolution than its sister subphylum, *Pezizomycotina*, it contains only one class compared to the 16 classes in *Pezizomycotina*. The third subphylum of *Ascomycota*, the *Taphrinomycotina*, consists of six classes and has approximately 10 times fewer species than the *Saccharomycotina*. These data indicate that the current classification of all these yeasts into a single class and a single order is an underappreciation of their diversity. Our previous genome-scale phylogenetic analyses showed that the *Saccharomycotina* contains 12 major and robustly supported phylogenetic clades; seven of these are current families (*Lipomycetaceae, Trigonopsidaceae, Alloascoideaceae, Pichiaceae, Phaffomycetaceae, Saccharomycodaceae*, and *Saccharomycetaceae*), one comprises two current families (*Dipodascaceae* and*Trichomonascaceae*), one represents the genus *Sporopachydermia*, and three represent lineages that differ in their translation of the CUG codon (CUG-Ala, CUG-Ser1, and CUG-Ser2). Using these analyses in combination with relative evolutionary divergence and genome content analyses, we propose an updated classification for the *Saccharomycotina*, including seven classes and 12 orders that can be diagnosed by genome content. This updated classification is consistent with the high levels of genomic diversity within this subphylum and is necessary to make the higher rank classification of the *Saccharomycotina* more comparable to that of other fungi, as well as to communicate efficiently on lineages that are not yet formally named.

11 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 40:msad111 - 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.

12 Parra M, Libkind D, Hittinger CT, Alvarez L, Bellora N. 2023. Assembly and comparative genome analysis of a Patagonian *Aureobasidium pullans* isolate reveals unexpected intraspecific variation. Yeast 40:197-213 - 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 taxogenomic 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.

III State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIgenetika), NRC "Kurchatov Institute", I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by E.S. Naumova <lena_naumova@yahoo.com>.

The following are papers for 2023 or in press.

1 Borovkova AN, Naumov GI, Shnyreva AV, Naumova ES. 2023. Genetically isolated population of *Saccharomyces bayanus* in New Zealand and Australia. Russian J Genet 59(4):403–416.

2 Lyutova LV, Naumova ES. 2023. Comparative analysis of lactose fermentation and its components, glucose and galactose, by inter-strain hybrids of dairy yeast *Kluyveromyces lactis.* Appl Biochem Microbiol 39(9):1–7.

3 Tuaeva AYu, Kachmazov GS, Naumova ES. 2023. Peculiarities of the yeast microflora of authentic Ossetia cheese. Biotekhnologiya (Moscow) 40 (1):(in press).

Yeast microflora of brined Ossetia cheeses made from raw milk by small farms in various regions of North and South Ossetia has been studied, for the first time. A total of 17 yeast species belonging to 10 genera were identified. It is shown that the composition of the yeast microflora of Ossetia cheeses depends on the place of production, the microflora of raw milk and starter culture, as well as on the type of milk. Most of the studied Ossetian cheeses were dominated by lactose-utilizing yeasts *Kluyveromyces lactis* and *Debaryomyces hansenii*, as well as by lactose-negative yeasts *Saccharomyces cerevisiae*, *Pichia kudriavzevii* and *P. fermentans*. The composition of the microflora of artisanal Ossetia cheeses is more diverse than that of commercial cheeses produced from pasteurized milk.

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 Kbayashi Y, Kayamori A, Aoki K, Shiwa Y, Matsutani M, Fujita N, Sugita T, Iwasaki W, Tanaka N, Takashima M. 2023. Chromosome-level genome assemblies of *Cutaneotrichosporon* spp. (Trichosporonales, Basidiomycota) reveal imbalanced evolution between nucleotide sequences and chromosome synteny. BMC Genomics. 24(1):609 - doi: 0.1186/s12864-023-09718-2

Background. Since DNA information was first used in taxonomy, barcode sequences such as the internal transcribed spacer (ITS) region have greatly aided fungal identification; however, a barcode sequence alone is often insufficient. Thus, multi-gene- or whole-genome-based methods were developed. We previously isolated Basidiomycota yeasts classified in the Trichosporonales. Some strains were described as *Cutaneotrichosporon cavernicola* and *C. spelunceum*, whereas strain HIS471 remained unidentified. We analysed the genomes of these strains to elucidate their taxonomic relationship and genetic diversity. **Results**. The long-read-based assembly resulted in chromosome-level draft genomes consisting of seven chromosomes and one mitochondrial genome. The genome of strain HIS471 has more than ten chromosome inversions or translocations compared to the type strain of C. cavernicola despite sharing identical ITS barcode sequences and displaying an average nucleotide identity (ANI) above 93%. Also, the chromosome synteny between *C. cavernicola* and the related species, *C. spelunceum*, showed significant rearrangements, whereas the ITS sequence identity exceeds 98.6% and the ANI is approximately 82%. Our results indicate that the relative evolutionary rates of barcode sequences, whole-genome nucleotide sequences, and chromosome synteny in *Cutaneotrichosporon* significantly differ from those in the model yeast *Saccharomyces*. **Conclusions**. Our results revealed that the relative evolutionary rates of nucleotide sequences and chromosome synteny are different among fungal clades, likely because different clades have diverse mutation/repair rates and distinct selection pressures on their genomic sequences and syntenic structures. Because diverse syntenic structures can be a barrier to meiotic recombination and may lead to speciation, the non-linear relationships between nucleotide and synteny diversification indicate that sequence-level distances at the barcode or whole-genome level are not sufficient for delineating species boundaries.

V Bioprocess and Metabolic Engineering Lab (LEMeB), University of Campinas (UNICAMP), Faculty of Food Engineering (FEA), Rua Monteiro Lobato, 80, 13083-862 Campinas - SP, Brazil. Communicated by Andreas Karoly Gombert <gombert@unicamp.br>.

I am pleased to announce my promotion to Full Professor at the University of Campinas, Brazil, as of May 2023.

Recent articles.

1 Nwaefuna AE, Garcia-Aloy M, Loeto D, Ncube T, Gombert AK, Boekhout T, Alwasel S, & Zhou N. 2023. Dung beetle-associated yeasts display multiple stress tolerance: a desirable trait of potential industrial strains. BMC Microbiology 23(1):309 - https://doi.org/10.1186/s12866-023-03044-z

2 Eliodório KP, Cunha GCGE, Lino FSO, Sommer MOA, Gombert AK, Giudici R, Basso TO. 2023. Physiology of *Saccharomyces cerevisiae* during growth on industrial sugar cane molasses can be reproduced in a tailor-made defined synthetic medium. Sci Rep 13(1):10567 - https://doi.org/10.1038/s41598-023-37618-8

3 Rego-Costa A, Huang IT, Desai MM, Gombert AK. 2023. Yeast population dynamics in Brazilian bioethanol production. G3 (Bethesda, Md) 13(7):jkad104 - https://doi.org/10.1093/g3journal/jkad104

VI Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, 31270-901, Brazil. Communicated by C.A. Rosa <carlrosa@icb.ufmg.br>.

The following papers have been recently published.

1 Rosa CA, Lachance MA, Limtong S, Santos ARO, Landell MF, Gombert AK, Morais PB, Sampaio JP, Gonçalves C, Gonçalves P, Góes-Neto A, Santa-Brígida R, Martins MB, Janzen DH, Hallwachs W. 2023. Yeasts from tropical forests: Biodiversity, ecological interactions, and as sources of bioinnovation. Yeast 40(11):511-539 - https://doi.org/10.1002/yea.3903

Tropical rainforests and related biomes are found in Asia, Australia, Africa, Central and South America, Mexico, and many Pacific Islands. These biomes encompass less than 20% of Earth's terrestrial area, may contain about 50% of the planet's biodiversity, and are endangered regions vulnerable to deforestation. Tropical rainforests have a great diversity of substrates that can be colonized by yeasts. These unicellular fungi contribute to the recycling of organic matter, may serve as a food source for other organisms, or have ecological interactions that benefit or harm plants, animals, and other fungi. In this review, we summarize the most important studies of yeast biodiversity carried out in these biomes, as well as new data, and discuss the ecology of yeast genera frequently isolated from tropical forests and the potential of these microorganisms as a source of bioinnovation. We show that tropical forest biomes represent a tremendous source of new yeast species. Although many studies, most using culture-dependent methods, have already been carried out in Central America, South America, and Asia, the tropical forest biomes of Africa and Australasia remain an under-explored source of novel yeasts. We hope that this review will encourage new researchers to study yeasts in unexplored tropical forest habitats.

2. Santos ARO, Barros KO, Batista TM, Souza GFL, Alvarenga FBM, Abegg MA, Sato TK, Hittinger CT, Lachance MA, Rosa CA. 2023. *Saccharomycopsis praedatoria* sp. nov., a predacious yeast isolated from soil and rotten wood in an Amazonian rainforest biome. Int J Syst Evol Microbiol 73(10) - https://doi.org/10.1099/ijsem.0.006125

Three yeast isolates were obtained from soil and rotting wood samples collected in an Amazonian rainforest biome in Brazil. Comparison of the intergenic spacer 5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that the isolates represent a novel species of the genus *Saccharomycopsis*. A tree inferred from the D1/D2 sequences placed the novel species near a subclade containing *Saccharomycopsis lassenensis*, *Saccharomycopsis fermentans*, *Saccharomycopsis javanensis*, *Saccharomycopsis babjevae*, *Saccharomycopsis schoenii* and *Saccharomycopsis oosterbeekiorum*, but with low bootstrap support. In terms of sequence divergence, the novel species had the highest identity in the D1/D2 domains with *Saccharomycopsis capsularis*, from which it differed by 36 substitutions. In contrast, a phylogenomic analysis based on 1061 single-copy orthologs for a smaller set of *Saccharomycopsis* species whose whole genome sequences are available indicated that the novel species represented by strain UFMG-CM-Y6991 is phylogenetically closer to *Saccharomycopsis fodiens* and *Saccharomycopsis* sp. TF2021a (=*Saccharomycopsis phalluae*). The novel yeast is homothallic and produces asci with one spheroidal ascospore with an equatorial or subequatorial ledge. The name *Saccharomycopsis praedatoria* sp. nov. is proposed to accommodate the novel species. The holotype of *Saccharomycopsis praedatoria* is CBS 16589^T. The MycoBank number is MB849369. *S. praedatoria* was able to kill cells of *Saccharomyces cerevisiae* by means of penetration with infection pegs, a trait common to most species of *Saccharomycopsis.*

3 Santa-Brígida R, Santos ARO, Martins MB, Rosa LH, Lachance MA, Rosa CA. 2023. *Teunomyces gombertii* f.a., sp. nov., *Teunomyces landelliae* f.a., sp. nov., *Teunomyces ledahaglerae* f.a., sp. nov. and *Teunomyces paulamoraisiae* f.a., sp. nov., four yeast species isolated from mushrooms and drosophilids in a Brazilian Amazonian rainforest biome. Int J Syst Evol Microbiol 73(9) - https://doi.org/10.1099/ijsem.0.006035

Ten yeast isolates representing four candidate novel species of the genus *Teunomyces* were obtained from different species of mushrooms and drosophilids collected in an Amazonian Forest biome in Brazil. Sequence analyses of the ITS 5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that four isolates were phylogenetically related to *Teunomyces stri*, two isolates related to *Teunomyces atbi*, two isolates related to *Teunomyces aglyptinius*, and another two isolates related to *Teunomyces aglyptinius*, *Teunomyces barrocoloradensis*, *Teunomyces gatunensis* and *Teunomyces stri*. The four novel species differ by 3 % or more of sequence divergence in D1/D2 domains from their closest relatives. These species were isolated from basidiocarps of the mushrooms *Marasmiellus volvatus*, *Tricholomopsis aurea*, *Hydropus* sp. And *Favolus tenuiculus*, or drosophilids feeding on these substrates. The names *Teunomyces gombertii* f.a., sp. nov. (holotype CBS 16168^T; Mycobank MB849065), *Teunomyces landelliae* f.a., sp. nov. (holotype =CBS 16169^T; Mycobank MB 849066), *Teunomyces ledahaglerae* f.a., sp. nov. (holotype CBS 16170^T; Mycobank MB 849067) and *Teunomyces paulamoraisiae* f.a., sp. nov. (holotype CBS 16120^T; Mycobank MB 849068) are proposed for these species.

4 de Paula GT, Melo WGDP, de Castro I, Menezes C, Paludo CR, Rosa CA, Pupo MT. Further evidences of an emerging stingless bee-yeast symbiosis. Front Microbiol 14:1221724 - https://doi.org/10.3389/fmicb.2023.1221724

Symbiotic interactions between microorganisms and social insects have been described as crucial for the maintenance of these multitrophic systems, as observed for the stingless bee *Scaptotrigona depilis* and the yeast *Zygosaccharomyces* sp. SDBC30G1. The larvae of *S. depilis* ingest fungal filaments of *Zygosaccharomyces* sp. SDBC30G1 to obtain ergosterol, which is the precursor for the biosynthesis of ecdysteroids that modulate insect metamorphosis. In this work, we find a similar insect-microbe interaction in other species of stingless bees. We analyzed brood cell samples from 19 species of stingless bees collected in Brazil. The osmophilic yeast *Zygosaccharomyces* spp. was isolated from eight bee species, namely *Scaptotrigona bipunctata*, *S. postica*, *S. tubiba*, *Tetragona clavipes*, *Melipona quadrifasciata*, *M. fasciculata*, *M. bicolor*, and *Partamona helleri*. These yeasts form pseudohyphae and also accumulate ergosterol in lipid droplets, similar to the pattern observed for *S. depilis*. The phylogenetic analyses including various *Zygosaccharomyces* revealed that strains isolated from the brood cells formed a branch separated from the previously described *Zygosaccharomyces* species, suggesting that they are new species of this genus and reinforcing the symbiotic interaction with the host insects.

VII VTTTechnical Research Centre of Finland, Tietotie 2, P.O. Box 1000, 02044 VTT, Espoo, Finland. Communicated by Kristoffer Krogerus <Kristoffer.Krogerus@vtt.fi>.

Recent publications.

1 Uotila I, Krogerus K. 2023. A simple and rapid CRISPR-Cas12a based detection test for diastatic *Saccharomyces cerevisiae*. J Inst Brewing 129:128–146 - DOI: 10.58430/jib.v129i2.21

2 Laurel M, Mojzita D, Seppänen-Laakso T, Oksman-Caldentey K-M, Rischer H. 2023. Raspberry ketone accumulation in *Nicotiana benthamiana* and *Saccharomyces cerevisiae* by expression of fused pathway genes. J Agric Food Chem 71:13391-13400 - DOI: 10.1021/acs.jafc.3c02097

3 Li Y, Lin P, Lu X, Yan H, Wei H, Liu C, Liu X, Yang Y, Molnár I, Bai, Z. 2023. Plasmid copy number engineering accelerates fungal polyketide discovery upon unnatural polyketide biosynthesis. ACS Synth Biol 12(8):2226-2235 - DOI: 10.1021/acssynbio.3c00178

4 Yue Q, Meng J, Qiu Y, Yin M, Zhang L, Zhou W, An Z, Liu Z, Yuan Q, Sun W, Li C, Zhao, H, Molnár, I, Xu, Y, Shi, S. (2023) A polycistronic system for multiplexed and precalibrated expression of multigene pathways in fungi. Nature Communications 14(1):4267 - DOI: 10.1038/s41467-023-40027-0

VIII Centro de Referencia en Levaduras y Tecnología Cervecera (CRELTEC), Instituto Andino-Patagónico de Tecnologías Biológicas y Geoambientales (IPATEC, CONICET-UNComahue), Quintral 1250, (8400), Bariloche, Argentina. Communicated by Diego Libkind <libkindfd@comahue-conicet.gov.ar>.

Recent publications.

1 Groenewald M, Hittinger CT, Bensch K, Opulente DA, Shen X-X, Li Y, Liu C, LaBella AL, Zhou X, Limtong S, Jindamorakot S, Gonçalves P, Robert V, Wolfe KH, Rosa CA, Boekhout T, Čadež N, Péter G, Sampaio JP, Lachance M-A, Yurkov AM, Daniel H-M, Takashima M, Boundy-Mills K, Libkind D, Aoki K, Sugita T, Rokas A. **2023**. A genome-informed higher rank classification of the biotechnologically important fungal subphylum Saccharomycotina. Studies in Mycology 105:1–22.
doi: 10.3114/sim.2023.105.01.

2 Parra M, Libkind D, Hittinger CT, Álvarez L, Bellora N. 2023. Assembly and comparative genome analysis of a Patagonian *Aureobasidium pullulans* isolate reveals unexpected intraspecific variation. Yeast 40:197-213 - http://dx.doi.org/10.1002/yea.3853

IX Department of Chemistry and Biotechnology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia. Communicated by Hidde Berg <hidde.berg@taltech.ee>.

Recent publication.

1 Berg HY, Arju G, Becerra-Rodríguez C, Galeote V, Nisamedtinov I. 2023. Unlocking the secrets of peptide transport in wine yeast: insights into oligopeptide transporter functions and nitrogen source preferences. Appl Environ Microbiol. Oct 16:e01141-23 - https://doi.org/10.1128/aem.01141

A limited nitrogen supply can prevent the completion of alcoholic fermentation, and supplementation through peptides as an alternative, natural source of nitrogen for yeast offers an interesting solution to this issue. For this reason, we studied yeast oligopeptide transporters from the Opt and Fot families in a set of CRISPR-Cas9-engineered *S. cerevisiae* wine strains. We demonstrated that Fot and Opt2 have a broader peptide length preference than previously reported, enabling yeasts to acquire sufficient nitrogen from peptides without requiring additional ammonia or amino acids to complete fermentation. The consumption order of peptides was determined by the peptide length as higher chain length peptides were taken up by Opt2 only after most ditetrapeptides were depleted from the media. Interestingly, Opt1 activity was not sufficient to support growth on peptides as nitrogen source. Altogether, this work provides further evidence of the importance of peptides as a nitrogen source for yeast and their consequent positive impact on fermentation kinetics.

X Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore. Communicated by Ee-Sin Chen<bchces@nus.edu.sg>.

Recent publications.

1 Lim KK, Koh NZH, Zeng YB, Chuan JK, Raechell R, Chen ES. 2023. Resistance to chemotherapeutic 5-fluorouracil conferred by modulation of heteochromatic integrity through Ino80 functions in fission yeast. Int J Mol Sci (MDPI) 24:10687 - https://doi.org/10.3390/ijms241310687

5-Fluorouracil (5-FU) is a conventional chemotherapeutic drug widely used in clinics worldwide, but development of resistance that compromises responsiveness remains a major hurdle to its efficacy. The mechanism underlying 5-FU resistance is conventionally attributed to the disruption of nucleotide synthesis, even though research has implicated other pathways such as RNA processing and chromatin dysregulation. Aiming to clarify resistance mechanisms of 5-FU, we tested the response of a collection of fission yeast (*Schizosaccharomyces pombe*) null mutants, which confer multiple environmental factor responsiveness (MER). Our screen identified disruption of membrane transport, chromosome segregation and mitochondrial oxidative phosphorylation to increase cellular susceptibility towards 5-FU. Conversely, we revealed several null mutants of Ino80 complex factors exhibited resistance to 5-FU. Furthermore, attenuation of Ino80 function via deleting several subunit genes reversed loss of chromosome-segregation fidelity in 5-FU in the loss-of-function mutant of the Argonaute protein, which regulates RNA interference (RNAi)-dependent maintenance of peri-centromeric heterochromatin. Our study thus uncovered a critical role played by chromatin remodeling Ino80 complex factors in 5-FU resistance, which may constitute a possible target to modulate in reversing 5-FU resistance.

2 Lam UT, Nguyen TTT, Raechell R, Yang J, Singer H, Chen ES. 2023. A normalization protocol reduces edge effect in high-throughput analyses of hydroxyurea hypersensitivity in fission yeast. Biomedicines (MDPI) 11:2829 - https://doi.org/10.3390/biomedicines11102829

Edge effect denotes better growth of microbial organisms situated at the edge of the solid agar media. Although the precise reason underlying edge effect is unresolved, it is generally attributed to greater nutrient availability with less competing neighbors at the edge. Nonetheless, edge effect constitutes an unavoidable confounding factor that results in misinterpretation of cell fitness, especially in high-throughput screening experiments widely employed for genome-wide investigation using microbial gene knockout or mutant libraries. Here, we visualize edge effect in high-throughput high-density pinning arrays and report a normalization approach based on colony growth rate to quantify drug (hydroxyurea)-hypersensitivity in fission yeast strains. This normalization procedure improved the accuracy of fitness measurement by compensating cell growth rate discrepancy at different locations on the plate and reducing false-positive and -negative frequencies. Our work thus provides a simple and coding-free solution for a struggling problem in robotics-based high-throughput screening experiments.

XI Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil. Communicated by Gabriela de Paula <gabriela.paula@usp.br>.

Recent publication.

1 de Paula GT, Melo WGP, Castro I, Menezes C, Paludo CR, Rosa CA and Pupo MT. 2023. Further evidences of an emerging stingless bee-yeast symbiosis. Front. Microbiol. 14:1221724 - doi: 10.3389/fmicb.2023.1221724

Symbiotic interactions between microorganisms and social insects have been described as crucial for the maintenance of these multitrophic systems, as observed for the stingless bee *Scaptotrigona depilis* and the yeast *Zygosaccharomyces* sp. SDBC30G1. The larvae of *S. depilis* ingest fungal filaments of *Zygosaccharomyces* sp. SDBC30G1 to obtain ergosterol, which is the precursor for the biosynthesis of ecdysteroids that modulate insect metamorphosis. In this work, we find a similar insect-microbe interaction in other species of stingless bees. We analyzed brood cell samples from 19 species of stingless bees collected in Brazil. The osmophilic yeast *Zygosaccharomyces* spp. was isolated from eight bee species, namely *Scaptotrigona bipunctata*, *S. postica*, *S. tubiba*, *Tetragona clavipes*, *Melipona quadrifasciata*, *M. fasciculata*, *M. bicolor*, and *Partamona helleri*. These yeasts form pseudohyphae and also accumulate ergosterol in lipid droplets, similar to the pattern observed for *S. depilis*. The phylogenetic analyses including various *Zygosaccharomyces* revealed that strains isolated from the brood cells formed a branch separated from the previously described *Zygosaccharomyces* species, suggesting that they are new species of this genus and reinforcing the symbiotic interaction with the host insects.

XII Lodz University of Technology. Faculty of Biotechnology and Food Sciences, Department of Environmental Biotechnology, Wolczanska 171/173, 90-530 Lodz, Poland. Communicated by Prof. Dorota Kregiel <dorota.kregiel@p.lodz.pl>.

The following papers have been published.

1 Kregiel D, Czarnecka-Chrebelska K, Schusterová H, Vadkertiová R, Nowak A. 2023. The *Metschnikowia pulcherrima* clade as a model for assessing inhibition of *Candida* spp. and the toxicity of its metabolite, pulcherrimin. Molecules (MDPI) 28(13):5064 - DOI: 10.3390/molecules28135064

Candidiasis is one of the most frequent infections worldwide. In this study, the antimicrobial properties of six strains belonging to the *Metschnikowia pulcherrima* clade were evaluated against twenty *Candida* and *Candida*-related *Filobasidiella neoformans* var. *bacillispora* (syn. *Cryptococcus neoformans*) of different origins, employing the agar cross method. The toxic effect of pulcherrimin, a red metabolite that is responsible for the antimicrobial activities of *Metschnikowia* spp., was evaluated in various experimental models. The results of agar tests showed that the selected *M. pulcherrima* strains inhibited the growth of the *Candida* and non-*Candida* strains. However, inhibition was dependent on the strain and the environment. The presence of peptone, sodium silicate, and a higher incubation temperature decreased the antifungal action of the *M. pulcherrima* strains. Pulcherrimin showed cytotoxic and antiproliferative activity, with oxidative stress in cells leading to apoptosis. More research is needed on the mechanism of action of pulcherrimin on somatic cells.

2 Dygas D, Liszkowska W, Steglińska A, Sulyok M, Kregiel D, Berłowska J. 2023. Rapeseed meal waste biomass as a single-cell protein substrate for nutritionally-enhanced feed components. Processes (MDPI) 11(5):1556 - DOI: 10.3390/pr11051556

Rapeseed meal (RM) is produced in large quantities as a byproduct of oil extraction from rapeseeds. However, the efficient utilization of RM as animal feed is limited by its low metabolizable energy, poor palatability, and high levels of fiber and anti-nutritional components. Here, we investigate the potential of enriching RM with single-cell protein through fermentation with conventional and unconventional yeasts. The process of simultaneous saccharification and fermentation improved the parameters of the waste biomass, especially the protein content, while reducing the amount of crude fiber and enhancing the biotransformation of isoflavone compounds present in the waste. Fermentation yielded the highest protein gain for the *Saccharomyces cerevisiae* Ethanol Red strain ($\Delta N = 2.38\%$) at a biomass load of 12.5 g and for *Scheffersomyces stipitis* ($\Delta N = 2.34\%$) at an enzyme dose of 0.125 mL/10 g DM. The crude fiber content (CF) was reduced by 2.55–7.18%. The simultaneous saccharification and fermentation (SSF) process resulted in the conversion of isoflavones to forms with fewer adverse effects and a lower estrogenic activity. The results show the potential of using RM as a substrate for making a nutritionally improved feed components.

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

Recent presentations/publications.

1 Walker G, Iannetta P, Black K. 2023. Legumes as novel brewing and distilling adjuncts. Presentation at the Institute of Brewing & Distilling Asia-Pacific conference, Adelaide, Australia, March 2023.

The legume plant family (Fabaceae) includes peas and beans which yield highly nutritious grains rich in starch, protein, and minerals. Legume cultivation also helps support more sustainable agrifood systems, since they require no synthetic (inorganic) nitrogen fertilisers. This is a function of legumes' symbiotic association with nitrogen fixing soil microbes, collectively termed *Rhizobia*. There are very few reports on the use of legume starch as adjuncts in brewing and distilling, and we report on our research exploring the potential benefits of dried peas (*Pisum sativum* L.) and faba beans (*Vicia faba* L.) for brewers and distillers - with a view to reducing overall production carbon footprints. We have successfully demonstrated that faba bean starch can be utilised as a novel adjunct for producing beers that have been favourably accepted by consumers. Furthermore, use of peas for distilled spirits has led to the introduction of the world's first *Climate Positive* spirits, including gin and vodka. This presentation will describe the research activities which led to the launch of these novel beverages. We contend that exploitation of legume starch as a novel adjunct is attractive to brewers and distillers interested in the development of products which help safeguard a more diverse, sustainable, and resilient drinks industry, and of the agricultural systems which provide their raw materials.

2 Walker G, Daute M, Harrison B, Baxter I, Jack F. 2023. Potential of Non-*Saccharomyces* yeasts for Scotch Whisky fermentations. Presentation at the Worldwide Distilled Spirits Conference, Edinburgh, Scotland (May, 2023).

This study demonstrates the potential of non-*Saccharomyces* yeast strains for diversifying malt whisky flavour characteristics. The Scotch Whisky industry only sources a limited range of commercial yeast strains, all belonging to the species *Saccharomyces cerevisiae*. The yeast converts the malt-derived sugars into alcohol, and variety of flavoursome secondary metabolites that contribute to the congener profile. The fermentation performance of 42 different yeast strains in distillers' malt wort was analysed. Fermentation parameters were measured, together with chemical and sensory analysis of congeners in low wines following copper-pot distillation. The fermentation progress was monitored by measuring CO_2 release during fermentation, residual carbohydrates, pH, yeast cell count and viability. The low wines were assessed for their flavour profile by *Napping* employing a trained sensory panel (n=20), and for their chemical congener composition by semi-quantitative analysis of 202 volatile compounds by gas chromatography-mass spectrometry. When comparing the fermentations, clear differences in fermentation performance and efficiency between yeast strains were observed. Some yeasts, notably *Schizosaccharomyces pombe* and *Dekkera bruxellensis* compared favourably with distillers' strains of *S. cerevisiae* (M-type). While it was observed that yeast strains with a high fermentation efficiency produced more esters and higher alcohols, this correlation was not stringent, and the yeast strain was the main driver for the flavour and congener differences. Other fermentation parameters such as temperature, original wort gravity, pH and pitching rate had a smaller impact on congener development. The diverse flavour profiles of non-*Saccharomyces* yeast strains were clearly differentiated on distillate profiles (both low wines and new make spirits) and showed promise for diversifying malt whisky flavour characteristics.

This presentation is a good fit for the session "*Innovation and diversity in fermentation*" because it shows a wide range of non-*Saccharomyces* yeast strains that influence Scotch Malt Whisky fermentation, flavour evolution, and flavour congener transfer into distillates.

3 Black K, Walker G. 2023. Yeast fermentation for the production of neutral distilled spirits. Appl Sci 13:4927 - https://doi.org/10.3390/app13084927

The production of neutral distilled spirits is increasing worldwide due to the popularity of beverages such as vodka and gin. Yeast fermentation lies at the heart of such production, but there are salient differences between the yeast strains employed for neutral spirits, as compared to those used in whisky, rum, and brandy fermentation. For example, the former white spirit processes aim to minimise the synthesis of flavour-active volatile compounds (or congeners), whilst the opposite is true for more flavoursome brown spirits such as whisky. This paper describes the raw materials, yeasts, and fermentation conditions involved in neutral spirit production processes and discusses challenges and opportunities in such technology, including exciting new developments regarding strategies to improve yeast strains.

XIV Department of Soil Biology, Faculty of Soil Science, Lomonosov Moscow State University, 119234, Leninskie gory, 1/12, Moscow, Russia; All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS, 142290, pr. Nauki 5, Pushchino, Russia. Communicated by A.V. Kachalkin <kachalkin_a@mail.ru>.

Recent publications.

1 Glushakova A, Kachalkin A, Rodionova E. 2023. Hydrolytic enzyme production and susceptibility to antifungal compounds of opportunistic *Candida parapsilosis* strains isolated from *Cucurbitaceae* and *Rosaceae* fruits. Appl Microbiol (MDPI) 3(1):199–211 - DOI: 10.3390/applmicrobiol3010014

Endophytic yeast species were studied in the internal tissues of ripe cultivated vegetables and fruits. The opportunistic yeast *Candida parapsilosis* was present in all plants studied. Several virulence factors (production of hydrolytic enzymes and sensitivity to antifungal agents) were examined in all 107 isolates of *C. parapsilosis* from the internal tissues of fruits. Virulent isolates of *Candida parapsilosis* have been found in vegetables and fruits.

2 Kachalkin AV, Glushakova AM, Tomashevskaya MA. 2023. *Leucosporidium egoroviorum* f.a, sp. nov, a new yeast species isolated from zucchini. Microbiology 92(1):30–35 - DOI: 10.1134/S0026261722602494

Leucosporidium egoroviorum f.a, sp. nov, a new anamorphic species of the basidiomycetous yeasts, was isolated as an endophyte from the fruits of *Cucurbita pepo* subsp. *pepo* (zucchini). According to its genetic, physiological, and morphological characteristics, the new species differed significantly from the closely related species *L. fellii*, *L. intermedium*, and *L. krtinense*.

3 Glushakova AM, Kachalkin AV. 2023. Yeast community succession in cow dung composting process. Fungal Biol 127(6):1075–1083 - DOI: 10.1016/j.funbio.2023.06.001

Yeast complexes in the composting process of cow dung prepared to fertilize the soil for growing vegetables and fruits were studied. The average abundance of yeasts changed during the four temperature stages of the composting process. The highest abundance of yeasts, 1.38 x 10^4 cfu/g, was observed in the second stage of heating from 20 to 40 °C; the lowest was studied in the stage with the highest temperature (65 °C), 1.68 x 10^3 cfu/g. A total of 19 yeast species were observed and identified. The greatest diversity of yeasts was found in the initial (20 °C) and second (heating up to 40 °C) temperature stages of composting (*Aureobasidium pullulans, Candida parapsilosis, Candida saitoana, Candida santamariae, Candida tropicalis, Curvibasidium cygneicollum, Cutaneotrichosporon moniliforme, Debaryomyces fabryi, Debaryomyces hansenii, Filobasidium magnum, Kazachstania sp, Moesziomyces bullatus, Naganishia globosa, Papiliotrema flavescens, Rhodotorula mucilaginosa, Scheffersomyces insectosa, Torulaspora delbrueckii, Vanrija musci*), and the lowest in the stage of maximum heating (65 °C) (*C. parapsilosis, C. tropicalis, Cyberlindnera jadinii*). The opportunistic yeasts *C. parapsilosis* and *C. tropicalis* were obtained not only in the initial, second and third temperature stages of the composting process, but also in mature compost in the final stage prepared for soil application. The reduction of opportunistic yeast species was not achieved with the composting method used. The likelihood of these species entering agricultural products via compost and soil and developing as endophytes in the internal tissues of fruits is very high.

4 Arzumanyan VG, Ozhovan IM, Kachalkin AV, Kolyganova TI, Vartanova NO, Ilyina IV. 2023. Detection of *Naganishia albida* yeasts in dermatological patients. Bull Exp Biol Med 174:616–622 - DOI: 10.1007/s10517-023-05757-7

The yeasts *Cryptococcus albidus* (*Naganishia albida*) usually occur on natural substrates and rarely are the etiological factor of different mycoses. In the present study, two yeast isolates from the skin of female patients (age 7 and 74 years) with infective dermatitis (ICD-10-CM Code L30.3) were studied. The sensitivity of the obtained strains to antimycotics of three different chemical groups, namely itraconazole, naftifine, and amphotericin B, determined by microdilution method in a synthetic medium showed the following minimum inhibitory concentrations: 64-128, 16, and 0.125-4 μg/ml, respectively. It was found that the sensitivity of this yeast to pooled human serum was 30-47%, *i.e.* lower by 1.9-2.9 times than the sensitivity of the collection strains of *C. albicans* and *C. neoformans*. This result could be explained by lower prevalence of *N. albida* in the human population in comparison with these species. However, the sensitivity of *N. albida* strains to the low-molecular-weight fraction of serum was approximately the same as in *C. albicans* and *C. neoformans*, which indicates their high sensitivity to antimicrobial peptides.

5 Glushakova A, Kachalkin A. 2023. Yeasts associated with mines on tree leaves in the urban areas. Int Microbiol 26:1113–1121 - DOI: 10.1007/s10123-023-00370-0

Mines on tree leaves and undamaged leaves were studied to investigate yeast complexes in urban areas (*Aesculus hippocastanum*, miner - *Cameraria ohridella*; *Betula verrucosa*, miner - *Caloptilia betulicola*; *Populus nigra*, miner - *Lithocolletis populifoliella*; *Quercus robur*, miner - *Tischeria companella*; *Salix caprea*, miner - *Trachys minuta*; *Syringa vulgaris*, miner - *Caloptilia syringella*; *Tilia cordata*, miner - *Phyllonorycter issikii*; *Ulmus laevis*, miner - *Carpatolechia fugitivella*). The abundance and taxonomic structure of yeasts were studied using a surface plating method on solid media (GPY agar). The average abundance of yeasts during the first stages of mine formation in the internal tissues of leaves was 10^3 cfu/g. After 23–25 days, during the last stage of larval metamorphosis before mine destruction, the abundance of yeasts in the mines increased by two orders of magnitude to 10^5 cfu/g. The fast-growing ascomycetous yeasts *Hanseniaspora uvarum* and *H. occidentalis* dominated the mines. On undamaged leaves, the basidiomycetous yeasts *Papiliotrema flavescens* and *Rhodotorula mucilaginosa*, typical in the phyllosphere, dominated. The opportunistic yeast *Candida parapsilosis* was detected in the yeast complexes of all mines examined and was not found on the surface of leaves.

6 Glushakova A, Kachalkin A, Rodionova E. 2023. The role of fruits as reservoirs for resistant and virulent strains of opportunistic yeasts. World J Microbiol Biotechnol 39(11):313:1–13 - DOI: 10.1007/s11274-023-03758-2

Strains of the *Candida parapsilosis* and *Meyerozyma guilliermondii* species complexes isolated from the internal tissues of 26 fruit species from 21 countries were evaluated for their susceptibility to conventional antifungal compounds (fluconazole, voriconazole, amphotericin B) and hydrolytic activity. A total of 144 strains were studied. Resistance to at least one of the antifungal compounds tested was found in 26.4% of the endophytic strains examined. Most of the strains were insensitive to fluconazole. Multiresistance was detected only in two strains of *C. parapsilosis* sensu stricto from tropical apples. Phospholipase production and proteolytic and hemolytic activities were significantly higher in endophytes from tropical fruits. Resistant and virulent strains of opportunistic yeasts can thus spread worldwide via purchased fruit, which can harm people with a weakened immune status and children whose immune systems are not yet fully developed.

7 Kachalkin AV, Lepeshko AA, Streletskii RA, Glushakova AM. 2023. Endophytic strains of the yeast *Metschnikowia pulcherrima* positive for phytohormones production. Biologia - DOI: 10.1007/s11756-023-01551-y

Strains of the ascomycetous yeast *Metschnikowia pulcherrima* isolated from the internal tissues of fruits were evaluated for phytohormonal activity. IAA production in the culture liquid was determined by HPLC-UV, and zeatin and gibberellin (GA3) production was determined by Q-TOF LC/MS. IAA and zeatin were detected in the culture liquid of all strains studied. Synthesis of gibberellin (GA3) was detected in only 5 of 18 strains examined. On average, the strains showed the highest activity in the synthesis of IAA (6.668 µg/mL and 2.135 mg/g). Zeatin content was 3 orders of magnitude lower (0.365 ng/mL and 0.129 µg/g) and GA3 content was 4 orders of magnitude lower (0.076 ng/mL and 0.027 µg/g). According to our results, the yeast *M. pulcherrima* is not an active producer of zeatin and GA3 in amounts relevant to the plant. On the other hand, the average level of IAA synthesis detected in *M. pulcherrima* strains in this study is within the range of concentrations that can stimulate plant development and growth.

8 Glushakova A, Kachalkin A. 2023. Wild and partially synanthropic bird yeast diversity, in vitro virulence, and antifungal susceptibility of *Candida parapsilosis* and *Candida tropicalis* strains isolated from feces. Int Microbiol - DOI: 10.1007/s10123-023-00437-y

Yeast complexes in the fecal samples of wild (*Dendrocopos major*, *Picus viridis*) and partially synanthropic (*Bombycilla garrulus*, *Garrulus glandarius*, *Pica pica*, and *Pyrrhula pyrrhula*) birds were studied in a forest ecosystem during winter. A total of 18 yeast species were identified: 16 ascomycetes and two basidiomycetes. Most yeast species were found in the fecal samples of *P. pyrrhula* (*Candida parapsilosis*, *C. zeylanoides*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Meyerozyma carpophila*, *M. guilliermondii*, *Rhodotorula mucilaginosa*); the lowest number of yeast species was observed in the feces of *B. garrullus* (*C. parapsilosis*, *C. zeylanoides*, *Met. pulcherrima*, and *Rh. mucilaginosa*). The opportunistic species of the genus *Candida* were found only in feces of partially synanthropic birds. Strains of *C. parapsilosis* and *C. tropicalis* isolated from the feces of partially synanthropic birds were evaluated for their susceptibility to conventional antifungal agents (fluconazole, voriconazole, amphotericin B) and hydrolytic activity. A total of 160 strains were studied. Resistance to fluconazole was detected in 86.8% of *C. parapsilosis* strains and in 87% of *C. tropicalis* strains; resistance to voriconazole was detected in 71.7% of *C. parapsilosis* and in 66.7% of *C. tropicalis* strains, and the lowest percentage of resistant strains was detected to amphotericin B, 2.8% and 3.7% in *C. parapsilosis* and *C. tropicalis* strains, respectively. Phospholipase and hemolysin activities in the strains of *C. parapsilosis* were low; protease activity was moderate. The ability to produce hydrolytic enzymes was higher in the isolated strains of *C. tropicalis*. Thus, partially synanthropic birds can be vectors of virulent strains of opportunistic *Candida* species from urban environments to natural biotopes.

9 Čadež N, Boundy-Mills K, Botha A, Kachalkin A, Dlauchy D, Pĭter G. 2023. Taxogenomic placement of *Rasporella oleae* and *Rasporella dianae* gen. and spp. nov, two insect associated yeast species. Yeast - DOI: 10.1002/yea.3904

During the course of independent studies in Europe, North America, and Africa, seven yeast strains were isolated from insect frass, decaying wood, tree flux, and olive oil sediment. Based on the phylogenetic analysis of two barcoding DNA regions and genomes, formal descriptions of the new genus *Rasporella* and of two new species are provided.

XV Wine Research Centre, Faculty of Land and Food Systems. Rm 325, Food, Nutrition and Health Building, 2205 East Mall, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4. Communicated by Vivien Measday <vivien.measday@ubc.ca>.

Recent publication.

1 Marr RA, Moore J, Formby S, Martiniuk JT, Hamilton J, Ralli S, Konwar K, Rajasundaram N, Hahn A, Measday V. 2023. Whole genome sequencing of Canadian *Saccharomyces cerevisiae* strains isolated from spontaneous wine fermentations reveals a new Pacific West Coast Wine Clade. G3: Genes, Genomes, Genetics. 13(8):jkad130 - https://doi.org/10.1093/g3journal/jkad130

Vineyards in wine regions around the world are reservoirs of yeast with oenological potential. *Saccharomyces cerevisiae* ferments grape sugars to ethanol and generates flavor and aroma compounds in wine. Wineries place a high-value on identifying yeast native to their region to develop a region-specific wine program. Commercial wine strains are genetically very similar due to a population bottleneck and in-breeding compared to the diversity of *S. cerevisiae* from the wild and other industrial processes. We have isolated and microsatellite-typed hundreds of *S. cerevisiae* strains from spontaneous fermentations of grapes from the Okanagan Valley wine region in British Columbia, Canada. We chose 75 *S. cerevisiae* strains, based on our microsatellite clustering data, for whole genome sequencing using Illumina paired-end reads. Phylogenetic analysis shows that British Columbian *S. cerevisiae* strains cluster into 4 clades: Wine/European, Transpacific Oak, Beer 1/Mixed Origin, and a new clade that we have designated as Pacific West Coast Wine. The Pacific West Coast Wine clade has high nucleotide diversity and shares genomic characteristics with wild North American oak strains but also has gene flow from Wine/European and Ecuadorian clades. We analyzed gene copy number variations to find evidence of domestication and found that strains in the Wine/European and Pacific West Coast Wine clades have gene copy number variation reflective of adaptations to the wine-making environment. The "wine circle/Region B", a cluster of 5 genes acquired by horizontal gene transfer into the genome of commercial wine strains is also present in the majority of the British Columbian strains in the Wine/European clade but in a minority of the Pacific West Coast Wine clade strains. Previous studies have shown that *S. cerevisiae* strains isolated from Mediterranean Oak trees may be the living ancestors of European wine yeast strains. This study is the first to isolate *S. cerevisiae* strains with genetic similarity to nonvineyard North American Oak strains from spontaneous wine fermentations.

XVI Microbial Biochemical and Food Biotechnology, Faculty of Natural and Agricultural Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, Republic of South Africa. Communicated by Carolina Pohl-Albertyn <PohlCH@ufs.ac.za>.

Recent publication.

1 Mokoena NZ, Steyn H, Hugo A, Dix-Peek T, Dickens C, Gcilitshana OMN, Sebolai O, Albertyn J, Pohl CH. 2023. Eicosapentaenoic acid influences the pathogenesis of *Candida albicans* in *Caenorhabditis elegans* via inhibition of hyphal formation and stimulation of the host immune response. Med Microbiol Immunol 212:349-368.

The intake of omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA), is associated with health benefits due to its anti-inflammatory properties. This fatty acid also exhibits antifungal properties in vitro. In order to determine if this antifungal property is valid in vivo, we examined how EPA affects *Candida albicans* pathogenesis in the *Caenorhabditis* *elegans* infection model, an alternative to mammalian host models. The nematodes were supplemented with EPA prior to infection, and the influence of EPA on *C. elegans* lipid metabolism, survival and immune response was studied. In addition, the influence of EPA on hyphal formation in *C. albicans* was investigated. It was discovered that EPA supplementation changed the lipid composition, but not the unsaturation index of *C. elegans* by regulating genes involved in fatty acid and eicosanoid production. EPA supplementation also delayed killing of *C. elegans* by *C. albicans* due to the inhibition of hyphal formation in vivo, via the action of the eicosanoid metabolite of EPA, 17,18-epoxyeicosatetraenoic acid. Moreover, EPA supplementation also caused differential expression of biofilm-related gene expression in *C. albicans* and stimulated the immune response of *C. elegans*. This provides a link between EPA and host susceptibility to microbial infection in this model.

XVII Department of Genetics and Applied Microbiology, University of Debrecen, H-4031 Debrecen, Hungary. Communicated by Matthias Sipiczki <gecela@post.sk, lipovy@gmx.com>.

Recent publications.

1 Sipiczki M. 2023. Identification of antagonistic yeasts as potential biocontrol agents: Diverse criteria and strategies. Int J Food Microbiol 406:110360 - doi: 10.1016/j.ijfoodmicro.2023.110360

2 Csoma H, Kállai Z, Czentye K, Sipiczki M. 2023. *Starmerella lactis-condensi*, a yeast that has adapted to the conditions in the oenological environment. Int J Food Microbiol 401:110282 - doi: 10.1016/j.ijfoodmicro.2023.110282

3 Brysch-Herzberg M, Jia GS, Sipiczki M, Seidel M, Li W, Assali I, Du LL. 2023. *Schizosaccharomyces lindneri* sp. nov., a fission yeast occurring in honey. Yeast 40(7):237-253 - doi: 10.1002/yea.3857

4 Csoma H, Acs-Szabo L, Papp LA, Kállai Z, Miklós I, Sipiczki M. 2023. Characterization of *Zygosaccharomyces lentus* yeast in Hungarian botrytized wines. Microorganisms (MDPI) 11(4):852. doi: 10.3390/microorganisms11040852

5 Brejová B, Hodorová V, Lichancová H, Peričková E, Šoucová VA, Sipiczki M, Vinař T, Nosek J. 2023. Chromosome-level genome assembly of the yeast *Candida verbasci*. Microbiol Resour Announc 12(3):e0000523 - doi: 10.1128/mra.00005-23

6 Sipiczki M, Hrabovszki V. 2023. *Galactomyces candidus* diversity in the complex mycobiota of cow-milk bryndza cheese comprising antagonistic and sensitive strains. Int J Food Microbiol 388:110088 - doi: 10.1016/j.ijfoodmicro.2023.110088

7 Antunovics Z, Szabo A, Heistinger L, Mattanovich D, Sipiczki M. 2023. Synthetic two-species allodiploid and three-species allotetraploid *Saccharomyces* hybrids with euploid (complete) parental subgenomes. Sci Rep 13(1):1112 - doi: 10.1038/s41598-023-27693-2

XVIII Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia. Communicated by Renáta Vadkertiová, <renata.vadkertiova@savba.sk>.

Recent publications.

1 Chrenkova V, Vadkertiová R, Vlachova K, Babjuk M, - Lischke R, Bebrova E, Hudacek P. 2022. *Candida sojae*: First report of a human infection. J Mycol Med 32:101309 - https://doi.org/10.1016/j.mycmed.2022.101309

2 Šuchová K, Chyba A, Hegyi Z, Rebroš M, Puchart V. 2022. Yeast GH30 xylanase from *Sugiyamaella lignohabitans* is a glucuronoxylanase with auxiliary xylobiohydrolase activity. Molecules (MDPI) 27: 751 - https://doi.org/10.3390/molecules27030751

Xylanases are the enzymes that catalyze the breakdown of the main hemicellulose present in plant cell walls. They have attracted attention due to their biotechnological potential for the preparation of industrially interesting products from lignocellulose. While many xylanases have been characterized from bacteria and filamentous fungi, information on yeast xylanases is scarce and no yeast xylanase belonging to glycoside hydrolase (GH) family 30 has been described so far. Here, we cloned, expressed and characterized GH30 xylanase SlXyn30A from the yeast *Sugiyamaella lignohabitans.* The enzyme is active on glucuronoxylan (8.4 U/mg) and rhodymenan (linear β-1,4-1,3-xylan) (3.1 U/mg) while its activity on arabinoxylan is very low

(0.03 U/mg). From glucuronoxylan SlXyn30A releases a series of acidic xylooligosaccharides of general formula $MeGlcA^2Xyl_n$. These products, which are typical for GH30-specific glucuronoxylanases, are subsequently shortened at the non-reducing end, from which xylobiose moieties are liberated. Xylobiohydrolase activity was also observed during the hydrolysis of various xylooligosaccharides. SlXyn30A thus expands the group of glucuronoxylanases/xylobiohydrolases which has been hitherto represented only by several fungal GH30-7 members.

3 Paulovičová E, Hrubiško M. 2022. Humoral immune responses against facultative pathogen *Candida utilis* in atopic patients with vulvovaginal candidiasis. *Candida utilis* glucomannan – New serologic biomarker. Immunobiol 227: 152154 - https://doi.org/10.1016/j.imbio.2021.152154

Vulvovaginal candidiasis is one of the most commonly reported female genital tract infections, affecting approximately 70-75% of childbearing age women at least once during their lifetime. Approximately 50% of patients have refractory episodes and in 5-10% of cases the disease has a chronic course. The fungal cell wall represents the important host-invader interface. Cell-wall polysaccharides represent biological response modifiers and the pathogen-associated molecular patterns and virulence factors. Glycans are sensed by germ-line encoded pattern recognition receptors and reactively participate in immune system cell signaling. The most dominant cell-wall antigenic structures of *Candida* species as ß-glucan, α- and ß-mannans, glucomannan and other immunogenic polysaccharides are of particular relevancy for specific in vitro diagnosis and long-term follow-up of the Candida infection. In this study we assessed the immunobiological activity of facultative pathogen *Candida utilis* cell glucomannan and its effectivity as in vitro serological marker for antibody testing. The novel serologic assay has been developed and optimized for *C. utilis* serodiagnosis. The comparison assays were performed to establish relationship between antibodies against *C. utilis*, *C. albicans* and *S. cerevisiae* main cell-wall antigens in patient sera. The study evaluates applicability of glucomannan as serodiagnostic antigen and as a trigger of antigenspecific IgG, IgM and IgA antibody isotypes in the cohort of 35 atopic female subjects with recurrent vulvovaginal candidiasis. Statistically significant sera values of specific anti-glycan IgM and IgA class antibodies were revealed. The results are suggestive for efficient serological application of *C. utilis* glucomannan as in vitro disease marker and prospectively for follow-up of the specific long-term antimycotic therapy.

4 Katrlík J, Holazová A, Medovarská I, Seilerová I, Gemeiner P, Bystrický S. 2022. SPR biosensor chip based on mannan isolated from *Candida dubliniensis* yeasts applied in immunization effectiveness testing. Sens. Actuators B Chem 350: 130883 - https://doi.org/10.1016/j.snb.2021.130883

Study of interactions of glycan structures with glycan binding proteins attracts considerable attention due to their key significance in the processes of viral and bacterial pathogenesis, the innate immunity and the protection against infectious and communicable diseases. Here we describe the development of SPR biosensor based on mannan isolated from *Candida dubliniensis* yeasts, biotinylated and immobilized on a streptavidin-modified SPR chip with polycarboxylate matrix. The biosensor response was linear up to 16 nM of Concanavalin A (Con A) lectin with the limit of detection of 0.1 nM and response time from 2 min. SPR biosensor was applied in evaluating the efficiency of the immunization against *C. dubliniensis* in the animal model by the measurement of rabbit sera taken from animals immunized by mannan, killed yeast cells and mannan-albumin conjugate. Anti-mannan antibodies were quantified by ELISA in microplate modified with Con A and mannan. Based on calculated apparent half-life ($t_{1/2app}$) for the interaction of antisera with mannan SPR biosensor we proved that the immunization with the mannan conjugate was the most efficient method producing mannan specific immunoglobulins with the highest affinity to mannan of *C. dubliniensis*.

5 Horváthová Á, Farkaš V. 2022. Effect of N-acetyl chito-oligosaccharides on the biosynthesis and properties of chitin in *Saccharomyces cerevisiae*. Folia Microbiol 67 (2): 285-289 - https://doi.org/10.1007/s12223-021-00933-6.

Chitin exists in yeast cells both as free and bound in a complex with β-1,3/β-1,6-glucan. The formation of covalent links between chitin and β-glucans is catalyzed by the enzymes Crh1 and Crh2, acting as transglycosylases. We found that N-acetyl-chito-oligosaccharides, as well as laminarioligosaccharides, the respective products of partial hydrolysis of chitin, and β-1,3-glucan, interfered with reactions catalyzed by Crh1p and Crh2p in vitro. However, the N-acetyl-chito-oligosaccharides did not influence the growth rate of the yeast, neither did they affect the yeast phenotype, but they prolonged the lag phase. Inhibition of Crh1 and Crh2 in vivo with oligosaccharides derived from chitin leads to an increase of alkali-soluble chitin and a decrease in the amount of chitin linked to β-glucans. In addition, yeast cells growing in the presence of N-acetyl-D-chito-oligosaccharides accumulated more chitin than control cells.

6 Pipiková J, Horváthová Á, Schusterová H, Vadkertiová R. 2023. *Moniliella zaluziensis* sp. nov., a black yeast related to fruit trees of the Rosaceae family. Int J Syst Evol Microbiol 73(2) - doi: 10.1099/ijsem.0.005719.

Our previous studies focused on the diversity of yeasts related to the aboveground parts of fruit trees, as well as the soil adjacent to these trees, located in the south-west of Slovakia. During these studies, we isolated two *Moniliella* strains: CCY 11-1-1^T from the blossoms of a peach tree (*Prunus persica*) and CCY 11-1-2 from the soil adjacent to a pear tree (*Pyrus communis*), both found in the Malé Zálužie locality. The sequences of the D1/D2 domain of the large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS) region were identical in both strains. They differed only by two nucleotide substitutions in the segment of the gene of translation elongation factor one alpha (*TEF-1α*). Phylogenetic analysis demonstrated that strains CCY 11-1-1^T and CCY 11-1-2 formed a separate species in the clade of insect-associated members of the genus *Moniliella*. The strains differed from the closest species *Moniliella oedocephalis* by 23 nucleotide substitutions and 12 indels in the D1/D2 domain, more than 6 % in the ITS region (31 nt and 25 indels) and by 44 nt in the segment of *TEF-1α*. Therefore, these two strains are recognized as belonging to a novel species, for which we have proposed the name *Moniliella zaluziensis* sp. nov., derived from the locality of their origin, Malé Zálužie. The type strain of *M. zaluziensis* sp. nov. is CCY 11-1-1^T.

7 Labancová E, Šípošová K, Kučerová D, Horváthová Á, Schusterová H, Vivodová Z, Vadkertiová R, Kollárová K. 2023. The tremellaceous yeast: *Papiliotrema terrestris* - as the growth stimulant of maize plants. J Plant Growth Regul 42:3835–3850 - https://doi.org/10.1007/s00344-022-10848-2

Yeasts play different roles in the environment and might be involved in plant development processes. However, only a few yeast species and strains have been investigated in relation to plant-growth promotion. In this study, *Papiliotrema terrestris*, a soil basidiomycetous yeast, was tested for its effects on maize (*Zea mays* L.) plants. The plant-growth promotion depended on the concentration of the yeasts. Overall, the application of *P. terrestris* strain CCY 17-29-1 to the maize grains in the concentrations of 10^4 and 10^5 cells ml^{-1} increased the growth of roots, shoots, the formation of lateral and adventitious roots and the concentration of photosynthetic pigments. The application of *P. terrestris* in the concentration of 10^5 cells ml^{-1} showed a positive effect on the germination rate. The application of yeasts in the concentrations of 10^4 and 10^5 cells ml^{-1} increased the concentrations of some macro-nutrients (P, K, Mg, Ca) and micro-nutrients (Fe, Mn, Cu, Zn) in the plant roots and shoots. We have verified that *P. terrestris* is able to produce IAA and change the endogenous concentrations of IAA in plants. The PCA showed that the application of *P. terrestris* to the maize grains in the concentrations of 10^4 and 10^5 cells ml^{-1} had the most impact on root characteristics.

8 Paulovičová E, Paulovičová L, Čížová A, Mečárová J, Vrzoňová R, Farkaš P, Bystrický S. 2023. Amphoteric mannan as an immune response modifier. New model immunobiologically active *Candida albicans* mannan-based formula. Immunol Investig 52(4):415 - 438 - https://doi.org/10.1080/08820139.2023.2186245

Native and ultrasonically treated mannan from the yeast *Candida albicans* were chemically modified to contain both positive and negative charges in a nearly equimolar ratio mimicking the zwitterionic polysaccharides. RAW 264.7 macrophages and Balb/c mice were subjected as *in vitro* and *in vivo* models. Macrophage exposure to the set of amphoteric derivatives of mannan induced a release of Th1, Th2, Th17, and Treg cytokine signature patterns. The functionality of the exposed macrophages was assayed by cell proliferation and phagocytosis. The Th1 and Th17 dominance was over Th2. The phagocytosis and respiratory burst, together with the viability based on cell proliferation supported the bioavailability of formulas. Mouse immunization induced humoral immune responses with high titers of the IgM isotype with the IgM/IgG shift. Our study demonstrated the immunobiological activities of amphoteric derivatives of mannan from *Candida albicans*. Amphoteric derivatives can be considered as bioavailable formulas with an effective immunomodulatory potency, prospectively applied as a subunit formula in the design of a mannan-based platform for drug and vaccine delivery systems.

XIX Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand, Biodiversity Center Kasetsart University (BDCKU), Bangkok 10900, Thailand Communicated by Pannida Khunnamwong < fscipik@ku.ac.th > and Savitree Limtong <fscistl@ku.ac.th>.

Our publications for 2022–2023 are the following.

1 Nualthaisong P, Sakolrak B, Panicharoen T, Limtong S, Khunnamwong P. 2023. *Kodamaea samutsakhonensis* f.a., sp. nov., a novel ascomycetous yeast species isolated from wild mushrooms in Thailand. Int J Syst Evol Microbiol 73(5) - https://doi.org/10.1099/ijsem.0.005840

2 Kajadpai N, Angchuan J, Khunnamwong P, Srisuk N. 2023. Diversity of duckweed (Lemnaceae) associated yeasts and their plant growth promoting characteristics. AIMS Microbiol 9(3):486–517 - https://doi.org/10.3934/microbiol.2023026

3 Poontawee R, Lorliam W, Polburee P, Limtong S. 2023. Oleaginous yeasts: Biodiversity and cultivation. Fungal Biol. Rev. 44:100295 - https://doi.org/10.1016/j.fbr.2022.11.003.

4 Kanpiengjai A, Kodchasee P, Unban K, Kumla J, Lumyong S, Khunnamwong P, Sarkar D, Shetty K, Khanongnuch C. 2023. Three new yeast species from flowers of *Camellia sinensis* var. assamica collected in Northern Thailand and their tannin tolerance characterization. Front Microbiol 14:1043430 - https://doi.org/10.3389/fmicb.2023.1043430.

5 Nasanit R, Jaibangyang S, Onwibunsiri T, Khunnamwong P. 2022. Screening of volatile organic compound-producing yeasts and yeast-like fungi against aflatoxigenic *Aspergillus flavus*. Microbiol Biotechnol Lett 50(2):202-2 - https://doi.org/10.48022/mbl.2202.02004.

6 Khunnamwong P, Savarajara A, Jindamorakot S, Limtong S. 2022. *Metahyphopichia suwanaadthiae* sp. nov., an anamorphic yeast species in the order Saccharomycetales and reassignment of *Candida silvanorum* to the genus *Metahyphopichia*. Int J Syst Evol Microbiol 72(1):005183 - https://doi.org/10.1099/ijsem.0.005183.

7 Khunnamwong P, Kingphadung K, Lomthong T, Kanpiengjai A, Khanongnuch C, Limtong S. 2022. *Wickerhamiella nakhonpathomensis* f.a. sp. nov., a novel ascomycetous yeast species isolated from a mushroom and a flower in Thailand. Int J Syst Evol Microbiol 72(1):005191 - https://doi.org/10.1099/ijsem.0.005191.

8 Punyauppa-Path S, Kiatprasert P, Punyauppa-Path P, Rattanachaikunsopon P, Khunnamwong P, Limtong S, Srisuk N. 2022. Distribution of *Kazachstania* yeast in Thai traditional fermented fish (Plaa-som) in northeastern Thailand. J Fungi (MDPI) 8(10):1029 - https://doi.org/10.3390/jof8101029.

9 Punyauppa-path S, Kiatprasert P, Sawaengkaew J, Mahakhan P, Phumkhachorn P, Rattanachaikunsopon P, Khunnamwong P, Srisuk N. 2022. Diversity of fermentative yeasts with probiotic potential isolated from Thai fermented food products. AIMS Microbiol. 8(4):575-594 - https://doi.org/10.3934/microbiol.2022037.

10 Nutaratat P, Boontham W, Khunnamwong P. 2022. A novel yeast genus and two novel species isolated from pineapple leaves in Thailand: *Savitreella phatthalungensis* gen. nov., sp. nov. and *Goffeauzyma siamensis* sp. nov. J Fungi (MDPI) 8(2):118 - https://doi.org/10.3390/jof8020118.

11 Punyauppa-Path S, Punyauppa-Path P, Tingthong S, Sakpuntoon V, Khunnamwong P, Limtong S, Srisuk N. 2022. *Kazachstania surinensis* f.a., sp. nov., a novel yeast species isolated from Thai traditional fermented food. Int J Syst Evol Microbiol 72(8):005488 - https://doi.org/10.1099/ijsem.0.005488.

12 Angchuan J, Sakpuntoon V, Limtong S, Srisuk N. 2022. *Pichia bovicola* sp. nov., a yeast species associated with small-intestinal content of cattle. Int J Syst Evol Microbiol 72(3):005269 - https://doi.org/10.1099/ijsem.0.005269.

13 Sakpuntoon V, Péter G, Groenewald M, Dlauchy D, Limtong S, Srisuk N. 2022. Description of *Crinitomyces reliqui* gen. nov., sp. nov. and reassignment of *Trichosporiella flavificans* and *Candida ghanaensis* to the genus *Crinitomyces*. J Fungi (MDPI) 8(3): 224 - https://doi.org/10.3390/jof8030224.

14 Sapsirisuk S, Polburee P, Lorliam W, Limtong S. 2022. Discovery of oleaginous yeast from mountain forest soil in Thailand. J Fungi (MDPI) 8(10): 1100 - https://doi.org/10.3390/jof8101100.

XX Portuguese Yeast Culture Collection, Life Sciences Department, NOVA School of Science and Technology, UNL, 2829-516 Caparica, Portugal. Communicated by J.P. Sampaio <jss@fct.unl.pt>.

We recently remodelled the collection website, which can be found here https://pycc.pt/. The collection aims to assemble the most diverse catalogue of yeast strains from all taxonomic groups. We invite yeast researchers to deposit at PYCC yeast cultures that are cited in publications, especially (but not restricted to) representatives of novel species. Ecologically relevant strains and cultures of rarely isolated species are most welcome. PYCC distributes cultures worldwide and follows current standard practices and regulations. The collection was founded more than 70 years ago and is certified according to the ISO 9001:2015 norm. It has a strong commitment to research, maintaining yeast isolation programs on a regular basis and participating in yeast research projects.

The following paper, authored by members of PYCC was recently published.

1 Aires A, Gonçalves C, Sampaio JP 2023. *Hannaella floricola* sp. nov., a novel basidiomycetous yeast species isolated from a flower of *Lantana camara* in Portugal. Int J Syst Evol Microbiol Feb;73(2) - https://doi.org/10.1099/ijsem.0.005740

XXI BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand, and Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan. Communicated by Y. Yamada <ymdy333@kdt.biglobe.ne.jp>.

Recent publications.

1 Yamada Y, Vu HTL, Yukphan P, Tanasupawat S. The revision of Lipomycetaceae. Int J Novel Res Life Sci 10(5)64-72 - https://doi.org/10.5281/zenodo.10057492

In the family Lipomycetaceae, the seven genera *Waltomyces*, *Zygozyma*, *Babjevia*, *Smithiozyma*, *Kawasakia*, *Limtongia*, and *Kockiozyma* were introduced. However, all of them were neither accepted nor recognized. This paper described the historical surveys in the systematics of the Lipomycetaceous yeasts and confirmed the total of ten genera within the family Lipomycetaceae, i.e., the genus *Lipomyces* sensu stricto (the type genus), the above-mentioned seven genera, the monotypic genus *Dipodascopsis*, and the new genus *Neeoaidaea* from the phylogenetic and the phenotypic points of view.

2 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Mikata K, Yamada Y. The revision of Schizosaccharomycetaceae. Int J Novel Res Phys Chem Math 10(3):100-106 - https://doi.org/10.5281/zenodo.10066158

Although the genus *Hasegawaea* was introduced along with the recognition of the genus *Octosporomyces* in the classification of fission yeasts, the two additional genera were neither accepted nor recognized. However, the genus *Schizosaccharomyces* sensu Kurtzman et Robnett was taxonomically heterogeneous-natured and corresponded to a higher-ranked taxon, i.e., a monotypic family. Thus, the following three genera were confirmed in the family Schizosaccharomycetaceae. The genus *Schizosaccharomyces* sensu stricto was comprised of *Schizosacchromyces pombe*, the genus *Octosporomyces* was of the four species, *Octosporomyces octosporus*, *Octosporomyces osmophilus*, *Octosporomyces lindneri* and *Octosporomyces cryophilus* and the genus *Hasegawaea* was of *Hasegawaea japonica*. In conclusion, the precise classification of microorganisms will not be able to be expected in the generic designation without the presence of taxonomic homogeneous-natured taxa. The phylogenetic distances have to be absolutely considered. Namely, the longer the distances are, the more taxonomic heterogeneous-natures will be increased in the resulting genus.

3 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. 2023. The generic circumscription of *Mrakia* and *Mrakiella*: The proposal of *Thomashallia* gen. nov. Int J Novel Res Life Sci 10(5):33-41.

In the family Mrakiaceae, the type genus *Mrakia* senseu stricto included five teleomorphic species with the type species, *Mrakia frigida*. In contrast, the anamorphic genus *Mrakiella* sensu stricto contained nine species with the type species, *Mrakiella cryoconiti*. Between the two genera, the completely separated clusters were shown respectively in the phylogenetic tree based on the 28S rRNA gene D1/D2 domain sequences derived from the neighbour-joining method. Between *Mrakia frigida* and *Mrakiella cryoconiti* as well as *Mrakiella aquatica*, the pair-wise sequence similarities were 98.6% and 97.4% (1.2% width) respectively. However, the similarities between *Mrakia frigida* and the remaining four *Mrakia* species were extremely high (99.4 - 100% with 0.6% width). On the other hand, the similarity was relatively low (98.2%) between *Mrakiella cryoconiti* and *Mrakiella aquatica*, showing the wide range or the diversity of the anamorphic genus phylogenetically. The teleomorphic genus *Thomashallia* was newly introduced based on the formation of basidia and basidiospores with the type species, *Thomashallia stelviica*. The genus *Krasilnikovozyma* contained four species with the type species, *Krasilnikovozyma curviuscula*. Thus, the three teleomorphic genera were respectively taxonomic homogeneous-natured, and the two of the three were surely characteristic of Q-8.

4 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. 2023. The generic circumscription of *Tetrapisispora* Ueda-Nishimura et Mikata (1999) (Saccharomycetaceae): the proposal of *Nishimuraea* gen. nov. Int J Novel Res Life Sci 10(5):48-52.

The genus *Tetrapisispora* was introduced with the following four species; *T. arboricola*, *T. iriomotenensis*, *T. nanseiensis* and *T. phaffii* (the type species). Later, the three species were additionally accommodated to the genus; *T. blattae*, *T. fleetii* and *T. namnaonensis*. Of all the seven species, *Tetrapisispora blattae* represented an abnormally long branch in the phylogenetic trees based on the 26S rRNA gene D1/D2 domain and the 18S rRNA gene sequences derived from the neighbour-joining method. The calculated pair-wise 26S rRNA gene D1/D2 domain sequence similarities were extremely low (81.2 - 95.7%) within the genus. Except for *T. blattae*, the similarities were 90.3 - 95.7% among them. From the phylogenetic data obtained, *Nishimuraea* gen. nov. was introduced along with *Nishimuraea blattae* comb. nov.

5 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. 2023. The genus *Hansenula* Sydow et Sydow (1919) nom. rev. and the genus *Wickerhamomyces* Kurtzman et al. (2008). Int J Novel Res Life Sci 10(5):42-47.

The genus *Wickerhamomyces* Kurtzman et al. (2008) was extremely diverse phylogenetically, since a large number of species were equipped with long branches. Of the genus, *Wickerhamomyces anomalus* (= *Hansenula anomala*) and the remaining eight species mutually produced a large cluster with short branches. The pair-wise sequence similarities of *H. anomala* were very high (96.5-99.1%) to the eight species. Thus, the genus *Hansenula* Sydow et Sydow (1919) was revived as a taxonomic homogeneous-natured taxon and phylogenetically and taxonomically separated from the genus *Wickerhamomyces*. In addition, the genus *Wickerhamomyces* was designated as the double generic-structured, along with the genus *Lipomyces* sensu Kurtzman et al. (as the multiple genericstructured) and the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (as the quadruple generic-structured). The three genera actually corresponded respectively to a higher-ranked taxon, i.e., the subfamily or the family.

6 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. 2023. The reinstatement of the genus *Kloeckeraspora* Niehaus (1932) (apiculate yeast). Int J Novel Res Life Sci 10(2):32-37.

The genus *Kloeckeraspora* Niehaus (1932) was not accepted for a long time. This paper has tried to reinstate the genus. Two phylogenetic trees were constructed for three *Kloeckeraspora* and four representative *Hanseniaspora* species. In addition, the pair-wise sequence similarities were calculated. In the phylogeneetic tree based on the 26S rRNA gene D1/D2 domain sequences derived from the neighbour-joining method, the three species of the genus *Kloeckeraspora* represented an extremely long branch. The calculated pair-wise sequence similarities were abnormally low (86.2 - 88.5%) between the two genera. Incidentally, the sequence similarity between *Vanderwatozyma polyspora* and *Saccharomyces cerevisiae* used as references was 94.0%. The genus *Kloeckeraspora* should be unequivocally accepted and retained. However, the two genera were not yet perfectly taxonomic homogeneous-natured.

7 Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. 2023. The subdivision of the genus *Eremothecium* Borzi emend. Kurtzman (1995). Int J Novel Res Life Sci 10(5):58-63 - https://doi.org/10.5281/zenodo.10041023

In the family Saccharomycetaceae, the genus *Eremothecium* Borzi emend. Kurtzman (1995) included the five species that were on the whole characterized by the complex distribution of isoprenoid quinone homologues (Q-5, Q-6, Q-7, Q-8 and Q-9) as well as of needle-shaped ascospore ornamentation within the emended genus. However, the calculated pair-wise sequence similarities among the five species were very low (94.7 - 96.5%, i.e., below 98%) in the 26S rRNA gene D1/D2 domain sequences. The experimental data obtained indicated that the emended genus was not taxonomic homogeneous- but heterogeneous-natured, showing that the five species appropriately accommodated to their own separate five genera as *Eremothecium cymbalariae*, *Nematospora coryli*, *Ashbya gossypii*, *Crebrothecium ashbyi* and *Holleya sinecauda*. In addition, the taxonomic reliability of isoprenoid quinone homologues was discussed in detail.

XXII Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by M.A. Lachance <lachance@uwo.ca>.

Recent publications. Some abstracts are given under Dr. Rosa's or Dr. Hittinger's communications.

1 Tiwari S, Lee DK, Lachance MA, Baghela A. 2023. *Metschnikowia ahupensis* f.a., sp. nov., a new yeast species isolated from the gut of the wood-feeding termite *Nasutitermes* sp. Int J Syst Evol Microbiol 73(8) - https://doi.org/10.1099/ijsem.0.006012

The gut of xylophagous insects such as termites harbours various symbiotic micro-organisms, including many yeast species. In a taxonomic study of gut-associated yeasts, two strains (ATS2.16 and ATS2.18) were isolated from the gut of the wood-feeding termite Nasutitermes sp. in Maharashtra, India. Morphological and physiological characteristics and sequence analyses of the ITS and D1/D2 region of the large subunit rRNA gene revealed that these two strains represent a novel asexual ascomycetous yeast species in the genus *Metschnikowia*. The species differs from some of its close affiliates in the genus in its inability to utilize ethanol and succinate as the sole carbon source and growth in high sugar concentrations (up to 50 % glucose). In contrast to most members of *Metschnikowia*, the formation of ascospores was not observed on various sporulation media. Moreover, whole-genome sequencing was used to further confirm the novelty of this species. When compared with other large-spored Metschnikowia species, average nucleotide identity values of 79-80 % and digital DNA-DNA hybridization values of 16-17 % were obtained. The name *Metschnikowia ahupensis* f.a., sp. nov. is proposed to accommodate this novel yeast species, with ATS2.16 as the holotype and strains NFCCI 4949, MTCC 13085 and PYCC 9152 as isotypes. The MycoBank number is MB 844210.

2 Santa-Brígida R, Santos ARO, Martins MB; Rosa LH, Lachance MA, Rosa CA. 2023. *Teunomyces gombertii* f. a., sp. nov., *Teunomyces landelliae* f.a., sp. nov., *Teunomyces ledahaglerae* f. a., sp. nov., and *Teunomyces paulamoraisiae* f. a., sp. nov., four yeast species isolated from mushrooms and drosophilids in a Brazilian Amazonian rainforest biome. Int J Syst Evol Microbiol 73(9) - https://doi.org/10.1099/ijsem.0.006035

3 Santos ARO, Barros KO, Batista TM, Souza GFL, Alvarenga FBM, Abegg MA, Sato TK, Hittinger CT, Lachance MA, Rosa CA. 2023. *Saccharomycopsis praedatoria* sp. nov., a predacious yeast isolated from soil and rotten wood in an Amazonian rainforest biome. Int J Syst Evol Microbiol. 73(10) - https://doi.org/10.1099/ijsem.0.006125

4 Groenewald M, Hittinger CT, Bensch K, Opulente DA, Shen XX, Li Y, Liu C, LaBella AL, Zhou X, Limtong S, Jindamorakot S, Gonçalves P, Robert V, Wolfe KH, Rosa CA, Boekhout T, Ĉadež N, Péter G, Sampaio JP, Lachance MA, Yurkov AM, Daniel HM, Takashima M, Boundy-Mills K, Libkind D, Aoki K, Sugita T, Rokas A. 2023. A genome-informed higher rank classification of the biotechnologically important fungal subphylum Saccharomycotina. Stud Mycol 105:1-22 - https://doi.org/10.3114/sim.2023.105.01

5 Rosa CA, Lachance MA, Limtong S, Santos ARO, Landell MF, Gombert AK, Morais PB, Sampaio JP, Gonçalves C, Gonçalves P, Góes-Neto A, Santa-Brígida R, Martins MB, Janzen DH, Hallwachs W. 2023. Yeasts from tropical forests: biodiversity, ecological interactions, and as sources of bioinnovation. Yeasts - https://doi.org/10.1002/yea.3903

GMVV - Italian Group on Vine and Wine Microbiology

Coordinated by Prof. Patrizia Romano <pot2930@gmail.com>

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

The following are papers on yeasts from the partners of GMVV for 2022-2023.

GMVV-I Faculty of Economy, Universitas Mercatorum, Piazza Mattei, 10, 00186 Rome, Italy. Communicated by Professor Patrizia Romano <pot2930@gmail.com>.

1 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) 8:59 - 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).

2 Romano P, Braschi G, Siesto G, Patrignani F, Lanciotti R. 2022. role of yeasts on the sensory component of wines. Foods (MDPI) 11:1921 - 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.

3 Gottardi, D, Siesto, G, Bevilacqua, A, Patrignani, F, Campaniello, D, Speranza, B, Lanciotti, R, Capece, A, Romano, P. 2023. Pilot scale evaluation of wild *Saccharomyces cerevisiae* strains in aglianico. Fermentation (MDPI) 9:245 - https://doi.org/10.3390/fermentation9030245

In winemaking, the influence of *Saccharomyces cerevisiae* strains on the aromatic components of wine is well recognized on a laboratory scale, but few studies deal with the comparison of numerous strains on a pilot scale fermentation. In this scenario, the present work aimed to validate the fermentative behavior of seven wild S. cerevisiae strains on pilot-scale fermentations to evaluate their impact on the aromatic profiles of the resulting wines. The strains, isolated from grapes of different Italian regional varieties, were tested in pilot-scale fermentation trials performed in the cellar in 1 hL of Aglianico grape must. Then, wines were analyzed for their microbiological cell loads, main chemical parameters of enological interest (ethanol, total sugars, fructose, glucose, total and volatile acidity, malic and lactic acids) and volatile aroma profiles by GC/MS/SPME. Seventy-six volatile compounds belonging to six different classes (esters, alcohols, terpenes, aldehydes, acids, and ketones) were identified. The seven strains showed different trends and significant differences, and for each class of compounds, high-producing and low-producing strains were found. Since the present work was performed at a pilot-scale level, mimicking as much as possible real working conditions, the results obtained can be considered as a validation of the screened *S. cerevisiae* strains and a strategy to discriminate in real closed conditions strains able to impart desired wine sensory features.

GMVV-II School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale Ateneo Lucano 10, 85100 Potenza (PZ), Italy. Communicated by Professor Angela Capece <angela.capece@unibas.it>.

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1 Caporusso A, De Bari I, Liuzzi F, Albergo R, Valerio V, Viola E, Pietrafesa R, Siesto G, Capece A. 2023. Optimized conversion of wheat straw into single cell oils by *Yarrowia lipolytica* and *Lipomyces tetrasporus* and synthesis of advanced biofuels. Renewable Energy 202:184-195 - https://doi.org/10.1016/j.renene.2022.11.059

This paper deals with the optimized conversion of undetoxified wheat straw hydrolysates into microbial lipids by two oleaginous yeasts, *Yarrowia lipolytica* and *Lipomyces tetrasporus*. Wheat straw were pretreated by steam explosion at 203 °C for 300 s and hydrolysed at 20% solid-to-liquid ratio by using an enzymatic loading of 15 FPU/g substrate. The mixed wheat straw hydrolysates (WHS) contained 86 gL-1 glucose and 22 gL-1 xylose, 2.3 gL-1 acetic acid, 0.9 gL-1 furanic compounds. The fermentation process was optimized in terms of the inoculum age and density, medium composition, and bioreactor feeding strategy. In particular, the different capacity of the two yeasts to overcome the toxic effect of the biomass degradation by-products, in different inoculum ages, was deeply investigated. Two hydrolysates concentration were tested: WSH containing 86 gL-1 glucose and 22 gL-1 xylose and the diluted medium containing 40 gL-1 glucose and 22 gL-1 xylose. The results indicated that both yeasts were able to detoxify WSH and grow on undetoxified hydrolysates as effect of the intrinsic capacity to metabolize the furan derivatives. *Y. lipolytica* was able to detoxify the medium in all the investigated set-ups, while *L. tetrasporus* was able to detoxify the medium only if inoculated in the stationary phase of growth. After the process optimization in shaken flasks, the production of Single Cell Oils (SCOs) by *L. tetrasporus* was carried out in a medium-scale bioreactor of 10L obtaining lipid yield and cell content of 21% and 62%, respectively. The extracted SCOs, with high oleic and palmitic acid content, were converted into biodiesel displaying overall features in accordance with international biodiesel standards, namely ASTM and EN 14214.

2 Siesto G, Pietrafesa R, Tufariello M, Gerardi C, Grieco F, Capece A. 2023. Application of microbial cross-over for the production of Italian grape ale (IGA), a fruit beer obtained by grape must addition. Food Bioscience, 52, 102487 - https://doi.org/10.1016/j.fbio.2023.102487

In the last years, the production of grape ale beer has become a trendy choice among brewers in Italy, resulting in a new beer type known as Italian Grape Ale (IGA), a sort of bridge beverage between beer and wine. In this study, three autochthonous *Saccharomyces cerevisiae* strains (CHE-3, P4 and TA4-10), previously isolated from different food types, were tested in comparison with the commercial strain US-05. Fermentations were conducted at a laboratory scale on malt extracts with 15% and 25% grape must adjuncts, and on malt wort (control). After fermentation, the CHE-3, P4 and TA4-10 strains showed significantly higher CO_2 production than US-05. The main analytical parameters (organic acids, real and apparent attenuation, ethanol content, glycerol level and carbohydrate profile) and the volatile compounds related to the organoleptic quality of the experimental beers were strongly influenced both by the grape must addition to the fermentation medium and the used strains. The addition of grape must enhanced the CO_2 production, especially when 25% of grape must was added to the wort. By comparing the tested strains, the highest aromatic expression was observed with 15% grape must adjunct. The present results confirm the importance of media composition and microbial biodiversity to gain more different beers especially in the craft beers sector. Moreover, the outcome data show the significance of microbial cross-over, a novel approach based on the exploitation of microorganisms traditionally used in other agro-food chains also for brewing novel beer types.

3 Torrellas M, Pietrafesa R, Ferrer-Pinós A, Capece A, Matallana E, Aranda, A. 2023. Optimizing growth and biomass production of non-*Saccharomyces* wine yeast starters by overcoming sucrose consumption deficiency. Frontiers in Microbiology 14:1209940 - https://doi.org/10.3389/fmicb.2023.1209940

The use of non-*Saccharomyces* yeasts as starters in winemaking has increased exponentially in the last years. For instance, non-conventional yeasts have proven useful for the improvement of the organoleptic profile and biocontrol. Active dry yeast starter production has been optimized for *Saccharomyces cerevisiae*, which may entail problems for the propagation of non-*Saccharomyces* yeasts. This work shows that the poor growth of *Hanseniaspora vineae* and *Metschnikowia pulcherrima* in molasses is related to a deficient sucrose consumption, linked to their low invertase activity. In order to address this issue, simple modifications to the cultivation media based hydrolysis and the reduction of sucrose concentration were performed. We performed biomass propagation simulations at a bench-top and bioreactor scale. The results show that cultivation in a hexose-based media improved biomass production in both species, as it solves their low invertase activity. The reduction in sugar concentration promoted a metabolic shift to a respiratory metabolism, which allowed a higher biomass yield, but did not improve total biomass production, due to the lower sugar availability. To evaluate the technological performance of these adaptations, we performed mixed grape juice fermentations with biomass produced in such conditions of *M. pulcherrima* and *S. cerevisiae*. The analysis of wines produced revealed that the different treatments we have tested did not have any negative impact on wine quality, further proving their applicability at an industrial level for the improvement of biomass production.

4 Pietrafesa R, Siesto G, Tufariello M, Palombi L, Baiano A, Gerardi C, Braghieri A, Genovese F, Grieco F, Capece, A. 2023. A multivariate approach to explore the volatolomic and sensory profiles of craft Italian Grape Ale beers produced with novel *Saccharomyces cerevisiae* strains. Frontiers in Microbiology 14:1234884 - doi: 10.3389/fmicb.2023.1234884

This study investigated the influence of three *Saccharomyces cerevisiae* strains, selected from different matrices - CHE-3 (cherry), P4 (sourdough) and TA4-10 (grape must) - on characteristics of Italian Grape Ale (IGA) beers obtained at microbrewery scale. A multidisciplinary approach, combining results from analysis of chemical, volatile and organoleptic profiles of the beers, was adopted to underline the relationships between yeast starter and the quality of final products. Detection volatile organic compounds (VOCs) by Gas-Chromatography coupled with Mass Spectrometry (GC-MS) after extraction carried out by head-space micro-extraction (HS-SPME) revealed that the beer obtained by P4 strain differed from the others for its higher concentrations of esters, alcohols, and terpenes as confirmed by PCA (principal component analysis) and Cluster heatmap. Furthermore, sensorial analysis and consumer test showed that this sample differed from others by more pronounced notes of "fruity smell and floral" and "olfactory finesse," and it was the most appreciated beer for smell, taste, and overall quality. Conversely, CHE-3 was the sample with the lowest concentrations of the identified volatiles and, together TA4-10, showed the highest scores for smoked, yeast, malt, and hop notes. As far as we know, these are the first results on the application of indigenous S. cerevisiae strains in the production of craft IGA beers analyzed through a complex multivariate approach.

GMVV-III Department of Agricultural, Forest and Food Sciences, University of Turin. Via Largo Braccini 2, 10095 Grugliasco, Italy. Communicated by Prof. Luca Simone Cocolin <lucasimone.cocolin@unito.it>.

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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. Amer J Enol Viticulture 73:293-306 - doi: 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 sulfur dioxide (SO_2) addition during winemaking is a possible intervention strategy that could be of interest to winemakers. A strain of *Pichia kluyveri* and one of *Lactiplantibacillus plantarum* were inoculated into a Nebbiolo red grape must, previously inoculated with a mix of microorganisms to mimic a grape must environment. The synergistic effect of the added strains with no, low, or moderate addition of total SO_2 was explored over two vintages. Wine fermentations were monitored for microbiological and physicochemical attributes. Microbiological analyses showed fewer undesired microorganisms, namely *Acetobacter aceti* and *Hanseniaspora uvarum*, independently from the strain used; this decrease occurred faster when starter cultures were combined with SO_2. Chemical analyses revealed less acetic acid and ethyl acetate in the bioprotected wines. The bioprotective ability of the starter cultures *P. kluyveri* and *L. plantarum* demonstrated in both vintages.

2 Englezos V, Jolly N.P, Di Gianvito P, Rantsiou K, Cocolin L. 2022. Microbial interactions in winemaking: Ecological aspects and effect on wine quality. Trends Food Sci Technol 127:99-113 - doi.org/10.1016/j.tifs.2022.06.015

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 species and strains within species during alcoholic and malolactic fermentation is greatly influenced by the complexity of the wine environment. Consequently, the successful prediction of wine characteristics is limited. 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 lactic acid bacteria throughout the alcoholic and malolactic fermentations 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 these processes. 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 Serafino G, Di Gianvito P, Giacosa S, Škrab D, Cocolin L, Englezos V, Rantsiou K. 2023. Survey of the yeast ecology of dehydrated grapes and strain selection for wine fermentation. Food Res Int 170:113005 - doi.org/10.1016/j.foodres.2023.113005

In this study we investigated the yeast population present on partially dehydrated Nebbiolo grapes destined for ‘Sforzato di Valtellina’, with the aim to select indigenous starters suitable for the production of this wine. Yeasts were enumerated, isolated, and identified by molecular methods. A genetic, physiological and oenological characterization was also carried out. Based on relevant physiological features, non-*Saccharomyces* strains were chosen for laboratory-scale fermentations, either in pure or in mixed-culture (with a commercial *Saccharomyces cerevisiae* strain. Finally, the best couples and inoculation strategy were further tested in mixed fermentations in winery. In both laboratory and winery, microbiological and chemical analyses were conducted during fermentation. The most abundant species on grapes were *Hanseniaspora uvarum*, followed by *Metschnikowia* spp. and *Starmerella bacillaris*. Technological characterization highlighted several inter- and intra-species differences. The best oenological aptitude was highlighted for species *Starm. bacillaris*, *Metschnikowia* spp., *Pichia kluyveri* and *Zygosaccharomyces bailli*. The best fermentation performances in laboratory-scale fermentations were found for *Starm. bacillaris* and *P. kluyveri*, due to their ability to reduce ethanol and enhance glycerol production. This behavior was further confirmed in winery. Results of this study contribute to the knowledge of yeast communities associated with a specific environment.

4 Englezos V, Mota-Gutierrez J, Simone G, Rio Segade S, Pollon M, Gambino G, Rolle L, Ferrocino I, Rantsiou K. 2022. Effect of alternative fungicides and inoculation strategy on yeast biodiversity and dynamics from the vineyard to the winery. Food Res Int 162:111935 - doi.org/10.1016/j.foodres.2022.111935.

Fungi and oomycetes found in vineyards cause diseases such as powdery and downy mildew. Consequently, conventional and alternative agronomical practices are widely used prior to harvest to protect grapes. Alternative products are considered more eco-friendly and environmentally sustainable in comparison to conventional chemical products. However, the effect of these alternative products on yeast ecology, from the vineyard to the winery, is poorly understood. This study compared the effect of alternative and conventional chemical antifungal compounds on grapes’ mycobiota in the vineyard and during subsequent winery fermentation using culture-dependent and -independent approaches. Culture-dependent data indicated a treatment-dependent effect on the load and diversity of yeast populations on grapes. It was found that the population of *Hanseniaspora uvarum* was higher on grapes previously treated with laminarin and copper, compared to the other levels registered on grapes previously treated with the rest of antifungal products tested in this study. Concerning, wine quality, the chemical composition was not correlated to the application of antifungal treatment in the vineyard. Understanding the effect of different antifungal products on grape and wine microbial communities may help in setting up guidelines for wine grape production.

GMVV-IV Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, SP6 Lecce-Monteroni, 73100 Lecce (LE), Italy. Communicated by Professor Massimiliano Cardinale <massimiliano.cardinale@unisalento.it>.

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1 Girelli CR, Papadia P, Pagano F, Miglietta PP, Fanizzi FP, Cardinale M, Rustioni L. 2023. Metabolomic NMR analysis and organoleptic perceptions of pomegranate wines: influence of cultivar and yeast on the product characteristics. Heliyon 6:e16774 - https://doi.org/10.1016/j.heliyon.2023.e16774.

Pomegranate (*Punica granatum* L.) fruits are a historical agricultural product of the Mediterranean basin that became increasingly popular in the latest years for being rich in antioxidants and other micronutrients, and are extensively commercialized as fruits, juice, jams and, in some Eastern countries, as a fermented alcoholic beverage. In this work, four different pomegranate wines specifically designed using combinations of two cultivars (Jolly Red and Smith) and two yeast starters with markedly different characteristics (*Saccharomyces cerevisiae* Clos and *Saccharomyces cerevisiae* ex-*bayanus* EC1118) were analyzed. The chemical characterization of the wines together with the originating unfermented juices was performed by ^{1}H NMR spectroscopy metabolomic analysis. The full spectra were used for unsupervised and supervised statistical multivariate analysis (MVA), namely Principal Component Analysis (PCA), Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), and sparse PCA (SPCA). The MVA of the wines showed a clear discrimination between the cultivars, and a smaller, yet significant, discrimination between the yeasts used. In particular, a higher content of citrate and gallate was observed for the Smith cv. and, on the contrary, a statistically significant higher content of fructose, malate, glycerol, 2,3 butanediol, trigonelline, aromatic amino acids and 4-hydrophenylacetate was observed in Jolly Red pomegranate wines samples. Significant interaction among the pomegranate cultivar and the fermenting yeast was also observed. Sensorial analysis was performed by a panel of testing experts. MVA of tasting data showed that the cultivar significantly affected the organoleptic parameters considered, while the yeast had a minor impact. Correlation analysis between NMR-detected metabolites and organoleptic descriptors identified several potential sensorially-active molecules as those significantly impacting the characteristics of the pomegranate wines.

GMVV-V Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy. Communicated by Professor Rosanna Tofalo <rtofalo@unite.it>.

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1 Rossetti AP, Perpetuini G, Battistelli N, Zulli C, Arfelli G, Suzzi G, Cichelli A, Tofalo R. 2023. Capturing the fungal community associated with conventional and organic Trebbiano Abruzzese grapes and its influence on wine characteristics. Food Bioscience 52:102382 - doi.org/10.1016/j.fbio.2023.102382.

In this study, the influence of conventional and organic managements on Vitis vinifera cv. Trebbiano Abruzzese on fungal community was investigated. Moreover, spontaneous vinifications were performed, and wines were characterized for some oenological parameters and aroma profiles. A core of 8 genera was detected (*Zygosaccharomyces* spp., *Cladosporium* spp., *Botrytis* spp., *Hanseniaspora* spp., *Pichia* spp., *Alternaria* spp., *Candida* spp., *Aureobasidium* spp.), while some genera were unique for each grape sample. *Saccharomyces* spp. and *Mucor* spp. were present only on conventional grapes, while *Penicillium* spp. and *Aspergillus* spp. only on organic ones. At the species level *Aureobasidium pullulans*, *Cladosporium cladosporioides* and *Pichia terricola* dominated both samples with varying abundance depending on the vineyard management. The fermentation performed with conventional grapes resulted in a faster rate than that performed with organic ones. No significative differences were observed for the oenological parameters analyzed with the only exception of ethanol content which was higher in conventional wines than in organic ones: 11.57 ± 0.10 g/L and 10.51 ± 0.09 g/L, respectively. Conventional wines were characterized by the presence of aldehydes, while organic ones by terpenes and β-damascenone. The obtained results suggested an effect of vineyard practices on grape fungal community which could in turn influence some characteristics of Trebbiano d'Abruzzo wines.

2 Perpetuini G, Rossetti AP, Giordano L, Pulcini M, Dufrusine B, Battistelli N, Zulli C, Arfelli G, Palliotti, A, Dainese E, Tofalo R. 2023. Characterization of Nero Antico di Pretalucente wine and grape fungal microbiota: an expression of Abruzzo region cultivar heritage. Fermentation (MDPI) 9:150 - doi.org/10.3390/fermentation9020150

The aim of this study was to characterize the ampelographic and genetic profiles of *Vitis vinifera* L. cv. Nero Antico di Pretalucente and to describe the grape-borne fungal communities. The oenological characteristics and the aroma profile of wine obtained by spontaneous fermentation were also investigated. Microsatellite profiles and ampelographic traits indicated that this cultivar presented a unique profile, and therefore it can be considered a cultivar in its own right and autochthonous of Gessopalena village. Next-generation sequencing analysis revealed that *Aureobasidium* spp. was the main genus detected on grapes. At the species level, *Aureobasidium pullulans* was the main species, followed by *Alternaria alternata*. Wines were characterized by a final ethanol content of 12.75% (v/v), a pH of 3.4, a volatile acidity lower than 0.6 g/L, a content of glycerol of 8.56 g/L, and a concentration of polyphenols and anthocyanins of 977 GAE/L and 266 mg/L, respectively. The intensity and tonality of the wine as well as the active odor compounds found were described. The results obtained could improve the knowledge concerning the agronomic traits and the wine obtained from this ancient and autochthonous grapevine variety cultivated in a foothill area, in order to offer consumers a wine with unique traits.

3 Perpetuini G, Rossetti AP, Battistelli N, Zulli C, Piva A, Arfelli G, Corsetti A, Tofalo R. 2023. Contribution of *Starmerella bacillaris* and oak chips to Trebbiano d'Abruzzo wine volatile and sensory diversity. Foods (MDPI) 12:1102 - doi.org/10.3390/ foods12051102.

In this study, six fermentation trials were carried out: co-inoculation and sequential inoculation of *Saccharomyces cerevisiae* and *Starmerella bacillaris* in the presence and absence of oak chips. Moreover, *Starm. bacillaris* strain was attached to the oak chips and co-inoculated or sequentially inoculated with *S. cerevisiae*. Wines fermented with *Starm. bacillaris* adhered to oak chips showed a higher concentration of glycerol (more than 6 g/L) than the others (about 5 g/L). These wines also showed a higher content of polyphenols (more than 300 g/L) than the others (about 200 g/L). The addition of oak chips induced an increase of yellow color (b* value of about 3). Oak-treated wines were characterized by a higher concentration of higher alcohols, esters and terpenes. Aldehydes, phenols and lactones were detected only in these wines, independently from the inoculation strategy. Significant differences ($p < 0.05$) were also observed in the sensory profiles. The fruity, toasty, astringency, and vanilla sensations were perceived as more intense in wines treated with oak chips. The white flower descriptor showed a higher score in wines fermented without chips. Oak surface-adhered *Starm. bacillaris* cells could be a good strategy to improve the volatile and sensory profile of Trebbiano d'Abruzzo wine.

4 Perpetuini G, Nzobouh Fossi PA, Kwak S, Namessi O, Petchkongkaew A, Tofalo R, Waché Y. 2023. Pesticides in foods: towards bioremediation biocatalysts? Catalysts (MDPI) 13:1055 - doi.org/ 10.3390/catal13071055

The presence of hazardous chemical compounds in foods is a growing concern in almost every country. Although some toxins come from microbial contamination, a major part comes from residues of pesticides used for plant health and food preservation. Despite plans to decrease their use, the concentration of hazardous residues encountered in food is growing. The societal solution to this issue is to find alternatives to chemicals and replace the most hazardous by biodegradable, fewer toxic compounds. However, as this greener transition takes some time, any transitory solution to decrease the risks of contamination is welcome. Among them the stimulation of microbial pesticide degradation in food in a similar way to bioremediation in the environment would be very positive. In this review, we present the problem of food contamination, focusing on organophosphates and organochlorines, and the various possibilities of microbial decontamination. We discuss the possible use of microbial biocatalysts as a biopreservation tool. We conclude that, although this process is very promising, it lacks research taking into account the various degradation products and the elaboration of screening procedures able to choose some rare, efficient biopreservation strains.

GMVV-VI School of Agriculture, Department of Agriculture, Food, Environmental and Forestry (DAGRI) – Agricultural Microbiology Section, University of Florence, via San Bonaventura, 13, 50145 Firenze, Italy - Communicated by Professor Lisa Granchi <lisa.granchi@unifi.it>.

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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. Int J Mol Sci (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 10^5 cells/mL), as well as at lower concentrations (10^3–10^2 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* and could allow preventive actions to reduce wine spoilage.

2 Galli V, Venturi M, Guerrini S, Mangani S, Barbato D, Vallesi G, Granchi L. 2023. Exploitation of selected sourdough *Saccharomyces cerevisiae* strains for the production of a craft raspberry fruit beer. Foods (MDPI) 12:3354 - https://doi.org/10.3390/foods12183354

Recent interest in the special beer category has encouraged the search for novel brewing materials, including new ingredients and novel yeast strains, in order to differentiate the finished products. The aim of this work was to select non-brewing *S. cerevisiae* strains for the production of a fruit beer with raspberry. The in vitro tests and the wort fermentations allowed the selection of two sourdough *S. cerevisiae* strains, showing high maltose and maltotriose consumption, high ethanol production, and high viability. Fruit beers (FB) and control beers (CB) without raspberries were prepared. Fruit addition accelerated sugar consumption (7 days compared to 13 days) and increased ethanol and glycerol production by yeasts. Raspberry addition and the inoculated yeast strongly affected the aroma profile of beers. FB samples showed a higher amount of volatile organic compounds (VOCs); the most represented classes were alcohols, followed by esters and acids. FB inoculated by the selected S. cerevisiae SD12 showed the highest VOCs concentration (507.33 mg/L). Results highlighted the possible application of sourdough yeast strains for the brewing process, which, combined with raspberry addition, can be exploited for the production of beers with enhanced aromatic features and suitable chemical properties.

3 Guerrini S, Barbato D, Mangani S, Ganucci D, Buscioni G, Galli V, Triossi A, Granchi L. 2023. Management of in-Amphora "Trebbiano Toscano" wine production: selection of indigenous *Saccharomyces cerevisiae* strains and influence on the phenolic and sensory profile. Foods (MDPI) 12:2372 - https://doi.org/10.3390/foods12122372

The use of earthenware amphorae in winemaking can give wines unique attributes enhancing their typicity. Therefore, in this study, spontaneous and inoculated in-amphora fermentations of Trebbiano Toscano grape must were monitored to assess the *Saccharomyces cerevisiae* strains occurring in each fermentation as well as the chemical characteristics of the wines. Strain typing via Interdelta analyses pointed out that the commercial starters did not dominate, showing 24% and 13% implantation percentages, and that 20 indigenous strains were present at different percentages, ranging from 2 to 20%, in inoculated and spontaneous fermentations. The assessment of the technical characteristics of the indigenous strains via fermentations at lab and pilot scale (20 L amphorae) and the sensory analysis of the experimental wines allowed for the selection of two indigenous strains to be used as starter cultures in comparison to a commercial strain in 300-L-amphorae vinifications in the cellar. The observed fermentative performances and sensory analysis of the experimental wines highlighted that one indigenous *S. cerevisiae* strain dominated the process and conferred distinctive sensory characteristics to the Trebbiano Toscano wine, demonstrating its effectiveness in managing the in-amphora fermentations. In addition, the results demonstrated the ability of amphorae to protect the polyphenolic compounds from oxidation during wine ageing. Indeed, the concentration of both hydroxycinnamic acids and flavonols decreased, with an average reduction of 30% and 14%, respectively, while hydroxybenzoic acids remained unchanged.

4 Guerrini S, Galli G, Barbato D, Facchini G, Mangani S, Pierguidi L, Granchi, L. 2023. Effects of *Saccharomyces cerevisiae* and *Starmerella bacillaris* on the physicochemical and sensory characteristics of sparkling pear cider (Perry). Eur Food Res Technol 249(2):341–352 - https://doi.org/10.1007/s00217-022-04119-3

This study was aimed to produce pear cider (Perry), using small caliber pears cv Abate Fètel, fermented by *Starmerella bacillaris* and *Saccharomyces cerevisiae* in co-inoculated (COF) and sequential (SEF) mixed cultures in comparison with *S. cerevisiae* monoculture fermentation (AXF), evaluating the infuence of yeast starter cultures on Perry characteristics. The perries were re-fermented in bottle by *S. cerevisiae* strain EC1118. During primary fermentation, growth and fermentation kinetics were diferent in the co-inoculated and sequential fermentations in comparison with pure *S. cerevisiae* fermentation; however, sugars were depleted, and 6% (v/v) ethanol was produced in all the trials. Glycerol content was significantly higher in mixed fermentations due to *Starm. bacillaris* metabolism (+20% in COF, and+42% in SEF conditions). After re-fermentation in bottle, higher levels of 3-Methyl-1-butanol, 1-propanol, acetaldehyde and esters were detected in Perry from the mixed fermentations. All the Perries were accepted by the consumers (general liking values from 6.01 to 6.26). Perries' appearance from mixed fermentations was described as less intense and more clear. The use of small caliber pears cv Abate Fètel and *Starm. bacillaris* in combination with *S. cerevisiae* in Perry production might be a suitable tool to obtain novel beverages with distinctive organoleptic features.

GMVV-VII Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100 Sassari, Italy. Communicated by Professor Marilena Budroni <mbudroni@uniss.it>.

1 Fancello F, Bianco A, Niccolai M, Zara G, Coronas R, Serra E, D'Hallewin G, Valentoni A, Santoru A, Pretti L, Budroni M. 2022. Fruit microbial communities of the bisucciu sardinian apricot cultivar (*Prunus armeniaca* L.) as a reservoir of new brewing starter strains. Fermentation (MDPI) 8:364 - https://doi.org/10.3390/fermentation8080364.

Local fruit cultivars may improve the originality of specialty beers both directly, by conferring peculiar tastes and flavors, and indirectly, as a reservoir of new starter strains. Accordingly, the fungal and bacterial communities of Bisucciu fruit, a Sardinian apricot cultivar used to produce a local fruit beer, were here investigated by culture-dependent methods. From the 16S rDNA and ITS sequence analyses of 68 epiphytic isolates, 5 bacterial species and 19 fungal species were identified. *Aureobasidium pullulans* and *Rhodotorula glutinis* were the dominant fungal species, while *Enterococcus mundtii* (Firmicutes) and *Frigoribacterium faeni* (Actinobacteria) were the most represented species among bacterial isolates. Enrichment cultures of fresh apricot puree, followed by fermentation trials in beer wort and beer wort added with apricot puree, allowed the selection of four isolates of *Pichia kudriavzevii*, *Hanseniaspora uvarum*, *H. pseudoguilliermondii*, and *H. clermontiae* able to dominate over the Bisucciu native microbiota and to produce from 0.57% to 0.74% (vol/vol) of ethanol. HS-SPMEGC/MS analysis highlighted a significant increase in the ester and alcohol fractions as well as a reduction in terpenes after fermentation with the selected yeasts. Results obtained suggest that the yeast isolates may contribute to the definition of the taste and flavor of beers when used in mixed fermentations with *Saccharomyces*.

2 Tkáčová J, Zara G, Ianiri G, Castoria R, Čertík M, Mannazzu I. 2022. Impairment of carotenoid biosynthesis through CAR1 gene mutation results in CoQ10, sterols, and phytoene accumulation in *Rhodotorula mucilaginosa*. Appl Microbiol Biotechnol 106(1):317-327 - doi: 10.1007/s00253-021-11673-5.

Red yeasts, mainly included in the genera *Rhodotorula*, *Rhodosporidiobolus*, and *Sporobolomyces*, are renowned biocatalysts for the production of a wide range of secondary metabolites of commercial interest, among which lipids, carotenoids, and other isoprenoids. The production of all these compounds is tightly interrelated as they share acetyl-CoA and the mevalonate pathway as common intermediates. Here, T-DNA insertional mutagenesis was applied to the wild type strain C2.5t1 of *Rhodotorula mucilaginosa* for the isolation of albino mutants with impaired carotenoids biosynthesis. The rationale behind this approach was that a blockage in carotenoid biosynthetic pathway could divert carbon flux toward the production of lipids and/or other molecules deriving from terpenoid precursors. One characterized albino mutant, namely, strain W4, carries a T-DNA insertion in the CAR1 gene coding for phytoene desaturase. When cultured in glycerol-containing medium, W4 strain showed significant decreases in cell density and fatty acids content in respect to the wild type strain. Conversely, it reached significantly higher productions of phytoene, CoQ10, and sterols. These were supported by an increased expression of CAR2 gene that codes for phytoene synthase/lycopene cyclase. Thus, in accordance with the starting hypothesis, the impairment of carotenoids biosynthesis can be explored to pursue the biotechnological exploitation of red yeasts for enhanced production of secondary metabolites with several commercial applications. KEY POINTS: • The production of lipids, carotenoids, and other isoprenoids is tightly interrelated. • CAR1 gene mutation results in the overproduction of phytoene, CoQ10, and sterols. • Albino mutants are promising tools for the production of secondary metabolites.

3 Portaro L, Maioli F, Canuti V, Picchi M, Lencioni L, Mannazzu I, Domizio P. 2022. *Schizosaccharomyces japonicus*/*Saccharomyces cerevisiae* mixed starter cultures: New perspectives for the improvement of Sangiovese aroma, taste, and color stability. LWT – Food Sci Technol 156:113009 - https://doi.org/10.1016/j.lwt.2021.113009.

Schizosaccharomyces japonicus and *Saccharomyces cerevisiae* were inoculated in Sangiovese grape must and the impact of simultaneous (co-inoculation) and sequential inoculation protocols on growth and fermentation kinetics, and on the analytical and sensory profiles of the experimental wines, was evaluated at the end of the alcoholic fermentation and after aging in bottle. While *Sch. japonicus* proved to affect *S. cerevisiae* growth and fermentative performances solely in sequential inoculation, the combined activity of the two yeasts always resulted in significantly higher total polysaccharides concentrations ($p < 0.05$), no matter the inoculation protocol utilized. Moreover, *Sch. japonicus* modulated the concentration of some volatile compounds when in mixed culture. In particular, ethyl acetate, that reached high levels at the end of alcoholic fermentation, showed a significant decrease after twenty-four months of bottle aging ($p < 0.05$), when the occurrence of hydrolysis/esterification reactions led to a significant increase of other acetate esters ($p < 0.05$). As a result, sensory analyses showed no significant differences for the ethyl acetate perception in mixed fermentation and control aged wines. Moreover, sequentially inoculated wines showed significantly higher sweetness compared to control wines, possibly due to the higher glycerol and polysaccharide content.

4 Budroni M, Torija M-J, Moreno-García J, Zara G. 2022. Editorial: Biofilm and Food: Well- and Lesser-Known Interactions. Frontiers in Microbiology 13:923021 - doi: 10.3389/fmicb.2022.923021.

GMVV-VIII Department of Agriculture, Food, Natural Resources and Engineering" (DAFNE) - Laboratory of Predictive Microbiology, University of Foggia, via Napoli 25, 71122 Foggia, Italy – Communicated by Professor Antonio Bevilacqua < antonio.bevilacqua@unifg.it>.

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1 Speranza B, Sinigaglia M, Corbo MR, D'Errico N, Bevilacqua A. 2022. A preliminary approach to define the microbiological profile of naturally fermented Peranzana Alta Daunia table olives. Foods (MDPI) 11(14):2100 - DOI: 10.3390/foods11142100.

Samples of brines from *Peranzana Alta Daunia* olives at the end of fermentation were analyzed; samples were taken in two different years from eight different locations (Torremaggiore, San Severo, San Paolo di Civitate, Lucera, Chieuti, Serracapriola, Gargano and Termoli in Southern Italy). Total aerobic count, enterobacteria, pseudomonads, staphylococci, lactic acid bacteria and yeasts (*Saccharomyces* and non-*Saccharomyces*) were assessed; moreover, presumptive lactobacilli were characterized in relation to their ability to grow with salt added, and at 10 and 45 °C. Yeasts were generally more abundant than lactic acid bacteria (LAB), but two clusters were found: one including the areas of Torremaggiore, San Severo, Apricena, Lucera and San Paolo di Civitate (area 1, A1), and another comprising Gargano, Termoli and Serracapriola (area 2, A2). Lactobacilli of A1 were more resistant to stress conditions (growth at 10% of salt and at 10 °C); moreover, A1 was characterized by a lower abundance of yeasts. In some areas (Lucera and San Severo), a higher abundance of non-*Saccharomyces* yeasts was found. This paper offers a first insight into the profile of *Peranzana Alta Daunia* olives at the end of fermentation, suggesting that some indices (technological traits of lactobacilli, ratio yeasts vs. LAB, abundance of non-*Saccharomyces* yeasts) could be useful to define a microbiological profile of the variety.

2 Petruzzi L, Campaniello D, Corbo MR, Speranza B, Altieri C, Sinigaglia M, Bevilacqua A. 2022. Wine microbiology and predictive microbiology: a short overview on application, and perspectives. Microorganisms (MDPI) 10(2):421 - doi: 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 Siesto G, Corbo MR, 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.

4 Muñoz R, Bevilacqua A, Pérez MS, Arévalo-Villena M. 2023. Influence of microwave treatments on viability and vitality of wine-origin microorganisms. Int J Food Sci Technol 58(7):3800–3809 - https://doi.org/10.1111/ijfs.16481

Microwave has been used in food industries to enhance or replace conventional techniques. In Enology, its potential use is not clear since references indicate that can be intended for both inactivation of microorganisms and maceration treatments. In this context, it is important to know the effect of microwaves on microorganisms. This study focuses on the application of microwave (MW) on *Saccharomyces* and non-*Saccharomyces* yeasts and *Lactiplantibacillus plantarum* (from oenological ecosystems) to evaluate their behaviour under different conditions of power, duration and presence of pulses on; viability and vitality were studied. MW affected microorganisms in different ways. In general, yeasts were less resistant than *L. plantarum*. Counts after treatments showed inactivation in some cases. Nevertheless, OD_{max} after 96 h presented values closer to the controls. This effect shows that in some cases the treatment exerts an antimicrobial effect after application, but after some time, microorganisms can restore their biological structures and growth, which is reflected in an increase in the lag phase. On a few occasions, treatments were able to improve the kinetic parameters. Thus, this technique could be useful for inactivation, attenuation or even activation of associated food microorganisms.

GMVV-IX Department of Agricultural and Food Sciences, Campus of Food Science, University of Bologna, p.zza Goidanich 60, 47521 Cesena, Italy. Communicated by Professor Francesca Patrignani <francesca.patrignani@unibo.it>.

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1 Gottardi D, Ciccone M, Siroli L, Lanciotti R, Patrignani F. 2022. Use of *Yarrowia lipolytica* to obtain fish waste functional hydrolysates rich in flavoring compounds Fermentation (MDPI) 8(12):708 - https://doi.org/10.3390/fermentation8120708

Fishery processing industries generate large amounts of by-products. These by-products come from fish heads, skin, bones, thorns, and viscera. The disposal of these wastes represents an increasing environmental and health problem. Nowadays, there is a growing interest in how to utilize fish materials that are not used for human consumption. Among the different solutions proposed, the use of proteolytic and lipolytic microorganisms represents a green solution for waste valorization. In this work, first we screened several conventional and non-conventional microorganisms for their proteolytic and lipolytic functions. Then, the most promising strains (*Yarrowia lipolytica* YL2, *Y. lipolytica* YL4, *Bacillus amyloliquefaciens* B5M and *B. subtilis* B5C) were tested on a fish waste-based solution. After 72 h incubation at room temperature, the supernatants obtained using the strains of *Y. lipolytica* showed the highest degree of hydrolysis (10.03 and 11.80%, respectively, for YL2 and YL4), the strongest antioxidant activity (86.4% in DPPH assay for YL2) and the highest formation of aldehydes (above 50% of the total volatile compounds detected). Hydrolysates of fish waste obtained with *Y. lipolytica* may be reused in feed and food formulations for their functional and flavoring characteristics.

2 Gottardi, D Siroli L, Braschi G, Rossi S, Serrazanetti D.I, Patrignani F, Lanciotti R. 2022. Sublethal HPH treatment is a sustainable tool that induces autolytic-like processes in the early gene expression of *Saccharomyces cerevisiae*. Food Res Int 159:111589 - doi: 10.1016/j.foodres.2022.111589.

Sublethal HPH treatments have been demonstrated to impact the technological properties and functions of treated microorganisms by inducing specific enzymes/genes or modulating membrane structures and inducing autolysis. In this work, the early effects of a 100 MPa HPH treatment on the winery starter *Saccharomyces cerevisiae* ALEAFERM AROM grown in synthetic must were assessed. While there were no differences in cell cultivability during the first 48 h between treated and untreated cells, a reduction in volatile metabolites released by HPH-treated cells during the first 2 h was observed. This reduction was only temporary since after 48 h, volatile molecules reached similar or even higher concentrations compared with the control. Moreover, the gene expression response of HPH-treated cells was evaluated after 1 h of incubation and compared with that of untreated cells. A massive rearrangement of gene expression was observed with the identification of 1220 differentially expressed genes (DEGs). Most of the genes related to energetic metabolic pathways and ribosome structure were downregulated, while genes involved in ribosome maturation, transcription, DNA repair, response to stimuli and stress were upregulated. These findings suggest that HPH induces or promotes an autolytic-like behaviour that can be exploited in winemaking.

3 Rossi S, Maares M, Kieserling H, Rohn S, Schlüter O, Patrignani F, Lanciotti R, Haase H, Keil C. 2023. Zinc tolerance of special yeasts and lactic acid bacteria for use in the food industry. Fermentation (MDPI) 9(6):521 - https://doi.org/10.3390/fermentation9060521

In order to address human zinc deficiency, fortifying staple foods with zinc is a safe and cost-effective solution. To ensure the nutritional properties and quality of a final product, zinc tolerance of the microorganisms involved in the fermentation is necessary. Bread, which is widely consumed, occupies a substantial place in many people's diets, and is often based on a sourdough making process; thus, it might be an important headlining product. This study investigated the zinc tolerance of yeasts and lactic acid bacteria that are specifically suited to produce sourdough bread made with cricket powder hydrolysate and wheat flour. Amongst the yeasts, *Kazachstania servazzii* KAZ2 and *Kazachstania unispora* FM2 were only slightly affected in regard to cell growth and colony-forming ability when cultured in YPD broth spiked with 0.5 or 1 mM $ZnSO_4$, respectively. *Yarrowia lipolytica* RO25 showed a higher tolerance for up to 2.5 mM zinc ($ZnSO_4$). All the yeast strains were capable of accumulating zinc in the range between 200 and 400 fg/cell. The heterofermentative lactic acid bacterium *Fructilactobacillus sanfranciscensis* DG1 appeared to have a moderate zinc requirement and was homeostatically balanced, even under a high 20 mM extracellular $ZnSO_4$ load. A better understanding of zinc homeostasis in yeast and lactic acid bacteria for food industry applications may lead to improvements in zinc fortification, which might contribute to diminishing Zn deficiencies, especially in vulnerable population groups.

4 Gottardi D, Siroli L, Braschi G, Rossi S, Bains N, Vannini L, Patrignani F, Lanciotti R. 2023. Selection of *Yarrowia lipolytica* strains as possible solution to valorize untreated cheese whey. Fermentation (MDPI) 9(1):51 - https://doi.org/10.3390/fermentation9010051

Cheese whey management and disposal is a major issue for dairy industries due to its high level of chemical and biochemical oxygen demand. However, it can still represent a source of nutrients (i.e, sugars, proteins and lipids) that can be applied, among other options, as substrate for microbial growth. *Yarrowia lipolytica* can grow in different environments, consuming both hydrophilic and hydrophobic substrates, and tolerates high salt concentrations. In this work, the lipolytic and proteolytic profile of 20 strains of *Y. lipolytica* were tested on caseins and butter. Then, their growth potential was evaluated in four types of whey (caciotta, ricotta, squacquerone and their mix). *Y. lipolytica* showed a very strain-dependent behavior for both hydrolytic profiles and growth capabilities on the different substrates. The best growers for all the types of whey tested were PO1, PO2, and RO2, with the first one reaching up to 8.77 log cfu/mL in caciotta whey after 72 h. The volatile molecule profile of the samples incubated with the best growers were characterized by higher amounts of esters, acids, ketones and alcohols. In this way, cheese whey can become a source of microbial cultures exploitable in the dairy sector.

GMVV-X Department of Pharmaceutical Sciences, University of Perugia, Via Borgo 20 Giugno 74, Perugia, Italy. Communicated by Professor Gianluigi Cardinali <gianluigi.cardinali@unipg.it>.

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1 Cattò C, Corte L, Roscini L, Cardinali G, Villa F, Cappitelli F. 2022. Metabolomic and proteomic changes in *Candida albicans* biofilm in response to zosteric acid treatment. Int J Mol Sci (MDPI) 23(22), 14067 - https://doi.org/10.3390/ijms232214067.

Zosteric acid (ZA) is a secondary metabolite of the seagrass *Zostera marina*, with antibiofilm activity against fungi. Information concerning its mechanisms of action is lacking and this limits the development of more potent derivatives based on the same target and activity structure. The aim of this work was to investigate the ZA mode of action by analyzing the metabolic status of *Candida albicans* biofilm and its protein expression profile upon ZA treatment. Fourier-Transform Infrared Spectroscopy confirmed that ZA modified the metabolomic response of treated cells, showing changes in the spectral regions, mainly related to the protein compartment. Nano Liquid Chromatography–High-Resolution Mass Spectrometry highlighted that 10 proteins were differentially expressed in the *C. albicans* proteome upon ZA treatment. Proteins involved in the biogenesis, structure and integrity of cell walls as well as adhesion and stable attachment of hyphae were found downregulated, whereas some proteins involved in the stress response were found overexpressed. Additionally, ZA was involved in the modulation of non-DNA-based epigenetic regulatory mechanisms triggered by reactive oxygen species. These results partially clarified the ZA mechanism of action against fungi and provided insight into the major *C. albicans* pathways responsible for biofilm formation.

2 Gupte AP, Pierantoni DC, Conti A, Donati L, Basaglia M, Casella S, Favaro L, Corte L, Cardinali G. 2023. Renewing lost genetic variability with a classical yeast genetics approach. J Fungi (MDPI) 9(2):264 - https://doi.org/10.3390/jof9020264

Due to their long domestication time course, many industrial *Saccharomyces cerevisiae* strains are adopted in numerous processes mostly for historical reasons instead of scientific and technological needs. As such, there is still significant room for improvement for industrial yeast strains relying on yeast biodiversity. This paper strives to regenerate biodiversity with the innovative application of classic genetic methods to already available yeast strains. Extensive sporulation was indeed applied to three different yeast strains, specifically selected for their different origins as well as backgrounds, with the aim of clarifying how new variability was generated. A novel and easy method to obtain mono-spore colonies was specifically developed, and, to reveal the extent of the generated variability, no selection after sporulation was introduced. The obtained progenies were then tested for their growth in defined mediums with high stressor levels. A considerable and strain-specific increase in both phenotypic and metabolomic variability was assessed, and a few mono-spore colonies were found to be of great interest for their future exploitation in selected industrial processes.

3 Conti A, Pierantoni DC, Robert V, Corte L, Cardinali G. 2023. MinION sequencing of yeast mock communities to assess the effect of databases and ITS-LSU markers on the reliability of metabarcoding analysis. Microbiol Spectrum 11(1) - https://doi.org/10.1128/spectrum.01052-22

Microbial communities play key roles both for humans and the environment. They are involved in ecosystem functions, maintaining their stability, and provide important services, such as carbon cycle and nitrogen cycle. Acting both as symbionts and as pathogens, description of the structure and composition of these communities is important. Metabarcoding uses ribosomal DNA (rDNA) (eukaryotic) or rRNA gene (prokaryotic) sequences for identification of species present in a site and measuring their abundance. This procedure requires several technical steps that could be source of bias producing a distorted view of the real community composition. In this work, we took advantage of an innovative "long-read" next-generation sequencing (NGS) technology (MinION) amplifying the DNA spanning from the internal transcribed spacer (ITS) to large subunit (LSU) that can be read simultaneously in this platform, providing more information than "short-read" systems. The experimental system consisted of six fungal mock communities composed of species present at various relative amounts to mimic natural situations characterized by predominant and low-frequency species. The influence of the sequencing platform (MinION and Illumina MiSeq) and the effect of different reference databases and marker sequences on metagenomic identification of species were evaluated. The results showed that the ITS-based database provided more accurate species identification than LSU. Furthermore, a procedure based on a preliminary identification with standard reference databases followed by the production of custom databases, including only the best outputs of the first step, is proposed. This additional step improved the estimate of species proportion of the mock communities and reduced the number of ghost species not really present in the simulated communities.

GMVV-XI Department of Agricultural Food and Forest Science, University of Palermo, Viale delle SCienze 4, 90128 Palermo, Italy. Communicated by Professor Nicola Francesca <nicola.francesca@unipa.it francesca>.

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1 Pirrone A, Prestianni R, Naselli V, Todaro A, Farina V, Tinebra I, Guzzon R, Badalamenti N, Maggio A, Gaglio R, Settanni L, Bruno M, Moschetti G, Alfonzo A, Francesca N. 2022. Influence of indigenous *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* from sugar-rich substrates on the aromatic composition of loquat beer. Int J Food Microbiol 379:109868 - https://doi.org/10.1016/j.ijfoodmicro.2022.109868

The demand for unique and exclusive food products and beverages is constantly on the increase. One of the products that mostly evolved to encounter market dynamics in the last decade is craft beer. For a long time, craft breweries have included fruit in beer production to enrich flavour and aroma profile of different beer styles. In this study, for the first time, the use of *Saccharomyces* and non-*Saccharomyces* yeast strains isolated from high sugar matrices (manna and fermented honey by-products) were investigated to diversify fruit craft beer production, in order to improve the fermentation process and highlight the complexity of aroma profiles generated during alcoholic fermentation. Two yeast strains, *Hanseniaspora uvarum* YGA34 and *Saccharomyces cerevisiae* MN113, were tested as co-starters and starters for their beer production capacity. Commercial yeast strain US-05 was used as control. Loquat juice was added at the end of primary alcoholic fermentation in all trials. Interestingly, *S. cerevisiae* MN113 consumed sugars faster than control strain *S. cerevisiae* US-05, including maltose, even in the case of sequential inoculation. This strain showed an excellent ability to consume rapidly sugars present. All strains showed their concentrations ranged between 5 and 8 Log cycles during fermentation. The absence of off-odours and the improvement of aromatic perception were observed in experimental trials involving the use of *S. cerevisiae* MN113 as a monoculture and in sequential combination with *H. uvarum* YGA34. Esters and alcohols were the most abundant compounds emitted from the beers. The beers produced with sequential inoculation of *H. uvarum* YGA34 and *S. cerevisiae* MN113 or US-05 are characterised by a higher ester and lower alcohol concentration. These two unconventional yeast strains from high sugar matrices showed great technological properties, representing promising co-starters and starter during craft fruit beer production.

2 Prestianni R, Matraxia M, Naselli V, Pirrone A, Badalamenti N, Ingrassia M, Gaglio R, Settanni L, Columba P, Maggio A, Bruno M, Francesca N, Moschetti G, Alfonzo A. 2022. Use of sequentially inoculation of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* strains isolated from honey by-products to improve and stabilize the quality of mead produced in Sicily. Food Microbiol 107:104064 - https://doi.org/10.1016/j.fm.2022.104064

Mead is a beverage produced by alcoholic fermentation of honey-must. The starter yeasts that are commonly used for the alcoholic fermentation of honey-must are oenological *Saccharomyces cerevisiae* strains. The objective of the present work was, for the first time, to apply yeasts of honey by-products origin to evaluate the influences the taste-olfactory attributes of mead. For this purpose, three experimental productions were set up, which included: (i) single inoculation of *S. cerevisiae*; (ii) single inoculation of *Hanseniaspora uvarum*; (iii) sequential inoculation of *H. uvarum/S. cerevisiae*. Two control trials were performed, using a commercial strain of *S. cerevisiae* of oenological origin and a spontaneous fermentation. The results of the chemical parameters showed differences between the trials in terms of residual sugars, acetic acid, glycerol, ethanol and volatile organic compounds. Sensorial analysis also showed a high heterogeneity among trials. The attributes of sweetness, honey and floral were found in mead fermented with *H. uvarum*, whereas all meads obtained with *S. cerevisiae* were dry, balanced and without off-odors and off-flavours. The results obtained showed that the controlled application of conventional and non-conventional yeast strains isolated from honey by-products origin could be a promising approach to improve the quality of meads.

3 Francesca N, Pirrone A, Gugino I, Prestianni R, Naselli V, Settanni L, Todaro A, Guzzon R, Maggio A, Porrello A, Bruno M, Farina V, Passafiume R, Alfonzo A, Moschetti G, Gaglio R. 2023. A novel microbiological approach to impact the aromatic composition of sour loquat beer. Food Bioscience 55:103011 - https://doi.org/10.1016/j.fbio.2023.103011

The growing interest in novel beer development determined the exploitation of unconventional yeasts isolated from novel ecological niches to generate unexplored sensory profiles. In recent years, there is an increasing interest in generating beers brewed with the addition of fruits. For the first time, *Lachancea thermotolerans* MNF105 and *Saccharomyces cerevisiae* MN113 isolated from manna, were tested as starter cultures to process loquat beer to improve the sensory profile. Innovatively, the yeast species *L. thermotolerans* was investigated for the production of sour fruit beer. Sour fruit beers produced with *L. thermotolerans* MNF105 were more balanced than the respective control, especially in terms of perceived acidity during sensory analysis. This could be due to the lower lactic acid production (0.49 g/L) compared to the respective control (1.74 g/L). The overall organoleptic investigation showed a preference for *S. cerevisiae* MN113 (TF1) isolated from manna. Experimental trials conducted with the selected strains demonstrated the absence of off-odour and off-flavour and improved aroma perception. Aldehydes and alcohols were the most abundant compounds emitted from the beers. *S. cerevisiae* MN113 and *L. thermotolerans* MNF105, manna related yeasts, showed great technological properties, representing promising starters for the production of fruit beer and sour fruit beer.

4 Naselli V, Prestianni R, Badalamenti N, Matraxia M, Maggio A, Alfonzo A, Gaglio R, Vagnoli P, Settanni L, Bruno M, Moschetti G, Francesca N. 2023. Improving the aromatic profiles of Catarratto wines: impact of *Metschnikowia pulcherrima* and glutathione-rich inactivated yeasts. Antioxidants (MDPI) 12:439 - https://doi.org/10.3390/antiox12020439

Catarratto is one of the most widely cultivated grape varieties in Sicily. It is an indigenous non-aromatic white grape variety. Despite its widespread use in winemaking, knowledge of the aroma and chemical and microbiological properties of Catarratto wines is quite limited. The influence of *Metschnikowia pulcherrima* combined with *Saccharomyces cerevisiae* on the aromatic expression of Catarratto wines was investigated with and without the addition of glutathione-rich inactivated yeast. The substance is a natural specific inactivated yeast with a guaranteed glutathione level used to limit oxidative processes. The aromatic profiles of the final wines were determined through analysis of the volatile organic compounds using a solid-phase microextraction technique that identified 26 aromatic compounds. The addition of *M. pulcherrima* in combination with the natural antioxidant undoubtedly increased the aromatic complexity of the wines. Dodecanal was exclusively detected in the wines processed with glutathione-rich inactivated yeasts. Furthermore, the presence of this natural antioxidant increased the concentration of six esters above the perception threshold. Sensory analysis was also performed with a panel of trained judges who confirmed the aromatic differences among the wines. These results suggest the suitability of glutathione-rich inactivated yeasts for determining the oxidative stability of Catarratto wines, thus preserving its aromatic compounds and colour.

GMVV-XI CREA - Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Conegliano (TV), Italy. Communicated by Dr. Tiziana Nardi <tiziana.nardi@crea.gov.it> https://orcid.org/0000-0003-2148-3112 http://www.crea.gov.it/.

1 Giovenzana V, Beghi R, Guidetti R, Luison M, Nardi T. 2023. Evaluation of energy savings in white winemaking: impact of temperature management combined with specific yeasts choice on required heat dissipation during industrial-scale fermentation. J Agric Engin 54(3) - doi: 10.4081/jae.2023.1523.

Heat removal significantly impacts energy requests in the winery and is related to the temperature control of wine tanks during the fermentation and wine maturation phases. This work aimed to determine the heat required to be dissipated from wine tanks under different temperature programmes to evaluate the potential effects on energy saving during industrial-scale fermentations of Glera and Pinot Grigio wines. Comparative tests were carried out by using properly chosen yeast strains during fermentation at the usual winery temperature (15°C or 17-15°C) and 19°C and verifying the quality of the resulting wines regarding sensory, chemical, and aromatic features. Fermentation required, on average, 7.0 Wh dm-3 must be at 19°C, and 10.3 Wh dm-3 must be at 15/17-15°C, reducing energy use by ~32% at the higher temperature. The tested fermentation protocols, coupled with the use of some specifically selected yeast strains, have positive energy saving effects without compromising the resulting wine's sensory, chemical, and aromatic profiles. This work suggests how wineries can adopt a more sustainable winemaking process with low energy consumption and consequently propose eco-labelling strategies and price-premium policies.

GMVV-XII Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Naples Federico II, Viale Italia, 83100 Avellino, Italy. Communicated by Professor Giuseppe Blaiotta <blaiotta@unina.it>.

1 Aponte M, Esposito F, Sequino G, Blaiotta G, De Filippis F. 2022. Stuck or sluggish fermentations in home-made beers: Beyond the surface. Int J Food Microbiol 383:109956 - https://doi.org/10.1016/j.ijfoodmicro.2022.109956.

In the last several years, the popularity of homebrewed beers has skyrocketed. However, this type of product is extremely vulnerable to microbial deterioration. Twelve homemade beers, some characterized by defects or stuck fermentation, were analysed by using a polyphasic approach encompassing culturomics and culture-independent techniques to better understand mechanisms that drive microbiota evolution throughout production and to highlight determinants responsible for crowning with success. Two sour beers, one apple-flavoured ale, two Italian grape ales, and seven standard ales were sampled. Microbiological characterization was obtained by plating on nine different media coupled with High-throughput sequencing analysis of fungal and bacterial communities by targeting ITS1–2 and the V3–V4 regions of the 16S rRNA, respectively. Total microflora on PCA largely varied among samples, ranging from <10^2 CFU/mL up to around 10^7 CFU/mL often reflecting yeast counts on WL and LM. LAB population's levels on MRS and SDBm did not overlap, with the counts on the latter being even 5 Log CFU/mL greater. Acetic Acid bacteria were retrieved in Sour beers, as well as in one IGA, even though acetic acid was not detectable by HPLC in this last sample. *Brettanomyces* spp. were only found in sour beers, as expected, whereas *Enterobacteriaceae* were never counted. A total of 63 yeasts were randomly isolated from countable plates. *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* were the most frequently isolated species. In many cases, Interdelta analysis biotyping of S. cerevisiae isolates consistently allowed the detection of the starter strain. By HST *S. cerevisiae* dominated the mycobiota in four samples, even if in one of them residual maltose and ethanol contents suggested a stuck fermentation. W. anomalus was found to be the dominant species in two beers. Fifty-five LAB cultures were isolated and identified. *Pediococcus damnosus* was the only species retrieved in sour beers and two Ales, while *Levilactobacillus* brevis was found in two Ale samples. HTS did not confirm this result in one Ale sample since the genus Panotea spp. accounted for over 90 % of the microbiota. Enterobacteriaceae which were never counted dominated the microbiome of two Ale beers. Biogenic amines content largely varied with three Ale samples greatly contaminated. Based on chemical and microbiological outcomes only one beer ASAle out of 12 could be considered acceptable. Furthermore, the widespread presence of LAB by culturomics and Enterobacteriaceae by HTS raises concerns about the final products' safety.

2 Caridi A, Sidari R, Pulvirenti A, Blaiotta G, Ritieni A. 2022. Clonal selection of wine yeasts with differential adsorption activities towards phenolics and ochratoxin A. Food Biotechnol 36(1):22–37 - doi.org/10.1080/08905436.2021.2006064

To design a rapid, simple, and low-cost procedure for yeast selection with differential adsorption activities toward phenolics and ochratoxin A, 284 yeast strains were screened. This was done by evaluating the type of growth during grape must fermentation, acetic acid production on Chalk agar, H_2S production on BiGGY agar, and spore-formation on acetate agar. After that initial step, 84 strains were pre-selected and further studied by Petri plate tests and to determine their wine-making ability in trials and evaluating their differential adsorption activities toward phenolics and ochratoxin A. Three yeast strains were selected based on the above evaluations. After confirming that they belonged to *Saccharomyces cerevisiae* species and were diploids, a spore clonal selection was performed. The strain Sc1741A_1D was selected and used in winemaking at six Calabrian wineries and found to be suitable as wine starter to improve quality and safety of red wines.

3 Petruzziello E, Blaiotta G, Pittari E, Piombino P, Aponte M. 2023. Isolation and characterization of cryotolerant yeasts from fiano di avellino grapes fermented at low temperatures. Foods (MDPI) 12(3):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.

GMVV-XII Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy. Communicated by Professor Viviana Corich <viviana.corich@unipd.it>.

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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) 8:252 - 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.

2 Nadai C, da Silva Duarte V, Sica J, Vincenzi S, Carlot M, Giacomini A, Corich V. 2023. *Starmerella bacillaris* released in vineyards at different concentrations influences wine glycerol content depending on the vinification protocols. Foods (MDPI) 12(1):3 - DOI: 10.3390/foods12010003.

Starmerella bacillaris is a non-*Saccharomyces* yeast proposed for must fermentation together with *Saccharomyces cerevisiae* because of its high glycerol and moderate volatile acidity production. Furthermore, it was demonstrated that the same *S. bacillaris* strains that possess interesting technological properties exhibited antifungal activity against *Botrytis cinerea*, suggesting the release of this yeast in the vineyard. To obtain a positive effect during the following winemaking process, the maintenance of suitable concentrations of *S. bacillaris* is essential. Therefore, to obtain information on the survival of *S. bacillaris*, a small-scale field trial was performed. One week before the harvest, two different concentrations of *S. bacillaris* (10^6 and 10^7 cells/mL) were sprayed on Pinot grigio bunches and the strain concentration was monitored by means of qPCR during the subsequent fermentation process. In addition, the combined effect of different winemaking techniques was evaluated, i.e, the vinification of juice, juice with marc and cryomaceration treatment. Results demonstrated that, under the tested conditions, *S. bacillaris* released in the vineyard remained viable for one week on grape bunches and increased glycerol content during the subsequent fermentation process. Different vinification protocols influenced cell concentrations. In particular, the cryomaceration treatment, due to the use of low temperature, supported *S. bacillaris* growth due to its cryotolerant aptitude. The collected data open new perspectives on the control of alcoholic fermentation, involving both vineyard and cellar management.

GMVV-XIII Department of Biotechnology & VUCC-DBT Verona University Culture Collection, University of Verona, Strada Le Grazie 15, Ca' Vignal 2, 37134, Verona, VR, Italy. Communicated by Professor Giovanna Felis <giovanna.felis@univr.it>.

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1 Binati RL, Maule M, Luzzini G, Martelli F, Felis GE, Ugliano M, Torriani S. 2023. From bioprotective effects to diversification of wine aroma: Expanding the knowledge on *Metschnikowia pulcherrima* oenological potential. Food Res Int 174:113550 - https://doi.org/10.1016/j.foodres.2023.113550

Microbial diseases are of major concern in vitiviniculture as they cause grape losses and wine alterations, but the prevention with chemical substances represents a risk to human health and agricultural ecosystem. A promising alternative is the biocontrol and bioprotection activity of non-Saccharomyces yeasts, such as *Metschnikowia pulcherrima*, which also presents positive oenological traits when used in multistarter fermentations. The aim of this study was to assess the impact of a selected *M. pulcherrima* strain in the post-harvest withering and vinification of Garganega grapes to produce the sweet 'passito' wine Recioto di Gambellara DOCG (Italy). *M. pulcherrima* was firstly inoculated on grape at the beginning of the withering process, and afterwards in must for multistarter sequential microfermentation trials with *Saccharomyces cerevisiae*. Microbiological, chemical, and sensory analyses were carried out to monitor the vinification of treated and control grapes. Grape bunches during withering were a suitable environment for the colonization by *M. pulcherrima*, which effectively prevented growth of molds. Differences in grape must composition were observed, and the diverse inoculation strategies caused noticeable variations of fermentation kinetics, main oenological parameters, wine aroma profile, and sensory perception. *M. pulcherrima* proved effective to protect grapes against fungal infections during withering and contribute to alcoholic fermentation generating wine with distinguished aromatic characteristics.

2 Binati RL, Ferremi Leali N, Avesani M, Salvetti E, Felis GE, Monti F, Torriani S. 2023. Application of FTIR microspectroscopy in oenology: shedding light on cell wall composition of *Saccharomyces cerevisiae* strains. Food Bioprocess Technol - https://doi.org/10.1007/s11947-023-03218-7.

The evaluation of cell parietal components of yeasts is an important criterium for the selection of wine starters since they play a key role in the vinification process. The aim of this study was to characterize and compare the cell wall composition of four industrial (BM45, D47, EC1118, K1) and three native *Saccharomyces cerevisiae* (MY8, MY11, PEDRO2000E) wine strains by means of scanning and transmission electron microscopy and ATR-FTIR microspectroscopy. A statistically significant variability in the cell wall thickness and cell diameter was observed among the yeast cells, with native strains showing higher cell diameter values. FTIR microspectroscopy applied on the intact cells without any previous invasive treatment and on the separated cell walls highlighted profound differences among the strains in terms of the overall content of parietal polysaccharides as related to the thickness of the cell walls and in terms of the relative concentration of β-glucans and mannans in the cell walls. The strains EC1118, MY11, and PEDRO2000E showed a higher overall content of β-glucans and mannans, whose lower relative concentration in PEDRO2000E was compensated by a thicker cell wall; BM45 and D47 were characterized by a high relative concentration of polysaccharides in a thinner wall, while K1 and MY8 displayed a low relative concentration of polysaccharides. ATR-FTIR microspectroscopy allows identifying polysaccharide-rich yeast strains and can become a smart option for the selection of starter cultures to be used in oenology and for other applications in food industry, thanks to the interesting technological properties of parietal polysaccharides.

3 Troiano E, Larini I, Binati RL, Gatto V, Torriani S, Buzzini P, Turchetti B, Salvetti E, Felis GE. 2023. Finding a correct species assignment for a *Metschnikowia* strain: Insights from the genome sequencing of strain DBT012. FEMS Yeast Res 23:foad024 - https://doi.org/10.1093/femsyr/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 protechnological 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 nonsequenced 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*.

GMVV-XIV CNR-ISPA (National Resaearch Council - Institute of Sciences of Food Production, via Prov.le, Lecce-Monteroni, 73100 Lecce, Italy. Communicated by Dr. Francesco Grieco <francesco.grieco@ispa.cnr.it> www.ispacnr.it.

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1 Palombi L, Tufariello M, Durante M, Fiore A, Baiano A, Grieco F. 2023. Assessment of the impact of unmalted cereals, hops, and yeast strains on volatolomic and olfactory profiles of Blanche craft beers: A chemometric approach. Food Chem 416:135783 - https://doi.org/10.1016/j.foodchem.2023.135783

This study investigated the impact of changes in craft beer formulation, by modifying the unmalted cereal [(durum (Da) and soft (Ri) wheat), emmer (Em)], hops [Cascade (Ca) and Columbus (Co)], and yeast strains [M21 (Wi) - M02 (Ci)], on volatolomic, acidic, and olfactory profiles. Olfactory attributes were evaluated by the trained panel. Volatolomic and acidic profiles were determined by GC–MS. The sensory analysis detected significant differences for 5 attributes, including olfactory intensity and finesse, malty, herbaceous, and floral notes. Multivariate analysis of volatiles data, showed significant differences among the samples ($p < 0.05$). DaCaWi, DaCoWi, and RiCoCi beers differ from the others by their higher concentrations of esters, alcohols, and terpenes. A PLSC analysis was carried out between volatiles and odour attributes. As far as we know, this is the first investigation that shed light on the impact of 3-factors interaction on the sensory-volatolomic profile of craft beers, through a comprehensive multivariate approach.

2 Baiano A, Fiore A, la Gatta B, Tufariello M, Gerardi C, Savino M, Grieco F. 2023. Single and interactive effects of unmalted cereals, hops, and yeasts on quality of white-inspired craft beers. Beverages (MDPI) 9(1):9 - https://doi.org/10.3390/beverages9010009

White beers owe their name to their straw yellow colour deriving from the use of unmalted wheat, which also supplies a relatively high protein content causing haze formation. This study aimed to develop white-inspired craft beers made with combinations of three mixtures of barley malt/unmalted wheat (alternatively durum-var. Dauno III, soft-var. Risciola, or emmer-var. Padre Pio), two hop varieties (Cascade or Columbus), and two *Saccharomyces cerevisiae* strains (Belgian yeast and a high-ester producing yeast); and assess the single and interactive effects of these ingredients on physical, chemical, and sensory characteristics of the beers. According to the graphical representation of the results for the Principal Component Analysis, most of the samples appear overlapped since they had similar characteristics, but it was possible to highlight two clusters of beers different from the others: those produced with (a) Risciola wheat and Columbus hop and (b) Dauno III wheat, Cascade hop, and the Belgian yeast. The beers of these clusters obtained the highest scores for their overall quality that, in turn, was positively correlated with concentrations of citric acid, 4-hydroxybenzoic acid, syringic acid, and epicatechin; alcohol %, colour, amount and persistence of foam, intensity of fruity flavour, and body.

3 Tufariello M, Palombi L, Rizzuti A, Musio B, Capozzi V, Gallo V, Mastrorilli P, Grieco F. 2023. 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. A correlation analysis was performed on 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.

GMVV-XIV Department of Life and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche, 60131 Ancona, Italy. Communicated by Professor Maurizio Ciani <mciani@univpm.it>.

1 Canonico L, Agarbati A, Galli E, Comitini F, Ciani M. 2023. Biocontrol using *Torulaspora delbrueckii* in sequential fermentation: new insights into low-sulfite verdicchio wines. Foods (MDPI) 12(15):2899 - https://doi.org/10.3390/foods12152899

Torulaspora delbrueckii has attracted renewed interest in recent years, for its biotechnological potential linked to its ability to enhance the flavor and aroma complexity of wine. Sequential fermentations with a selected native strain of *T. delbrueckii* (DiSVA 130) and low-sulfite native strain of *Saccharomyces cerevisiae* (DiSVA 709) were carried out to establish their contribution in biocontrol and the aroma profile. A first set of trials were conducted to evaluate the effect of the sulfur dioxide addition on pure and *T. debrueckii/S. cerevisiae* sequential fermentations. A second set of sequential fermentations without SO_2 addition were conducted to evaluate the biocontrol and aromatic effectiveness of *T. delbrueckii*. Native *T. delbrueckii* showed a biocontrol action in the first two days of fermentation (wild yeasts reduced by c.a. 1 log at the second day). Finally, trials with the combination of both native and commercial *T. delbrueckii/S. cerevisiae* led to distinctive aromatic profiles of wines, with a significant enhancement in isoamyl acetate, phenyl ethyl acetate, supported by positive appreciations from the tasters, for ripe and tropical fruits, citrus, and balance. The whole results indicate that native *T. delbrueckii* could be a potential biocontrol tool against wild yeasts in the first phase of fermentation, contributing to improving the final wine aroma.

2 Comitini F, Canonico L, Agarbati A, Ciani M. 2023. Biocontrol and probiotic function of non-*Saccharomyces* yeasts: new insights in agri-food industry. Microorganisms (MDPI) 11:1450 - https://doi.org/10.3390/microorganisms11061450

Fermented food matrices, including beverages, can be defined as the result of the activity of complex microbial ecosystems where different microorganisms interact according to different biotic and abiotic factors. Certainly, in industrial production, the technological processes aim to control the fermentation to place safe foods on the market. Therefore, if food safety is the essential prerogative, consumers are increasingly oriented towards a healthy and conscious diet driving the production and consequently the applied research towards natural processes. In this regard, the aim to guarantee the safety, quality and diversity of products should be reached limiting or avoiding the addition of antimicrobials or synthetic additives using the biological approach. In this paper, the recent re-evaluation of non-*Saccharomyces* yeasts (NSYs) has been reviewed in terms of bio-protectant and biocontrol activity with a particular focus on their antimicrobial power using different application modalities including biopackaging, probiotic features and promoting functional aspects. In this review, the authors underline the contribution of NSYs in the food production chain and their role in the technological and fermentative features for their practical and useful use as a biocontrol agent in food preparations.

3 Agarbati A, Canonico L, Ciani M, Comitini F. 2023. *Metschnikowia pulcherrima* in cold clarification: biocontrol activity and aroma enhancement in Verdicchio wine. Fermentation (MDPI) 9:302 - https://doi.org/10.3390/fermentation9030302

Non-*Saccharomyces* wine yeasts are not only proposed to improve the sensory profile of wine but also for several distinctive promising features. Among them, biocontrol action at different steps of the wine production chain could be a suitable strategy to reduce the use of sulfur dioxide. In this work, the activity of a selected strain of *Metschnikowia pulcherrima* was evaluated as inoculum in cold clarification with the aim to reduce SO_2 and improve the aromatic profile of the wine. Fermentation processes were carried out at the winery level for two consecutive vintages using a *pied de cuve* as the starter inoculum coming from indigenous *Saccharomyces cerevisiae* strains. *M. pulcherrima* revealed an effective bio-protectant action during the pre-fermentative stage even if the timely and appropriate starter inoculum in the two years permitted the effective control of wild yeasts during the fermentation also in the control trials. In general, the main oenological characters did not show differences if compared with an un-inoculated trial, while the inoculum of *M. pucherrima* in cold clarification determined an enhancement of ethyl hexanoate, isobutanol, acetaldehyde, and geraniol even if they are considered in different amounts for each year. Indeed, the analytical and sensory profiles of wines were also influenced by the vintage and variation *pied the cuve* population. Nonetheless, the overall results indicated that *M. pulcherrima* led to biocontrol action and an improvement of the aromatic and sensory profile of the wine.

4 Agarbati A, Ciani M, Esin S, Agnolucci M, Marcheggiani F, Tiano L, Comitini F. 2023. Comparative zymocidial effect of three different killer toxins against *Brettanomyces bruxellensis* spoilage yeasts. Int J Mol Sci (MDPI) 24:1309 - https://doi.org/10.3390/ijms24021309

Three killer toxins that were previously investigated, one excreted by *Kluyveromyces wickerhamii* and two by different strains of *Wickerhamomyces anomalus*, were produced at the pilot scale, lyophilized and characterized, and the formulates were assessed for their zymocidial effect against *Brettanomyces bruxellensis* spoilage yeast. A comparative analysis allowed the evaluation of the minimum inhibitory concentration (MIC) against a sensitive strain. Fungicidal and fungistatic concentrations were used to evaluate the cytocidal effect using a cytofluorimetric approach that confirmed the lethal effect of all lyophilized formulates against *B. bruxellensis* spoilage yeasts. Moreover, the potential killer toxins' cytotoxicity against human intestinal cells (Caco-2) were evaluated to exclude any possible negative effect on the consumers. Finally, the effective effect lethal of all three lyophilized killer toxins toward *B. bruxellensis* sensitive strain were tested. The results indicated that all of them acted without dangerous effects on the human epithelial cells, opening the way for their possible commercial application. In particular, D15 showed the lowest MIC and the highest activity, was evaluated also in wine, revealing a strong reduction of *Brettamonyces* yeast growth and, at the same time, a control of ethyl phenols production.

GMVV-XI Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio 2/a, 33100 Udine, Italy. Communicated by Professor Giuseppe Comi <giuseppe.comi@uniud.it>.

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1 Voce S, Iacumin L, Comuzzo P. 2022. Characterization of non-*Saccharomyces* yeast strains isolated from grape juice and pomace: production of polysaccharides and antioxidant molecules after growth and autolysis. Fermentation (MDPI) 8:450 - https://doi.org/10.3390/fermentation8090450

Non-*Saccharomyces* yeasts (NSY) represent a relevant part of must and wine microbiota, contributing remarkably to the composition of lees biomass. Despite a number of studies indicate their capacity to increase wine polysaccharide content, their contribution to wine quality during aging on lees (AOL) has not been well elucidated yet. In the present study, twenty yeast strains (13 non-*Saccharomyces* and 7 *Saccharomyces*) were isolated from grape must and pomace and identified by morphologic and genetic characterization. Biomass production, cell growth and the release of soluble molecules (polysaccharides, amino acids, thiol compounds and glutathione) were evaluated after growth and after autolysis induced by β-glucanases addition. Differences between strains were observed for all parameters. Strains that produced higher amounts of soluble compounds during growth also showed the highest release after autolysis. *Hanseniaspora* spp. showed the greatest production of polysaccharides and antioxidant molecules, and biomass production and cell viability comparable to the commercial *S. cerevisiae* and *T. delbrueckii* used as reference. The aptitude of certain NSY to release antioxidants and polysaccharides is an interesting feature for managing AOL through sequential or mixed fermentations or for the production of inactive autolyzed yeasts for winemaking.

2 Iacumin L, Colautti A, Comi G. 2022. *Zygosaccharomyces rouxii* is the predominant species responsible for the spoilage of the mix base for ice cream and ethanol is the best inhibitor tested. Food Microbiol 102:103929 - https://doi.org/10.1016/j.fm.2021.103929

A mix base for ice cream (MBIC) is used to produce artisanal or industrial ice creams and desserts and consists of a mixture of different ingredients, including sugar, egg yolk, natural flavors, starch and milk proteins. MBICs, which have chemical-physical characteristics that include a pH of 5.61 and an activity water (A_w) less than or equal to 0.822, are packaged in tin boxes and stored at ambient temperature. Despite the low A_w, MBIC can support osmotolerant and osmophilic yeast growth. The aim of our work was to study the behavior of *Zygosaccharomyces rouxii*, the main microorganisms responsible of MBIC spoilage, either in the vivo or in a model system in order to inhibit its growth by the selection of antimicrobial agents. Different osmotolerant yeasts belonging to the genus Zygosaccharomyces were isolated and identified from spoiled and unspoiled lots of MBICs. In particular, Z. rouxii was the predominant species responsible for the spoilage, which depended on the high temperature of storage (>20 °C) and was highlighted by the presence of alcohol, esters, acids and gas (CO_2), which blew open the tin boxes. To stop spoilage, different antimicrobial compounds were tested: sulfur dioxide, sorbic and benzoic acids and ethanol. However, only 2% v/v ethanol was required to achieve the total inhibition of the Z. rouxii cocktails tested in this work. The use of other antimicrobials cannot be recommended because they were not able to stop yeast spoilage and changed the color and flavor of the products. Conversely, the use of ethanol is suggested because of its extreme effectiveness against osmotolerant yeasts, and the added amount was less than or equal to the taste threshold limit. The MBICs, treated with ethanol, were stable till the end of their shelf-life (6 months).

3 Colautti A, Civilini M, Contin M, Celotti E, Iacumin L. 2023. Organic vs. conventional: impact of cultivation treatments on the soil microbiota in the vineyard. Front Microbiol 14:1242267 - doi: 10.3389/fmicb.2023.1242267.

The aim of this study was to compare the effects of two vineyard management practices on the soil and its associated microbiota. The experiments were conducted in two adjacent plots, one completely organically managed and the other conventionally managed in terms of phytosanitary treatments but fertilized with organic amendments. The chemical soil analyses were correlated to the prokaryotic and fungal communities, which were studied using the metabarcoding technique. The main difference between the two treatments was a significantly higher amount of Cu in the organic managed vineyard soil, while conventional managed soil presented higher concentration of Na and Mg and was also associated with higher pH values. Despite these differences, no significant diversities were observed on soil biodiversity and microbial composition considering alpha and beta diversity metrics. However, the percentages of some phyla analyzed individually differed significantly between the two managements. Analyzing the metabolisms of these phyla, it was discovered an increment of species correlated to soils with higher organic matter content or land not used for agricultural purposes in the organic treated soil. The findings indicate that, despite the use of copper-based phytosanitary products, there was no degradation and loss of biodiversity in the organic soil microbial population compared to conventional management with the same type of fertilization, and the observed microbial population was more similar to that of natural soils.

GMVV-XVI National Research Council of Italy - Institute of Sciences of Food Production (ISPA), c/o CS-DAT, via Protano, 71121 Foggia, Italy. Communicated by Dr. Vittorio Capozzi <vittorio.capozzi@ispa.cnr.it>.

1 Morata A, Arroyo T, Bañuelos MA, Blanco P, Briones A, Cantoral JM, Castrillo D, Cordero-Bueso G, Del Fresno J.M, Escott C, Escribano-Viana R, Fernández-González M, Ferrer S, García M, González C, Gutiérrez AR, Loira I, Malfeito-Ferreira M, Martínez A, Pardo I, Ramírez M, Ruiz-Muñoz M, Santamaría P, Suárez-Lepe JA, Vilela A, Capozzi V. 2022. Wine yeast selection in the Iberian Peninsula: *Saccharomyces* and non-*Saccharomyces* as drivers of innovation in Spanish and Portuguese wine industries. Crit Rev Food Sci Nutrition 1–29 - https://doi.org/10.1080/10408398.2022.2083574

There is a profound connection between the geography of wine production and the selection of microorganisms of protechnological interest in wine. In this study, *Saccharomyces* and non-*Saccharomyces* selections associated with Spanish and Portuguese wine productions are used as models to explore the biotechnological potential connected to the wine industries of different nations. The review refers to an effort to isolate, identify and evaluate thousands of yeast strains from the relevant areas of interest for quality wine productions in the Iberian Peninsula (i.e. Douro, Extremadura, Galicia, La Mancha and Uclés, Ribera del Duero, Rioja, Sherry area, and Valencia). Overall, these selection programs have led to the *ex-situ* preservation of strains of 12 genera and 22 species of oenological importance. Through an in-depth evaluation of different selection criteria for starter cultures in wine, a panel of yeasts was selected to improve the quality and safety of regional wine production, enlightening aspects related to organoleptic characteristics and providing solutions to specific needs in winemaking. The valorisation of eukaryotic microbial diversity to modulate wine quality represents the hot topic of the manuscript, underlining case-studies on the development of specific microbial-based solutions tailored for the needs of certain geographical contexts.

2 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. Appl Sci (MDPI) 12:12760 - https://doi.org/10.3390/app122412760

Fermented products represent microbial ecosystems of interest for the study of microbial interactions and biocontrol. Studies on the wine microbial consortium are of great importance for the evaluation of microbial diversity in specific ecological niches, for the management of fermentations in oenology, and for the production of safe and quality wines. Microbial interactions between different species and strains are interesting for understanding the dynamics within the microbial consortium and for the selection of strains of interest for biocontrol applications. This study proposes a plate-based evaluation of the interactions between yeasts and bacteria of oenological interest. The plates were prepared by agarising culture medium, must and wine to evaluate several couples of interaction belonging to four categories: yeast-yeast, yeast-bacteria, bacteria-yeast, and bacteria-bacteria. 45 strains belonging to 15 species have been used, including yeasts (*Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia fermentans*, *Brettanomyces bruxellensis*, *Hanseniaspora guillermondii*, *Issatchenkia terricola*, *Starmerella bacillaris*) and bacteria (*Oenococcus oeni*, *Lactiplantibacillus plantarum*, *Pediococcus* spp, *Levilactobacillus brevis*, *Pediococcus parvulus*, *Lentilactobacillus hilgardii).*

3 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) 8:275 - https://doi.org/10.3390/fermentation8060275

The sparkling wine sector represents one of the most dynamic sectors of international wine production. In these productions, there are two main fermentation phases, one linked to the production of the base wine (primary fermentation) and one linked to the re-fermentation in sparkling wine production (secondary fermentation). The review offers an overview of the significance of microbial resources in this specific sector, highlighting the main selection criteria on the main interesting characteristics to screen *Saccharomyces* and non-*Saccharomyces* of interest for the design of starter cultures. Highlighting the opportunities for diversification in the use of eukaryotic resources allows us to present a perspective of how starter cultures can respond to the need for innovation in this field. In the manuscript, particular emphasis is given to the valorisation of native microbial resources for the production of regional sparkling wines in order to improve the typicality and uniqueness factors that can be perceived by the consumer and which can support a greater relationship between the productions and the geographical context. of reference.

GMVV-XVII Fondazione Edmund Mach - Istituto Agrario San Michele All'Adige, Fondazione Mach Council of Italy Fondazione Edmund Mach - · Center of Technology Transfer (CTT), Via Mach 1. 38010 San Michele all'Adige, Trento, Italy. Communicated by Dr. Raffaele Guzzon <raffaele.guzzon@fmach.it>.

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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. Eur Food Res Technol 248(3):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 specifc 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 micro-organisms is necessary.

3 Gallo A, Guzzon R, Paolini M, Nardin T, Malacarne M, Ongaro M, Roman T. 2023. Biological acidification of "Vino Santo di Gambellara" by mixed fermentation of *L. thermotolerans* and *S. cerevisiae*. Role of nitrogen in the evolution of fermentation and aroma profile. Oeno One 57(3):205–217 - DOI:10.20870/oeno-one.2023.57.3.7376.

In this work, *Lachancea thermotolerans* was exploited as a biological acidifier of Vino Santo di Gambellara, a traditional Italian wine made from long-dried grapes. The drying of grapes before winemaking is a traditional technique widely applied in Italy in the production of sweet wines, but in recent years, global warming reduces dramatically the acidity of grapes, causing microbial instability during drying and winemaking. The ability of *L. thermotolerans* to convert sugars in lactic acid was already applied in the acidification of red wines, but the peculiar features of dried grape (e.g, osmotic stress, lack of nutrients, presence of mould-derived toxic compounds during drying) impose specific tests. *L. thermotolerans* was employed in sequential fermentation in combination with a strain of *Saccharomyces cerevisiae*. Considering the lack of information about the nutritional requirement of the non-*Saccharomyces* yeast, three protocols of nitrogen supplementation (mineral, organic and organic at high dosage) were tested. Alcoholic fermentation experiments were followed by plate counts onto differential media to discriminate between the two yeast species and by chemical analysis. Moreover, a GC-MS-MS approach carried out a complete characterisation of the volatile profile of wines. Results evidenced a long permanence of *L. thermotolerans* during alcoholic fermentation, which remained over the 7 log units until the 14th day of fermentation. The nitrogen supplementation protocol influenced cell growth and fermentative activity. Inorganic nitrogen supplementations allowed the accomplishment of alcoholic fermentation and the maintenance of pH below 3.35, with respect to the control wine (made only by *S. cerevisiae*), which was over pH 3.50. *L. thermotolerans* also influenced the wine's volatile aroma profile. Statistical differences were found in the main families of the yeast-derived aroma: acetate, esters, lactates, fatty acids and C6 compounds.

GMVV-XVII Department of Biomedical, Surgical and Dental Sciences - One Health Unit, University of Milano, Via della Commenda, 10, 20122 Milan, Italy. Communicated by Professor Ileana Vigentini <ileana.vigentini@unimi.it>.

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1 Tronchoni J, Setati ME, Fracassetti D, Valdetara F, Maghradze D, Foschino R, Curiel JA, Morales P, Gonzalez R, Vigentini I, Bauer FF. 2022. Identifying the main drivers in microbial diversity for Cabernet Sauvignon cultivars from Europe to South Africa: evidence for a cultivar-specific microbial fingerprint. J Fungi (MDPI) 8(10):1034 - https://doi.org/10.3390/jof8101034

Microbial diversity in vineyards and in grapes has generated significant scientific interest. From a biotechnological perspective, vineyard and grape biodiversity has been shown to impact soil, vine, and grape health and to determine the fermentation microbiome and the final character of wine. Thus, an understanding of the drivers that are responsible for the differences in vineyard and grape microbiota is required. The impact of soil and climate, as well as of viticultural practices in geographically delimited areas, have been reported. However, the limited scale makes the identification of generally applicable drivers of microbial biodiversity and of specific microbial fingerprints challenging. The comparison and meta-analysis of different datasets is furthermore complicated by differences in sampling and in methodology. Here we present data from a wide-ranging coordinated approach, using standardized sampling and data generation and analysis, involving four countries with different climates and viticultural traditions. The data confirm the existence of a grape core microbial consortium, but also provide evidence for country-specific microbiota and suggest the existence of a cultivar-specific microbial fingerprint for Cabernet Sauvignon grape. This study puts in evidence new insight of the grape microbial community in two continents and the importance of both location and cultivar for the definition of the grape microbiome.

2 Cordero-Bueso G, Vigentini I, Foschino R, Maghradze D, Ruiz-Muñoz M, Benitez-Trujillo F, Cantoral JM. 2022. Culturable yeast diversity of grape berries from *Vitis vinifera* ssp. *sylvestris* (Gmelin) Hegi. J Fungi (MDPI) 8:410 - https://doi.org/10.3390/jof8040410

Vitis vinifera L. ssp. *sylvestris* (Gmelin) Hegi is recognized as the dioecious parental generation of today's cultivars. Climatic change and the arrival of pathogens and pests in Europe led it to be included on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species in 1997. The present work focused on the study of culturable yeast occurrence and diversity of grape berries collected from wild vines. Sampling was performed in 29 locations of Azerbaijan, Georgia, Italy, Romania, and Spain. In total, 3431 yeast colonies were isolated and identified as belonging to 49 species, including *Saccharomyces cerevisiae*, by 26S rDNA D1/D2 domains and ITS region sequencing. Isolates of *S. cerevisiae* were also analyzed by SSR–PCR obtaining 185 different genotypes. Classical ecology indices were used to obtain the richness (S), the biodiversity (H'), and the dominance (D) of the species studied. This study highlights the biodiversity potential of natural environments that still represent a fascinating source of solutions to common problems in winemaking.

3 Di Canito A, Altomare A, Fracassetti D, Messina N, Tirelli A, Foschino R, Vigentini I. 2023. The riboflavin metabolism in four *Saccharomyces cerevisiae* wine strains: assessment in oenological condition and potential implications with the light-struck taste. J Fungi (MDPI) 9(1):78 - https://doi.org/10.3390/jof9010078

Riboflavin (RF), or vitamin B2, is an essential compound for yeast growth and a precursor of the flavin coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), involved in redox and non-redox processes. RF is a photosensitive compound involved in the light-struck taste (LST), a fault causing the formation of off-flavors that can develop when the wine is exposed to light in the presence of methionine (Met), as well. As both RF and Met can be associated with detrimental changes in wines, a better comprehension of its yeast-mediated production is relevant to predict the maintenance of the desired character of the wine. This study aims at assessing the production of flavin derivatives (FDs) and Met by *S. cerevisiae* oenological starters under laboratory conditions. The results showed the presence of extra- and intracellular FDs, and Met is a strain-dependent characteristic being also affected by the initial content of RF in the medium. This finding was confirmed when the winemaking was carried out in a relevant environment. Our results evidenced the important impact of the yeast strain on the content of RF and its derivatives.

GMVV-XVIII Istituto Regionale del Vino e dell'Olio (IRVO), Via Libertà 66, 90143 Palermo, Italy. Communicated by Dr. Daniele Oliva <daniele.oliva@regione.sicilia.it>.

1 Forgia M, Chiapello M, Daghino S, Pacifico D, Crucitti D, Oliva D, Ayllon M, Turina M. 2022. Three new clades of putative viral RNA-dependent RNA polymerases with rare or unique catalytic triads discovered in libraries of ORFans from powdery mildews and the yeast of oenological interest *Starmerella bacillaris*. Virus Evolution 8(1):veac038 - https://doi.org/10.1093/ve/veac038

High throughput sequencing allowed the discovery of many new viruses and viral organizations increasing our comprehension of virus origin and evolution. Most RNA viruses are currently characterized through similarity searches of annotated virus databases. This approach limits the possibility to detect completely new virus-encoded proteins with no detectable similarities to existing ones, i.e. ORFan proteins. A strong indication of the ORFan viral origin in a metatranscriptome is the lack of DNA corresponding to an assembled RNA sequence in the biological sample. Furthermore, sequence homology among ORFans and evidence of co-occurrence of these ORFans in specific host individuals provides further indication of a viral origin. Here, we use this theoretical framework to report the finding of three conserved clades of protein-coding RNA segments without a corresponding DNA in fungi. Protein sequence and structural alignment suggest these proteins are distantly related to viral RNA-dependent RNA polymerases (RdRP). In these new putative viral RdRP clades, no GDD catalytic triad is present, but the most common putative catalytic triad is NDD and a clade with GDQ, a triad previously unreported at that site. SDD, HDD, and ADD are also represented. For most members of these three clades, we were able to associate a second genomic segment, coding for a protein of unknown function. We provisionally named this new group of viruses ormycovirus. Interestingly, all the members of one of these sub-clades (gammaormycovirus) accumulate more minus sense RNA than plus sense RNA during infection.

2 Crucitti D, Chiapello M, Oliva D, Forgia M, Turina M, Carimi F, La Bella F, Pacifico D. 2022. Identification and molecular characterization of novel mycoviruses in *Saccharomyces* and non-*Saccharomyces* yeasts of oenological interest. Viruses (MDPI) 14(1):52 - https://doi.org/10.3390/v14010052

Wine yeasts can be natural hosts for dsRNA, ssRNA viruses and retrotransposon elements. In this study, high-throughput RNA sequencing combined with bioinformatic analyses unveiled the virome associated to 16 *Saccharomyces cerevisiae* and 8 non-*Saccharomyces* strains of oenological interest. Results showed the presence of six viruses and two satellite dsRNAs from four different families, two of which—Partitiviridae and Mitoviridae—were not reported before in yeasts, as well as two ORFan contigs of viral origin. According to phylogenetic analysis, four new putative mycoviruses distributed in Totivirus, Cryspovirus, and Mitovirus genera were identified. The majority of commercial S. cerevisiae strains were confirmed to be the host for helper L-A type totiviruses and satellite M dsRNAs associated with the killer phenotype, both in single and mixed infections with L-BC totiviruses, and two viral sequences belonging to a new cryspovirus putative species discovered here for the first time. Moreover, single infection by a narnavirus 20S-related sequence was also found in one S. cerevisiae strain. Considering the non-*Saccharomyces* yeasts, *Starmerella bacillaris* hosted four RNAs of viral origin—two clustering in Totivirus and Mitovirus genera, and two ORFans with putative satellite behavior. This study confirmed the infection of wine yeasts by viruses associated with useful technological characteristics and demonstrated the presence of complex mixed infections with unpredictable biological effects.

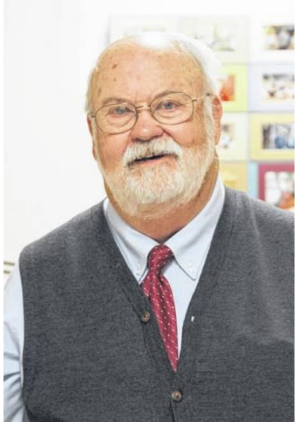
Obituaries

Jack D. Rogers 1937-2021

Dr. Jack D. Rogers, 83, WSU Regents Professor Emeritus, died peacefully at his home in Pullman, Wash., on Monday June 14 **2021** after a long illness. Jack was a nationally and internationally renowned mycologist, dedicated and inspirational educator, avid hunter and fly fisherman, a wonderful humorist, and most of all a dedicated husband to his wife Belle and father to his twin daughters Rebecca Ann (Hines) and Barbara Lee (Cooper). He will be sorely missed by his family and international network of colleagues, friends, and former students.

Jack was born on September 3, 1937 to Jack Rogers (Youkobis Uzskuriatis) and Thelma Rogers (née Coon), both school teachers in Point Pleasant. After graduating from Point Pleasant High School in 1955, Jack attended Davis and Elkins College, and in a five-year cooperative program with Duke University (North Carolina) earned a Bachelor of Science from Davis and Elkins and a Master of Science in Forest Management from Duke. He went on to earn his Ph.D. in Plant Pathology from the University of Wisconsin–Madison in 1963.

At the start of his junior year at Davis and Elkins, Jack met fellow student Belle Clay Spencer, daughter of University of Virginia chemistry professor Dr. Hugh M. Spencer and Thomasia Spencer (née Hancock). They married in June 1958 at Belle's family home in Charlottesville, Va., and began a 63-year long devoted marriage. They were blessed with twin daughters Rebecca and Barbara in June 1964.



In 1963, Jack joined the faculty of Washington State University's (WSU) College of Agriculture, beginning an educational career in the Plant Pathology and Forestry Departments which spanned half a century. He taught Forest Tree Pathology and Advanced Mycology, and served as a Major Professor and Advisor for graduate and post-graduate students. He rapidly rose through the ranks to become a full professor, and served as the Chairman of the Department of Plant Pathology from 1985 until 1999. An international authority on Xylariaceous fungi, he authored and co-authored more than 230 scientific papers and 2 mycological books. Jack traveled the world on mycological research and specimen-collecting trips, but far and away his first priority and passion was teaching his students. His many awards for educational and scientific excellence included the R.M. Wade Award for Instruction (1967); serving as President of the Mycological Society of America (1978); the Sahlin Faculty Excellence Award for Research, Scholarship, and Arts (1986); and the Mycological Society of America Weston Award for Teaching Excellence (1992). In 2004, he was honored with the Mycological Society of America's Distinguished Mycologist Award. Additionally, he received the Library Excellence Award for Service to WSU Libraries in 2005. Jack received the WSU Eminent Faculty Award in 2006, and was promoted to Regents Professor in 2007. He was the subject of a book of tributes from colleagues edited by former student and retired WSU professor Dr. Dean A. Glawe.

In addition to Jack's professional accomplishments, he was also an enthusiastic outdoorsman, a friend to many, and a legendary humorist. He particularly enjoyed hunting pheasant and quail on the breaks of the Snake River, and fly fishing in rivers and lakes across the Pacific Northwest. Jack was a regular at his two coffee groups as well as the Pullman Presbyterian Church's Men's Bible Study, and enjoyed visiting with his many colleagues and friends. Jack loved a good joke or humorous story, and had the rare ability to remember and re-tell every entertaining anecdote he ever heard. Whenever he said "that reminds me of a story…" indicated that whomever he was with would soon be laughing until their sides ached.

Jack is survived by Belle, his loving wife and constant companion of 63 years, and his daughters and their families: Becky and husband Warren of Papillion, Neb., with twin grandsons Ethan and Gareth; and Barbie and husband Brad of Martinez, Calif. He is also survived by his sister Mary Ann Mansour and husband George of Lansing, Mich.; his sister Nancy Sanders and husband Larry of Williamsport, Pa.; and many nieces and nephews.

Reproduced from https://www.kimballfh.com/obituary/jack-rogers

Byron F. Johnson 1928-2023

The Department of Biology at Carleton University mourns the passing of Dr. Byron Johnson, dear friend, esteemed scientific colleague, and passionate researcher. Byron's obtained his undergraduate degree from Penn State University and his Msc and PhD from UCLA, after which he was a postdoctoral fellow at the University of Edinburgh. He was based at the National research Council of Canada in Ottawa for nearly three decades, working chiefly with the fission yeast *Schizosaccharomyces pombe* as research model for eukaryotic development. His prime interests were cell wall synthesis, cell division, sex interconversion, cell-cell aggregation, conjugation, meiosis, and spore development. He is remembered as a generous and effective mentor to his younger co-workers. After major funding cuts to the research institute, Byron joined the Department of Biology at Carleton University in Ottawa as Adjunct Professor, where he continued his research on extensile growth, cell division, morphometric analysis, cell-cell interactions, and sexual differentiation, publishing his findings up until a few years ago. Dr. Johnson is listed in the peer-reviewed article (Yeast 2002; 19: 745-772) as one of ten major contributors to yeast cytology in the second half of the twentieth century.

Beyond his scientific achievements, Dr. Johnson was a great friend and mentor to many faculty, staff, and students at Carleton. His enthusiasm for research inspired countless students and colleagues to pursue their own passions in the world of science. Byron taught several courses for the Biology Department and supervised many thesis students. His door was always open, and he willingly shared his knowledge and expertise with anyone who sought his guidance. Byron also set the record for attendance at our Departmental seminars, always sitting at the front, listening attentively, and asking very probing questions of the speakers.

Outside the lab, Byron enjoyed traveling (a lot) and spending time with family and friends. Some of the Biology Department faculty members have great memories of cutting firewood with Byron at his rural property south of Ottawa. He was very skilled with the chain saw.

Byron Johnson left us on April 26, 2023, at the age of 94. Though we mourn Byron's passing, let us also honour and celebrate his contributions to the scientific community and his positive impact on the lives of all those fortunate enough to have known and worked alongside him. Rest in peace, dear friend, and colleague. Your contributions to the world of science and the lives you touched will never be forgotten.

James J. Cheetham
Gode B. Calleja

Enrico Cabib 1925-2023

It is our sad duty to announce to the international yeast community that Dr. Enrico Cabib, the prominent yeast researcher, passed away in Potomac, Maryland, USA on 24 February 2023, at age 98. He was one of the great scientists of the last century who laid down the foundations of modern molecular glycobiology.

Enrico was born in Genoa, Italy, on January 11, 1925. When he was sixteen, in 1941, his family emigrated to Argentina to escape anti-Semitic persecution. In 1944, he enrolled in chemistry at the School of Exact, Physical, and Natural Sciences at the University of Buenos Aires. After obtaining a degree in chemistry, he received a fellowship from the Institute for Biochemical Research, Campomar Foundation, in Buenos Aires. Working on his doctoral thesis, he experienced the thrill and satisfaction that scientific research brings sometimes to its fans; he realized that basic research was what he wanted to do for the rest of his life. At that time, the group led by his mentor, Dr. Luis Leloir, had discovered uridine diphosphate glucose (UDPG), the first sugar nucleotide identified, and found to act as a glucose donor in the synthesis of trehalose and sucrose. After defending his thesis on paper chromatography of proteins, Enrico continued working under the guidance of Leloir. He succeeded in the isolation and characterization of two new sugar nucleotides, uridine diphosphate-N-acetyl glucosamine (UDPAG) and guanosine diphosphate mannose (GDPM), and proved that they served as glycosyl donors in the biosynthesis of chitin and yeast mannan respectively. For the discovery of sugar nucleotides and their role in the biosynthesis of carbohydrates, Leloir obtained the Nobel Prize for chemistry in 1970.

In 1967, Enrico with his family (wife Amalia, daughters Claudia, Leila, and Cintia) moved to the United States. He joined the National Institutes of Health (NIH) in Bethesda, Maryland, as a principal investigator. Having at hand the three sugar nucleotides (UDPG, GDPM, and UDPAG) isolated from yeast, he showed that they were functioning as the respective glycosyl donors in the biosynthesis of yeast cell wall polysaccharides.

Enrico dedicated whole his life to yeast research studying cell wall biogenesis, particularly focusing on the main components of the cell walls – β-1,3 glucan, and chitin. He was intrigued by the questions of how these components are synthesized, and how their synthesis is regulated. Since the cell wall determines the shape of the cell, Enrico hoped to find an explanation of how the activities of individual enzymes are co-ordinated during morphogenesis. Collaborating with the authors of this article, Enrico identified and functionally characterized the cell wall transglycosylases that transfer chitin chains to glucan and proved that linkage between chitin and β-1,3-glucan is necessary to maintain shape at the mother–bud neck. His fundamental studies aimed at understanding how the yeast cell wall and septum function suggested these structures as potential targets for antifungal agents, as later confirmed with the development of echinocandins, inhibitors of β-1,3 glucan synthase activity. A detailed autobiographic story about Enrico's life and scientific achievements has also been published in FEMS Yeast Research.

Enrico Cabib belonged to the generation of scientists who grew up in the absence of economic prosperity. The special chemicals, materials, and instruments needed for the research had to be prepared by the investigators themselves. As he used to say, unexpected difficulties challenged the ingenuousness and manual skills of researchers. He devised a number of special analytical methods used to study the structure and composition of the yeast cell walls. Laboratories throughout the world have adopted many methods that he invented. As a mentor, Enrico always advised his students not to get discouraged if something doesn't work as we expect it should. He emphasized that in science, persistence is as important as intelligence.

Enrico was a very sympathetic person, always ready to help if someone needed it. His work and personality attracted many international students and researchers (including the authors of this article) who went to Bethesda to work under his guidance. Enrico had a good sense of humor and cheered his coworkers with entertaining stories. Following a 63-year research career, including 45 years in NIDDK's Laboratory of

Biochemistry and Genetics, Morphogenesis Section, Dr. Enrico Cabib retired in November 2012. His little car with the license plate YEAST no longer parks in front of Building 8. With Enrico's death, the international yeast community has lost one of its prominent figures. Everyone who studies yeast has been in some way influenced by his work and is profiting from the results of his long scientific research career. Over the years, his discoveries and conclusions are finding their place in Biochemistry textbooks, thus making Enrico Cabib immortal.

Vladimír Farkaš, Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Slovakia)
Javier Arroyo, Facultad de Farmacía, Universidad Complutense, Madrid (Spain)

Reproduced from https://fems-microbiology.org/in-memoriam-enrico-cabib/

Forthcoming Meetings

48th Annual Conference on Yeasts
Smolenice, Slovakia 14 May-17 May, 2024

It is our great pleasure to invite you to the 48th Annual Conference on Yeasts (ACY). The 48th ACY is organized by the Commission on Yeasts of the Czechoslovak Society of Microbiology and the Institute of Chemistry SAS, and will take place on May 14 - 17, 2024 in Smolenice Castle, Slovakia.
The main topics of the 48th ACY will include:

- Molecular and Cell Biology of Yeasts
- Biotechnology of Yeasts
- Yeast Diversity and Interactions
- Community Resources

The conference has been regularly held in the Congress Centre of the Slovak Academy of Sciences in the beautiful Smolenice Castle, located in the Small Carpathians (about 50 km from the capital city Bratislava). The splendid interiors of the castle and the beautiful natural surroundings contribute to a relaxing atmosphere at the meetings and provide a lot of space for informal and stimulating discussions of scientists. Thanks to the speakers, friendly atmosphere and unique location, the yeast conference is also exciting for students and young scientists.For more information:
https://yeastconference.sk

Milan Čertík
Renáta Vadkertiová

International Mycological Congress (IMC12)
Maastricht, Netherlands, 11-15 August 2024

On behalf of all Dutch mycologists, we are delighted and very honoured to host the 12th edition of the International Mycological Congress (IMC12), one of the most prestigious international mycological meetings. The congress will be held in the city of Maastricht, Netherlands, from 11-15 August 2024.

This World Congress represents a unique opportunity for mycologists from around the world to get together and enjoy excellent science in one of Europe's most impressive cities. We hope you will join us in creating an inspiring and diverse scientific programme.

Besides offering an excellent, inclusive and comprehensive scientific programme, currently under construction, we will support the training of young researchers from countries with limited resources, making it accessible at preferential prices for attendees from developing countries. In the spirit of the International Mycological Association (IMA), we are truly committed to the present and future of mycology, fostering the training and building of a strong mycological community worldwide.

This four-day meeting will include keynote lectures by scientific leaders, bridging sessions and workshops in seven themes: Cell biology, biochemistry and physiology;

Environment, ecology and interactions; Evolution, biodiversity and systematics; Fungal pathogenesis and disease control; Genomics, genetics and molecular biology; Applied Mycology and Nomenclature.

In addition to excellent science, the participants will also be able to enjoy many networking opportunities with colleagues.

We look forward to meeting you in August 2024!
For additional information: https://imc12.org/

Wieland Meyer, President of the International Mycological Association
Pedro Crous, Chair of IMC12

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

The 16th International Congress on Yeasts is the premier gathering of local and international academic and researchers, industry scientists and students working in the field of yeast biodiversity and biotechnology. It is intended to share the latest developments in fundamental yeast research linked to biotechnological applications as well as to enable more interaction between African and international partners.

Join us at the Cape Town International Convention Centre in South Africa, from 29 September to 03 October 2024 for this groundbreaking in-person event, where the contribution of yeast science and technology will be of great value to the continent, region, the local industry, academic scholars, innovators as well as the Southern African industry sectors of food and beverage, health and medicine, energy and environment.

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
16-17th of November 2024 in Bariloche, Patagonia, Argentina

The International Workshop on Brewing Yeasts is planned for 16-17th of November 2024 in Bariloche, Patagonia, Argentina. Stay tuned for news! Check the latest update at https://iwoby.com.ar

IWOBY
International Workshop on Brewing Yeasts
16 - 17th November 2024
Bariloche, PATAGONIA, ARGENTINA

Brief News Items

INCT Yeasts: Biodiversity, preservation and biotechnological innovation

As already informed in the previous Yeast Newsletter, a research network involving several universities in different regions of Brazil (and also some of our international colleagues) 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). Information on all the developments, including field work, scientific publications and science outreach activities, can be found on our Instagram profile:
https://www.instagram.com/inctleveduras/

We will soon release our official website.

A. Gombert

Andlid Bio Solutions – Consulting & Strains

As everyone here knows yeasts are not only interesting life forms but also highly useful in old and new ways. I would like to draw some attention to the newly started company "Andlid Bio Solutions – consultancy and strains" in which yeasts are central (but not exclusive, LAB and bifidobacteria are included too).

A long-lasting academic yeast passion led to the urge to implement some of the applied ideas and our collected strains. We have for instance yeasts proven to be efficient probiotics for many fish and shrimps in aquaculture, and for improving important human nutritional problems such as iron, zinc and folate deficiencies. We have yeasts producing high levels of phytase as well as folate (vitamin B9) and oil, and the knowledge on how to increase the production. The strain collection comes for example from traditionally fermented foods, mainly from African villages (co-evolved for maybe thousands of years to function with humans), from gut biotas, food and aquatic environments. It would be a privilege to collaborate with food/feed and biotech companies or researchers, to help creating new sustainable nutritious foods and feed, or produce valuable bio-compounds or develop new probiotics. If any interest, please look at the home page: Andlid Bio Solutions.

Thomas Andlid

PhD Position, European Doctoral network ECO2wine

The Eco2Wine project aims at providing a new generation of PhD graduated employees for the wine sector who will be able to manage winemaking-associated ecosystems, protect and control the biodiversity, and use this knowhow to reduce unsustainable interventions in natural environments while improving wine sustainability and "natural wine" production.

Project Title: Learning microbial interactions in wine from metabolomics

Project summary:

Characterization of the nature of yeast-yeast interactions, during must fermentation

In order to characterize the nature of the interactions among microorganisms must fermentation, we will study the physiology of growth of selected microorganisms in in co-culture and will follow the dynamic of the population during mixed culture fermentation. This will be achieved using multiparametric flow cytometry methods. Indeed, fluorescent labelling methods will be used to distinguish yeast species in a consortium. Fluorescent labelling of different yeast strain would be performed using CRISPR/Cas tools (UMIL). Combining the use of various fluorescent probe will allow to follow some physiological yeast parameters (i.e. membrane integrity, viability). The nature of the interactions between microorganisms will be assessed by comparing single to mixed cultures. The influence of several physicochemical parameters on the interactions (i.e. temperature, pH, O2), and aromatic profile in the case of must fermentations, will be investigated.

Another part of the study aim at determining the impact of interactions on yeast metabolism. Co-cultivating different microbial species results in dramatic physiological changes in comparison to yeasts growing as pure cultures. Outcomes of such interactions are mostly determined by metabolite production. Given that each microorganism produces hundreds of secreted metabolites and that interspecies interactions may influence production of these molecules, we will design an experimental framework that would allow to systematically examine changes in the secreted metabolome during multiple pairwise interspecies interactions. To that purpose, a non-targeted metabolomic approach using ultra-high-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometric analysis will be used in parallel to a targeted approach using HPLC-MS/MS analysis and RMN. These tools will be used to analyze instantaneous changes in the secreted metabolome during cell cultures, through the comparison of complex metabolites fingerprints between monocultures and co-cultures. Complementary UHPLC-MS/MS separation strategies will add structural information to FTICR-MS data. Interspecies interactions causing differential secondary metabolite production are expected to be identified.

Objectives:

1) Characterization of the nature of yeast-yeast interactions;
2) impact of biotic and abiotic factors on yeast-yeast interactions;
3) unravelling yeast-yeast interactions mechanisms by exometabolome study;
4) modelling interactions to better control co-fermentation.

Expected Results:

1) identification of yeast-yeast interaction mechanisms;
2) identification of biotic and abiotic factors that could be used to control co-fermentation.

Candidate profile:

The proposed subject deals with the study of the yeast exo-metabolome. The candidate recruited should therefore come from a background in either analytical chemistry or microbiology. Proficiency in certain biostatistical analyses is a plus. The candidate must be rigorous and dynamic. Analytical chemistry, interactions, metabolism, yeasts, fermentations, HPLC-MS, GC-MS

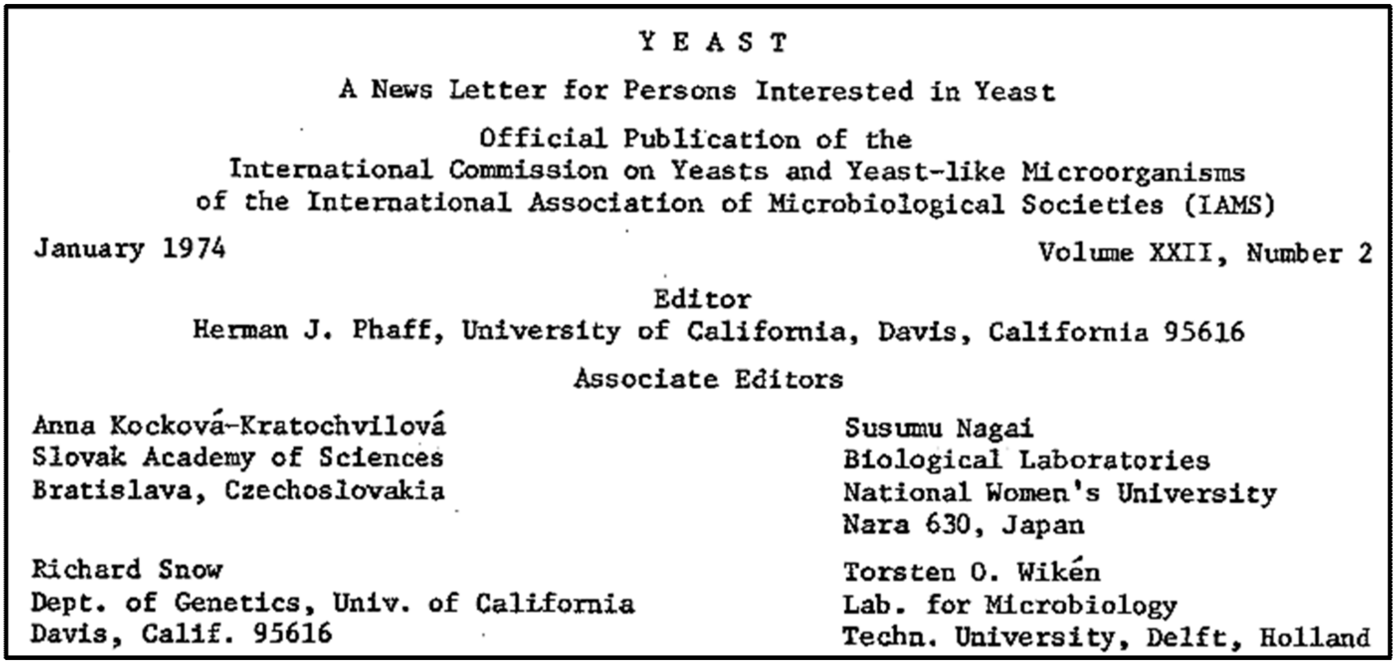
Enrolment in Doctoral degree: University of Burgundy

Hervé Alexandre
Institut Universitaire de la Vigne et du Vin Jules Guyot
Rue Claude Ladrey
Université de Bourgogne
BP 27877-21078 Dijon Cedex

rvalex@u-bourgogne.fr

http://iuvv.u-bourgogne.fr/
http://www.umr-pam.fr
http://www.u-bourgogne.fr/chaireunesco-vinetculture

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)

January 1974 Volume XXII, Number 2

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

Associate Editors

Anna Kocková-Kratochvilová
Slovak Academy of Sciences
Bratislava, Czechoslovakia

Susumu Nagai
Biological Laboratories
National Women's University
Nara 630, Japan

Richard Snow
Dept. of Genetics, Univ. of California
Davis, Calif. 95616

Torsten O. Wikén
Lab. for Microbiology
Techn. University, Delft, Holland

Four students of **Professor Boidin** of Université Calude Bernard, Lyon, France successfully defended their doctoral theses. **S. Poncet** used numerical analysis of GC content to divide *Kluyveromyces, Pichia* and *Hansenula* into two to three clusters each. **J. B. Fiol** sought to improve the systematics of several sporogenous genera using GC content, oxidase assays, vitamin requirements and other tests to propose more natural grouping of species. **F. Jacob** characterized 680 strains of yeasts isolated from plant-based tanning liquors from several regions of France, including five new yeast species. **M. C. Pignal** isolated 250 yeasts from tree-associated coleoptera beetles, found a few stable yeast-insect associations, and proposed possible advantages to the insect including vitamin synthesis, growth factors, degradation of sugars and tannins, and nitrogen sources.

P. Galzy of I.N.R.A, Montpellier, France listed nine recent or in-press publications including four doctoral theses on cheese-associated yeasts, sporulation, carbon metabolism, and lipid composition.

Luiz R. Travassos of Universidade Federal do Rio de Janeiro, Brasil published a carnitine assay method, identified a carnitine-requiring strain of *Torulopsis bovina*, and accessioned the strain in the CBS collection, CBS 6471.

In their studies of the life cycle of the yeastlike anther smut fungus *Ustilago violacea* using wild-type and UV-sensitive mutants and radioactive tracer studies, **A. W. Day** and **J. E. Cummins** of University of Western Ontario, London, Canada identified a single, possibly complex, locus with two alleles responsible for conjugation.

In their studies of the life cycle of the yeast *Saccharomyces rouxii*, **Haruhiko Mori** marked strains with auxotrophic genetic traits to determine that two allelic genes, which they designated a and alpha, controlled the two mating types. Strains NRRL-2547 and NRRL-2548 were designated as the mating type standards.

Nasim A. Khan shared results of UV-mutagenesis, crossing, tetrad dissection, and invertase activity to study the structural nature of the SUC 3 gene responsible for sucrose fermentation in *Saccharomyces cerevisiae*.

In their studies of the regulation of maltose and sucrose uptake and fermentation in *Saccharomyces cerevisiae*, **F. K. Zimmermann** of Technische Hochschule, Darmstadt, German Federal Republic isolated and used maltose non-fermenting mutants, crossing, and tetrad analysis.

Eckhart Schweizer of Universität Würzburg, Germany analyzed the genetics of fatty acid synthetase mutants of *Saccharomyces cerevisiae*. Using X-ray induced mitotic recombination to construct a genetic fine structure map, they determined that fas 1 encodes only one, functionally complex, poly-peptide chain.

R. Mortimer of the University of California Berkeley announced that an updated stock list for the Yeast Genetics Stock Center would be published in mid-1974, and listed five publications on temperature sensitivity, mating type, enzyme expression, and radiosensitivity.

S. R. Snow of the University of California Davis shared abstracts of two publications in Genetics on allelic complementation in histidine biosynthesis genes in *Saccharomyces cerevisiae.* Gamma rays, X-rays and MMS were used to order 64 his1 alleles and place them on chromosome V. The genetic and allelic complementation data led to models of the tertiary and quaternary structure of the enzyme.

Takashi Ito, University of Tokyo, Japan summarized two recent papers on diffusion of acridine orange to the nucleus of yeast cells, and the genetic effects of freeze- and vacuum-drying.

M. Brendel, Goethe Universität, Germany established a yeast research lab, and published work on DNA synthesis, DNA labelling using ^{32}P-5'-dTMP, and genes controlling sensitivity to radiation and alkylation in S. cerevisiae.

Claire D. Goldthwaite, Albert Einstein College of Medicine, Bronx, NY used antifungal markers, incorporation of radioactivity into DNA and DNA buoyancy in CsCl gradients to determine that *Saccharomyces cerevisiae* grown in glycerol contained two to three times as much mitochondrial DNA as the same culture grown in glucose.

P. Slonimski, Centre De Génétique Moléculaire, Gif-Sur-Yvette, France offered reprints to anyone interested in 33 papers published between 1971 and 1973 related to *S. cerevisiae* lactate dehydrogenase, antibiotic resistance, mitochondrial DNA, petite colony mutants, mitotic recombination, mutant ribosomes, cytochrome structure, and other topics.

W. Gajewski, Polish Academy of Sciences listed yeast work published or in progress on chemical mutagenesis, the physiology of the conjugation process, a method of synchronous mass production of zygotes, interactions between nuclear and mitochondrial genes.

V. K. Jain became an assistant professor in the department of biophysics, All India Institute, New Delhi, India, and studied the effects of the glucose antimetabolite 2-deoxy-D-glucose on respiratory deficient *S. cerevisiae* mutants.

Byron Johnson, National Research Council of Canada, Ottawa used irradiation of synchronized cultures of *S. cerevisiae* with a UV-microbeam to characterize the target for induction of the cytoplasmic petite mutation.

J. G. Little, York University, Ontario, Canada addressed the problem that wild-type *S. cerevisiae* lacks thymidine kinase, and thus could not efficiently incorporate labelled dTMP. By screening for aminopterin sensitivity, they isolated a mutant that required dTMP for growth. Radioactive dTMP was incorporated into both nuclear and mitochondrial DNA, allowing studies of DNA replication and repair.

Marjorie Crandall, Indiana University listed six publications on the genetics of *Hansenula wingei*. They identified a factor, which they named 5I, that induced synthesis of 5-factor, the agglutination factor from strain 5 of this sexually agglutinative yeast species.

Eric Zeuthen, Carlsberg Foundation, Copenhagen, Denmark used electron microscopy of thin sections to reveal that the membrane of the fission yeast *Schizosaccharomyces pombe* consists of triple-layered membranes, each 14-20 nm in dimension with complicated structures.

Marie Kopecka, Purkyne University, Brno, Czechoslovakia listed six papers on *S. cerevisiae* and *Nadsonia elongate* plasma membranes, cell envelopes, protoplasts, and sphaeroplasts. **Mirozlav Gabriel** completed their Ph.D. dissertation under guidance of **O. Necas**, studying regeneration of protoplasts of fungi *Rhizopus* and *Trichosporon*, the green alga *Uronema*, and blue-green alga *Cylindrospermum*.

V. Farkaš, Slovak Academy of Sciences, Bratislava, Czechoslovakia listed five publications on cell wall composition, degradation and synthesis in *S. cerevisiae* and *Schizosaccharomyces pombe*.

H. J. Phaff, university of California Davis shared the summary of the Ph. D. dissertation of **Graham H. Fleet** on the beta-glucan hydrolases of *Schizosaccharomyces versatilis.* **F. M. Rombouts** of the Agricultural University, Wageningen joined the Phaff group for a year. **Leda C. Mendonca** returned to Rio de Janeiro after a 2-year stay.

H. Suomalainen and **Chr. Waller**, Research Laboratories State Alcohol Monopoly, Helsinki, Finland found differences in the amounts of fatty acids C_4-C_{10} in fermentation of beer wort inoculated with wild yeasts *S. cerevisiae* var. *ellipsoideus*, *S. pastorianus, S. diastaticus* and *Brettanymyces anomala*, or *S. carlsbergensis* fermented at 8C or 12C. They also summarized work on *S. cerevisiae* alpha-glucosidase formation, nucleic acid composition, lipid composition of cell envelope fractions, and plasma membrane structure.

F. Schlenk, Argonne National Laboratory, Illinois, USA studied uric acid crystals in vacuoles of *Candida utilis*, and used differential centrifugation to isolate vacuoles.

P. A. D. Rickard, University of New South Wales, Australia studied the impacts of anaerobic conditions, ergosterol and Tween 80 on mitochondriogenesis and cytochrome concentration in *S. cerevisiae*.

H. Van den Bossche, Janssen Pharmaceutica, Belgium published a study of the effects of miconazole on uptake or utilization of purines, pyrimidines, nucleosides, amino acids and glucose in *Candida albicans*.

A. Fiechter, Swiss Federal Institute of Technology, Switzerland published work on hydrocarbon fermentation, malate dehydrogenase in *Schizosaccharomyces pombe* and Saccharomyces cerevisiae.

C. Gancedo, Universidad Autonoma, Madrid, Spain presented their work on hexose kinases of *Rhodotorula glutinis* at the Third International Specialized Symposium on Yeasts, June 1973, Otaneimi, Finland. The published work using labelled glucose to study the pentose-phosphate pathway in *S. cerevisiae*, intermediary metabolites, and transhydrogenation from NADH to NADP.

P. Babyeva, Moscow State University, USSR presented a paper at the Fifteenth Plenary Meeting of COSPAR, Madrid, Spain in 1972 on the mechanism of adaptation of *Cryptococcus albidus* var. *diffluens* to extreme low humidity.

R. R. Davenport, Cider and Fruite Juices Section, University of Bristol, England performed an environmemtal study of an English vineyard, and developed techniques and identification keys to handle the large numbers of isolates. New species were discovered in genera *Endomycopsis, Sporobolomyces, Sporidiobolus, Rhodosporidium, Bullera, Leucosporidium, Lipomyces, Candida, Pichai, Nadsonia,* and *Schizosaccharomyces*.

M. A. Lachance, McGill University, Quebec, Canada completed his M. Sc. Dissertation on production of single-cell-protein from waste pasta products under Dr. Blackwood, and shared his present address at the University of California Davis.

Myung Sam Park, Chonnam National University, Korea isolated 35 strains of yeast, and used two of them, *H. anomala* var. *anomala* No. 225 and *Candida utilis* No. 400*,* in fermentations of feeds using rice, barley, wheat, and defatted rice bran.

J. Jakubowska, Technical University of Lódz, Poland presented results at the 3rd ISSY meeting in Finland indole that acetic acid stimulated growth of *S. cerevisiae* by 20 to 60%, and increased oxygen uptake in *Schizosaccharomyces acidodevoratus*. They also studied fermentation properties of cryophilic wine yeasts.

Graham G. Stewart, Labatt Breweries, London, Ontario, Canada published papers on spheroplast formation and on maltose and glucose fermentation in *S. cerevisiae*.

R. J. Anderson, Allied Breweries, Burton-On-Trent, England completed their Ph.D. thesis on production of sulphur volatiles by brewery microorganisms.

E. Minárik, Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia published two papers (in German) on the influence of cysteine and methionine on sulphate uptake and sulphite formation in some species of *Saccharomyces*.

Herman J. Phaff joined the Editorial Board of the International Journal of Systematic Bacteriology, which issued a policy to publish papers dealing with systematics and taxonomy of yeasts and yeast-like organisms.

The **Fourth International Symposium on Yeasts** was scheduled for July 1974 in in Vienna, Austria.

The text of a speech given by **T. O. Wiken,** Chairman of the IUMS Commission on Yeasts and Yeast-like Microorganisms, at the farewell party of the Third International Specialized Symposium on Yeasts, held in Otaniemi, June 1973, thanking the host institution and organizers. The symposium was attended by about 200 persons from 24 countries.

H. A. Koch, Hautklinik Med Akad. Erfurt, D.D.R. gathered a community of researchers interested in yeast, which met in May 1973 in Erfurt to discuss yeast taxonomy, and in Leipzig in September 1973 to discuss karyokinesis and host-parasite relations.

A. Stenderup formed the Danish Mycology Club, and held a symposium on yeast infections in Copenhagen.

Wilfred Arnold University of Kansas hosted the first national meeting of the Society for Complex Carbohydrates in Chicago in November 1973, with a program focusing on yeast cell wall chemistry and structure.

Obituaries shared life stories of **Rudolf Müller**, head of the Institute of Microbiology and Experimental Therapy, German Academy of Sciencies, Jena, German Democratic Republic, and of **Lloyd K. Riggs**, Professor, Rutgers University.

Francis M. Clark, Professor Emeritus*,* University of Illinois opened a lyophilized vial of *Candida guilliermondii* received from L. J. Wickerham, NRRL in 1949. After 24 years stored in his desk, the culture was still viable.

A. Stenderup returned from a sabbatical year at the University of the South Pacific, Fiji and listed four publications on repetitive DNA in yeast.

A. Kocková-Kratochvilová was elected to the Executive Board of the World Federation for Culture Collections, which was established within the International Association of Microbiological Societies.

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