

# Yeast

**A Newsletter for Persons Interested in Yeast**

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Marc-André Lachance, Editor  
University of Western Ontario, London, Ontario, Canada N6A 5B7  
<[lachance@uwo.ca](mailto:lachance@uwo.ca)>

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

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#### Associate Editors

Patrizia Romano  
School of School of Agricultural, Forestry, Food  
and Environmental Sciences  
Università della Basilicata  
Via Nazario Sauro, 85, 85100 Potenza, Italy

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

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## **Editorial**

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Like every other area of human activity, yeast research has had to yield to the fickle temperament of the Covid-19 pandemic. The much anticipated ICY meetings in Vienna and Vancouver are on hold and for the moment re-scheduled for a year later. With university services shut down, the printed version of this issue was produced at a commercial venue and the postage determined and affixed in an artisanal setting. My own laboratory activities have been limited to topping up the liquid nitrogen over my yeast culture collection, as is probably the case for many of our readers. My heartfelt thanks go to those who have communicated information for this issue, with a special tip of the hat to Patrizia Romano, who is reporting on behalf of her newly created Italian wine yeast research network.

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## **Erratum**

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In the December 2019 issue, the printed version, I erroneously reported Matti Korhola's birth year as 1949 instead of 1946, in both the editorial and the obituary. My apologies. The error has been corrected in the online version.

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M.A. Lachance, Editor

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**I 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](mailto:gombert@unicamp.br)>.**

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In December 2019 I completed a one-year sabbatical with Prof. Michael Desai (Harvard University), where work on the molecular dynamics of industrial fermentations was carried out. During the Open Meeting of the European COST Action CA18113 (Understanding and exploiting the impacts of low pH on micro-organisms), I gave the opening talk, entitled

“What can the industrial production of fuel ethanol from sugarcane teach us about tolerance to low pH?.” The meeting took place in Lisbon between February 12 and 14, 2020, and several European microbiologists were present, among them some of our colleagues in yeast research. Finally, I have recently joined the editorial board of FEMS Yeast Research.

The following mini-review article was recently accepted for publication in FEMS Yeast Research.

- 1 Bermejo PM, Raghavendran V, Gombert AK. 2020. Neither 1 G nor 2 G fuel ethanol: setting the ground for a sugarcane-based biorefinery using an iSUCCELL yeast platform [published online ahead of print, 2020 May 13]. *FEMS Yeast Res.* 2020;foaa027. doi:10.1093/femsyr/foaa027.

The work describes a new paradigm for a sugarcane-based biorefinery, in which engineered strains of *Saccharomyces cerevisiae* convert sucrose

and cellobiose simultaneously into ethanol via intracellular disaccharide hydrolyses.

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**II Lodz University of Technology, Faculty of Biotechnology and Food Sciences, Department of Environmental Biotechnology, Wolczanska 171/173, 90-924 Lodz, Poland. Communicated by Dorota Kregiel <[dorota.kregiel@p.lodz.pl](mailto:dorota.kregiel@p.lodz.pl)>.**

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The following papers have been published.

- 1 Ewelina Pawlikowska, Monika Szymanska, Joanna Berlowska, Dorota Kregiel. 2020. Lysates of *Metschnikowia* yeast with higher content of hydroxyproline. *BioResources* 15(3):3228-3236.

The chemical characteristics of lysates obtained from yeasts belonging to *Metschnikowia* spp. were determined. Cell lysis was induced using saponin from *Q. saponaria* or 5% NaCl. The process was conducted at 50 °C for 48 h. The enzymatic profiles of the resulting lysates were analyzed. The mannose and glucose contents were also investigated, as well as the concentrations of proteins, free amino nitrogen (FAN), and free amino acids. The results were compared to the characteristics of lysates from conventional industrial strains of *Saccharomyces* spp. obtained under

analogous conditions. The *Metschnikowia* lysates showed different chemical profiles and the pool of individual amino acids was generally smaller. However, the content of hydroxyproline was 4 to 5 times higher. The results of this study show that yeast lysates are an attractive supplement for numerous applications.

Keywords: *Metschnikowia*; autolysates; amino-acid profile; hydroxyproline

- 2 Bahare Salehi, Dorota Kregiel, Gail Mahady, Javad Sharifi-Rad, Natália Martins, Célia F. Rodrigues. 2020. Management of *Streptococcus mutans* – *Candida* spp. oral biofilms’ infections: paving the way for effective clinical interventions. *Journal of Clinical Medicine* 9:517.

Oral diseases are considered the most common noncommunicable diseases and are related to serious local and systemic disorders. Oral pathogens can grow and spread in the oral mucosae and frequently in biomaterials (e.g., dentures or prostheses) under polymicrobial biofilms, leading to several disorders

such as dental caries and periodontal disease. Biofilms harbor a complex array of interacting microbes, increasingly unapproachable to antimicrobials and with dynamic processes key to disease pathogenicity, which partially explain the gradual loss of response towards conventional therapeutic regimens. New drugs

(synthesized and natural) and other therapies that have revealed promising results for the treatment or control of these mixed biofilms are presented and discussed here. A structured search of bibliographic databases was applied to include recent research. There are several promising new approaches in the treatment of *Candida* spp.–*Streptococcus mutans* oral mixed biofilms that could be clinically applied in the near

future. These findings confirm the importance of developing effective therapies for oral *Candida*-bacterial infections.

Key words: oral biofilm; infection control; *Streptococcus mutans*; *Candida* spp.; natural compounds; antimicrobial resistance

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

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Recent publication.

- 1 Golubev WI. 2020. Mycocinogeny in fission yeast. *Mykologia i Fitopatologia* 54(2):150-152.

A total of 39 *Schizosaccharomyces* strains have been examined for antagonistic activity, including the nomenclature types of the species with the names considered presently synonymous. Two strains of

*S. pombe* exhibited fungistatic activity. They are not active against any budding yeasts (Saccharomycotina) but act against some species of the genera *Protomyces* and *Taphrina* (Taphrinomycotina).

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**IV 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](mailto:cthittinger@wisc.edu)>.

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Recent publications.

- 1 Peris D, Alexander WG, Fisher KJ, Moriarty RV, Basuino MG, Ubbelohde EJ, Wrobel RL, Hittinger CT. 2020. Synthetic hybrids of six yeast species. *Nat Commun* 11:2085.  
<https://doi.org/10.1038/s41467-020-15559-4>

Allopolyploidy generates diversity by increasing the number of copies and sources of chromosomes. Many of the best-known evolutionary radiations, crops, and industrial organisms are ancient or recent allopolyploids. Allopolyploidy promotes differentiation and facilitates adaptation to new environments, but the tools to test its limits are lacking. Here we develop an iterative method of Hybrid Production (iHyPr) to combine the genomes of multiple budding yeast species, generating

*Saccharomyces* allopolyploids of at least six species. When making synthetic hybrids, chromosomal instability and cell size increase dramatically as additional copies of the genome are added. The six-species hybrids initially grow slowly, but they rapidly regain fitness and adapt, even as they retain traits from multiple species. These new synthetic yeast hybrids and the iHyPr method have potential applications for the study of polyploidy, genome stability, chromosome segregation, and bioenergy.

- 2 Dharampala PS, Diaz-Garcia L, Haase MAB, Zalapa J, Currie CR, Hittinger CT, Steffan SA. 2020. Microbial diversity associated with the pollen stores of captive-bred bumble bee colonies. *Insects* 11: 250. <https://doi.org/10.3390/insects11040250>

The pollen stores of bumble bees host diverse microbiota that influence overall colony fitness. Yet, the taxonomic identity of these symbiotic microbes is relatively unknown. In this descriptive study, we characterized the microbial community of pollen

provisions within captive-bred bumble bee hives obtained from two commercial suppliers located in North America. Findings from 16S rRNA and ITS gene-based analyses revealed that pollen provisions from the captive-bred hives shared several microbial

taxa that have been previously detected among wild populations. While diverse microbes across phyla Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Ascomycota were detected in all commercial hives, significant differences were detected at finer-scale taxonomic resolution based on the supplier source. The causative agent of chalkbrood disease in honey bees, *Ascosphaera apis*, was detected

in all hives obtained from one supplier source, although none of the hives showed symptoms of infection. The shared core microbiota across both commercial supplier sources consisted of two ubiquitous bee-associated groups, *Lactobacillus* and *Wickerhamiella/Starmerella* clade yeasts that potentially contribute to the beneficial function of the microbiome of bumble bee pollen provisions.

- 3 Landgon QK, Peris D, Eizaguirre JI, Opulente DA, Buh KV, Sylvester K, Jarzyna M, Rodríguez ME, Lopes CA, Libkind D, Hittinger CT. 2020. Postglacial migration shaped the genomic diversity and global distribution of the wild ancestor of lager-brewing hybrids. PLoS Genet 16: e1008680. <https://doi.org/10.1371/journal.pgen.1008680>

The wild, cold-adapted parent of hybrid lager-brewing yeasts, *Saccharomyces eubayanus*, has a complex and understudied natural history. The exploration of this diversity can be used both to develop new brewing applications and to enlighten our understanding of the dynamics of yeast evolution in the wild. Here, we integrate whole genome sequence and phenotypic data of 200 *S. eubayanus* strains, the largest collection known to date. *S. eubayanus* has a multilayered population structure, consisting of two major populations that are further structured into six subpopulations. Four of these subpopulations are found exclusively in the Patagonian region of South America; one is found predominantly in Patagonia and sparsely in Oceania and North America; and one is specific to the Holarctic ecozone. Plant host associations differed between subpopulations and between *S. eubayanus* and its sister species, *Saccharomyces uvarum*. *S. eubayanus* is most abundant and genetically diverse in northern

Patagonia, where some locations harbor more genetic diversity than is found outside of South America, suggesting that northern Patagonia east of the Andes was a glacial refugium for this species. All but one subpopulation shows isolation-by-distance, and gene flow between subpopulations is low. However, there are strong signals of ancient and recent outcrossing, including two admixed lineages, one that is sympatric with and one that is mostly isolated from its parental populations. Using our extensive biogeographical data, we build a robust model that predicts all known and a handful of additional regions of the globe that are climatically suitable for *S. eubayanus*, including Europe where host accessibility and competitive exclusion by other *Saccharomyces* species may explain its continued elusiveness. We conclude that this industrially relevant species has rich natural diversity with many factors contributing to its complex distribution and natural history.

- 4 Libkind D, Peris D, Cubillos FA, Steenwyk JL, Opulente DA, Langdon QK, Rokas A, Hittinger CT. 2020. Into the wild: new yeast genomes from natural environments and new tools for their analysis. FEMS Yeast Res 20:foaa008. <https://doi.org/10.1093/femsyr/foaa008>

Genomic studies of yeasts from the wild have increased considerably in the past few years. This revolution has been fueled by advances in high-throughput sequencing technologies and a better understanding of yeast ecology and phylogeography, especially for biotechnologically important species. The present review aims to first introduce new bioinformatic tools available for the generation and analysis of yeast genomes. We also assess the accumulated genomic data of wild isolates of industrially relevant species, such as *Saccharomyces* spp., which provide unique opportunities to further

investigate the domestication processes associated with the fermentation industry and opportunistic pathogenesis. The availability of genome sequences of other less conventional yeasts obtained from the wild has also increased substantially, including representatives of the phyla Ascomycota (e.g. *Hanseniaspora*) and Basidiomycota (e.g. *Phaffia*). Here, we review salient examples of both fundamental and applied research that demonstrate the importance of continuing to sequence and analyze genomes of wild yeasts.

- 5 Hittinger CT. 2020. Evaluation of Kuroda et al.: insight into yeast isobutanol tolerance with advances still needed. *Cell Syst* 10: 124. <https://doi.org/10.1016/j.cels.2020.01.005>

One snapshot of the peer review process for "Critical Roles of the Pentose Phosphate Pathway and

*GLN3* in Isobutanol-Specific Tolerance in Yeast" (Kuroda et al., 2019).

- 6 Shen XX, Steenwyk JL, LaBella AL, Ofulente DA, Zhou X, Kominek J, Li Y, Groenewald M, Hittinger CT, Rokas A. Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota. *bioRxiv* submitted. <https://doi.org/10.1101/2020.05.11.088658>

Ascomycota, the largest and best-studied phylum of fungi, contains three subphyla: Saccharomycotina (budding yeasts), Pezizomycotina (filamentous fungi), and Taphrinomycotina (fission yeasts); organisms from all three subphyla have been invaluable as models in diverse fields (e.g., biotechnology, cell biology, genetics, and medicine). Despite its importance, we still lack a comprehensive genome-scale phylogeny or understanding of the similarities and differences in the mode of genome evolution within this phylum. To address these gaps, we examined 1,107 genomes from Saccharomycotina (332), Pezizomycotina (761), and Taphrinomycotina (14) species to infer the Ascomycota phylogeny, estimate its timetree, and examine the evolution of key genomic properties. We inferred a robust genome-wide phylogeny that resolves several contentious relationships and estimated that the Ascomycota last common ancestor likely originated in the Ediacaran (~563 ± 68 million years ago). Comparisons of

genomic properties revealed that Saccharomycotina and Pezizomycotina, the two taxon-rich subphyla, differed greatly in their genome properties. Saccharomycotina typically have smaller genomes, lower GC contents, lower numbers of genes, and higher rates of molecular sequence evolution compared to Pezizomycotina. Ancestral state reconstruction showed that the genome properties of the Saccharomycotina and Pezizomycotina last common ancestors were very similar, enabling inference of the direction of evolutionary change. For example, we found that a lineage-specific acceleration led to a 1.6-fold higher evolutionary rate in Saccharomycotina, whereas the 10% difference in GC content between Saccharomycotina and Pezizomycotina genomes stems from a trend toward AT bases within budding yeasts and toward GC bases within filamentous fungi. These results provide a robust evolutionary framework for understanding the diversification of the largest fungal phylum.

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**V Instituto Andino Patagónico de Tecnologías Biológicas y Geoambientales (IPATEC), CONICET – UNComahue, Bariloche, Argentina. Communicated by Diego Libkind <[diego.libkind@gmail.com](mailto:diego.libkind@gmail.com)>.**

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Recent publications.

- 1 Steenwyk JL, Ofulente DA, Kominek J, Shen X-X, Zhou X, LaBella AL, Bradley NA, Eichman BF, Čadež N, Libkind D, DeVirgilio J, Hulfachor AB, Kurtzman CP, Hittinger CT, Rokas A. 2019. Extensive loss of cell cycle and DNA repair genes in an ancient lineage of bipolar budding yeasts. *PLoS Biol.* 17(5):e3000255. doi: 10.1371/journal.pbio.3000255.
- 2 Langdon Q, D Peris, EP Baker, DA Ofulente, Huu-Vang Nguyen, U Bond, P Gonçalves, JP Sampaio, D Libkind, CT Hittinger. 2019. Fermentation innovation through complex hybridization of wild and domesticated yeasts. *Nature Ecology & Evolution* 3:1576–1586. doi:10.1038/s41559-019-0998-8.
- 3 Langdon QK, Peris D, Eizaguirre JI, Ofulente D, Buh KV, Sylvester K, Jarzyna M, Rodríguez ME, Lopes CA, Libkind D, Hittinger CT. 2020. Postglacial migration shaped the genomic diversity and global distribution of the wild ancestor of lager-brewing hybrids. *PLOS Genetics*.16(4):e1008680. doi: 10.1371/journal.pgen.
- 4 Libkind D, Peris D, Cubillos FA, Steenwyk JL, Ofulente DA, Langdon QK, Rokas A, Hittinger CT. 2020. Into the wild: new yeast genomes from natural environments and new tools for their analysis. *FEMS Yeast Res* 20(2):foaa008. doi: 10.1093/femsyr/foaa008.

- 5 Latorre M, Hutzler M, Michel M, Zarnkow M, Jacob F, Libkind D. 2020. Genotypic diversity of *Saccharomyces cerevisiae* spoilers in a community of craft microbreweries. *Brewing Science*. In revision.
- 6 Libkind D, Čadež N, Opulente DA, Langdon QK, Rosa CA, Sampaio JP, Gonçalves P, Hittinger, CT, Lachance, M.A. Yeast taxogenomics: description of novel species based on complete genome sequences. *FEMS Yeast Research*. Submitted.
- 7 de Garcia, V.; Trochine, A.; J. Uetake; N. Bellora; D. Libkind. Novel yeast taxa from the cold: Description of *Cryolevonia giraudoi* sp. nov. and *Camptobasidium gelus* sp. nov. IJSEM-D-19-01013. Submitted.

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**VI State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by E.S. Naumova <[lenna\\_naumova@yahoo.com](mailto:lenna_naumova@yahoo.com)>.**

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The following are papers for 2019 and 2020 or in press.

- 1 Naumova ES, Shalamitskiy MYu, Naumov GI. 2019. Molecular polymorphism of pectinase genes PGU of *Saccharomyces bayanus* var. *uvarum* yeast. *Appl Biochem Microbiol* 55(9):723–729.
- 2 Korhola M, Naumova ES, Partti E, Aittamaa M, Turakainen H, Naumov GI. 2019. Exploiting heterozygosity in industrial yeasts to create new and improved baker's yeasts. *Yeast* 36(9):571–587.

The main aim of the work was to utilize heterozygosity of industrial yeast strains to construct new baker's yeast strains. Commercial baker's yeast strain ALKO 743, its more ethanol tolerant descendant ALKO 554 selected initially for growth over 300 generations in increasing ethanol concentrations in a glucose medium, and ALKO 3460 from an old domestic sour dough starter were used as starting strains. Isolated meiotic segregants of the strains were characterized genetically for sporulation ability and mating type, and the ploidy was determined physically. Heterozygosity of the segregant strains was estimated by a variety of molecular characterizations and fermentation and growth assays. The results

showed wide heterozygosity and that the segregants were clustered into subgroups. This clustering was used for choosing distantly or closely related partners for strain construction crosses. Intrastrain hybrids made with segregants of ALKO 743 showed 16–24% hybrid vigour or heterosis. Interstrain hybrids with segregants of ALKO 743 and ALKO 3460 showed a wide variety of characteristics but also clear heterosis of 27–31% effects as assayed by lean and sugar dough raising. Distiller's yeast ALKO 554 turned out to be a diploid genetic segregant and not just a more ethanol tolerant mutant of the tetraploid parent strain ALKO 743.

- 3 Chang ChF, Liu YR., Naumov GI, Naumova ES, Lee Ch-Fu. 2020. Taxonomy of the yeast genus *Vanderwaltozyma* and proposal of *Vanderwaltozyma meishanica* sp. nov., *Vanderwaltozyma huisunica* sp. nov., and *Vanderwaltozyma molinica* sp. nov. *Antonie van Leeuwenhoek* 113(5):663–676.

Using electrophoretic karyotyping, RAPD fingerprinting and phylogenetic analysis of ribosomal RNA gene sequences, twenty-six *Vanderwaltozyma* strains were studied. Out of 19 strains isolated in mountainous areas of Taiwan, eighteen strains were isolated from soil and one strain was isolated from the fruiting body of mushroom, six were identified as *V. polyspora* and three as *V. verrucispora*. Based on the results of a multigene sequence analysis (D1/D2, ITS and mitochondrial *COX II* gene) and DNA–DNA

reassociation, three new ascosporic members of the genus *Vanderwaltozyma* are formally described: *V. huisunica* sp. nov. (GA1S06<sup>T</sup> = CBS 12250<sup>T</sup> = BCRC 23260<sup>T</sup>), *V. meishanica* sp. nov. (EN4S02<sup>T</sup> = CBS 12249<sup>T</sup> = BCRC 23255<sup>T</sup>) and *V. molinica* sp. nov. (GJ8S05<sup>T</sup> = CBS 12251<sup>T</sup> = BCRC 23264<sup>T</sup>), and the holotypes of these novel species are assigned as BCRC 23260<sup>T</sup>, BCRC 23255<sup>T</sup> and BCRC 23264<sup>T</sup>, respectively.

- 4 Naumova ES, Boundy-Mills KL, Naumov GI. 2020. Phylogenetics and biogeography of biotechnologically important methylotrophic yeasts *Komagataella*. *Microbiology (Moscow)* 89(3):294–300.

Molecular genetic study of 22 methanol assimilating *Komagataella* strains maintained at the UCDFST collection (United States) was carried out. Multigene phylogenetic analysis revealed that strains previously assigned to “*Pichia pastoris*” based on the standard taxonomic tests actually belong to four different species: *K. pastoris*, *K. phaffii*, *K. mondavi-orum*, and *K. ulmi*. Seven *Komagataella* species are practically indistinguishable by the

standard morphological and physiological tests, and have similar D1/D2 sequences. Multigene phylogenetic analysis should be applied for reliable delineation of all seven *Komagataella* species. The biogeography of *Komagataella* yeasts is noteworthy. *K. mondavi-orum*, *K. kurtzmanii*, *K. phaffii*, *K. populi*, and *K. ulmi* have been found only in North America, while *K. pastoris* and *K. pseudopastoris* are characteristic of Europe.

- 5 Borovkova AN, Michailova YuV, Naumov GI. 2020. Molecular genetic characteristics of the *Saccharomyces* biological species. *Microbiology (Moscow)* 89 (4) (in press).

Using molecular karyotyping and comparative analysis of a number of nuclear and mitochondrial genes, we have conducted molecular genetic study of *Saccharomyces* yeasts of different species belonging. Multigene phylogenetic analysis showed that recently described species *S. jurei* is phylogenetically closest to *S. mikatae*, while *S. bayanus* and *S. arboricola* are the most divergent species within the genus *Saccharomyces*. Comparative analysis of amino acid sequences of  $\beta$ -fructosidase *SUC* genes also indicates a close genetic relationship between the species *S. jurei* and *S. mikatae*. Biological species *S. cerevisiae*,

*S. kudriavzevii* and *S. paradoxus* have collinear karyotypes. There are two reciprocal translocations in the *S. jurei* karyotype, one of which is unique (between chromosomes I and XIII), and the second is common with *S. mikatae*: VI/VII. Reciprocal translocations in the karyotypes of *S. arboricola*, *S. bayanus* and *S. cariocanus* are species-specific. It was established that only chromosome III carrying the mating type locus *MAT* has approximately the same size in all eight species of the genus *Saccharomyces*. The adaptive role of reciprocal translocations of chromosomes is discussed.

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**VII Section for Genetics and Evolutionary Biology, Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, NO-0316 Oslo, Norway. Communicated by David Peris Navarro <[d.p.navarro@ibv.uio.no](mailto:d.p.navarro@ibv.uio.no)>.**

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New papers. See abstracts under Dr. Hittinger’s communication.

- 1 Langdon QK, Peris D, Eizaguirre JI, Ofulente DA, Buh KV, Sylvester K, Jarzyna M, Rodríguez ME, Lopes CA, Libkind D, Hittinger CT. 2020. Postglacial migration shaped the genomic diversity and global distribution of the wild ancestor of lager-brewing hybrids. *Plos Genetics* 16 (4): e1008680
  - 2 Libkind D, Peris D, Cubillos FA, Steenwyk JL, Ofulente DA, Langdon QK, Bellora N, Rokas A, Hittinger CT. 2020. Into the wild: new yeast genomes from natural environments and new tools for their analysis. *FEMS Yeast Research* 20(2):foaa008
  - 3 Peris D, Alexander WG, Fisher KJ, Moriarty RV, Basuino MG, Ubbelohde EJ, Wrobel RL, Hittinger CT. 2020. Synthetic hybrids of six yeast species. *Nature Communications* 11(1):2085
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**VIII Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Dubravská cesta 9, 845 38 Bratislava, Slovak Republic. Communicated by Hana Schusterova <[hana.schusterova@savba.sk](mailto:hana.schusterova@savba.sk)>**

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Recent publications.

- 1 Vadkertiová R, Dudášová H, Stratilová E, Balaščáková M. 2019. Diversity of yeasts in the soil adjacent to fruit trees of the Rosaceae family. *Yeast* 2019, 1-14. DOI: 10.1002/yea.3430

Yeasts are common constituents of different types of soil. Their diversity depends on the season, the type and depth of the soil, the plant species, and the locality. In this study, diversity of yeasts isolated from the soil adjacent to five fruit trees (apple, apricot, peach, pear, and plum) in two localities (in Slovakia) in four sampling periods was examined. Our results demonstrated differences in the species richness and evenness among the yeast populations, which inhabited the soil beneath individual fruit tree species in both localities. Altogether, 32 ascomycetous and 27 basidiomycetous yeast species were discovered. The highest species richness was found in the soil adjacent to the apricot trees. *Galactomyces candidum*,

*Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Schwanniomyces capriottii*, and *Tausonia pullulans*, as well as the genus *Apiotrichum*, were present in soil samples in all samplings. Two species of the genus *Holtermanniella* (*H. festucosa* and *H. takashimae*) were exclusively isolated during Sampling IV in April. *Cyberlindnera* spp., *Clavispora reshetovae*, *S. capriottii*, and *Trichosporon asahii* were found only in one of two localities. Ascomycetous yeasts were present more frequently than their basidiomycetous counterparts in the three samplings (one in June and two in October); they formed from 65.6% to 70.8% of the total yeast population, whereas basidiomycetous yeasts prevailed in the April sampling (61.2%).

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**IX Canadian Institute of Fermentation Technology, Dalhousie University, Halifax, NS B3J 2X4, Canada and International Centre of Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland. Alex Speers <[Alex.Speers@Dal.Ca](mailto:Alex.Speers@Dal.Ca)>**

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Recent publications.

- 1 Speers RA and Mishra A. 2020. Wort boil time and trub effects on fermentability. Submitted to the J. ASBC.
- 2 Huisman M, Gormley F, Dzait D, Speers RA and Maskell DL. 2020. Identifying and diagnosing the source of unfilterable beer haze. Submitted to J. ASBC.
- 3 Reid SJ, Speers RA, Lumsden WB and Maskell DL. 2020. Pre-fermentation of malt whisky wort using *Lactobacillus plantarum* and its influence on new-make spirit character. *Food Chem.* 320 - online and 1 Aug 2020.
- 4 Rudolph A, MacIntosh AJ, Speers RA, St. Mary C. 2019. Modelling yeast in suspension during laboratory and commercial fermentations to detect aberrant fermentation processes. *J. ASBC.* 78:63-73

Presentations *Subject to potential delay due to Covid-19*

- 5 Speers RA, Reid S J and Maskell DL. 2020. Characterising fermentation behaviour Submitted to the World Brewing Congress, St. Paul, MN. Aug. 2020.
- 6 Reid SJ, Speers RA, Lumsden WB and Maskell DL. 2020. Scotch malt whisky new make spirit quality: The application of mathematical models. Submitted to the Young Scientist Symposium Cambridge, ENG. Sept. 27-30.
- 7 Reid SJ, Speers RA, Lumsden WB and Maskell DL. 2020. The application of high gravity fermentations for Scotch malt whisky production. Submitted to the Worldwide Distilled Spirits Conference. Edinburgh, SCT.

- 8 Reid SJ, Speers RA, Lumsden WB and Maskell DL. 2020. Modeling the effects of yeast format and pitching rate for Scotch malt whisky fermentations. Submitted to the Worldwide Distilled Spirits Conference. Edinburgh, GBR.

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**X Communication by Nguyen Huu-Vang <[vangng08@yahoo.fr](mailto:vangng08@yahoo.fr)>.**

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Recent publications.

- 1 Langdon QK, Peris D, Baker EP, Opulente DA, Nguyen HV, Bond U, Gonçalves P, Sampaio JP, Libkind D, Hittinger CT. 2019. Fermentation innovation through complex hybridization of wild and domesticated yeasts. *Nat Ecol Evol* 3:1576–1586. doi: 10.1038/s41559-019-0998-8.
- 2 Thomas S, Sanya DRA, Fouchard F, Nguyen HV, Kunze G, Neuvéglise C, Crutz-LeCoq AM. 2019. *Blastobotrys adenivorans* and *B. raffinosifermentans*, two sibling yeast species which accumulate lipids at elevated temperatures and from diverse sugars. *Biotechnol Biofuels* 12:154. doi: 10.1186/s13068-019-1492-x.

**Some brief comments.**

Retired since 2013, I continue to be interested in yeast classification and the new developments in genomics. In 1991 when I started with Yeast Taxonomy as Curator of the French Yeast Collection named CLIB (Collection de Levures d'Intérêt Biotechnologique) I had to face the difficulty of identifying by classical techniques yeast species designated as *Saccharomyces sensu stricto*, in particular *S. bayanus*, which included *S. uvarum*.

Langdon et al. 2019 (above) explored genomes of 122 hybrids of which 38 are *S. eubayanus* × *S. uvarum*, including CBS 380, which was sequenced three times with strains kept in different collections. In all cases CBS 380 carries a nuclear genome composed of *S. eubayanus*-*S. uvarum* subgenomes (ca. 33% *eubayanus*-67% *uvarum*) and also the *S. uvarum* mitochondrion.

CBS 380, the type strain of *S. bayanus*, is reclassified as a contaminant of the brewing environment. Its genome contains two functional *S. uvarum* *FDC1* and *PAD1* alleles which were known to cause undesired flavors as remarked by Will in 1891. The genomic data revealed that *S. bayanus* is a hybrid and so its species status became obsolete. One of its two parental species is *S. uvarum*, a pure species that is no longer a synonym. See review:

Borneman AR, Pretorius IS. Genomic insights into the *Saccharomyces sensu stricto* complex. *Genetics*. 2015 199:281-91.

In an article from Gallone et al. (below) 13 other *S. uvarum* × *S. eubayanus* hybrids from Lambic (Belgian Beer) and a brewing contaminant were also examined by whole genome sequencing and pan-

genome analyses:

Gallone B, Steensels J, Mertens S, Dzialo MC, Gordon JL, Wauters R, Theßeling FA, Bellinazzo F, Saels V, Herrera-Malaver B, Prah T, White C, Hutzler M, Meußdoerffer F, Malcorps P, Souffriau B, Daenen L, Baele G, Maere S, Verstrepen KJ. 2019. Interspecific hybridization facilitates niche adaptation in beer yeast. *Nat Ecol Evol*. 3:1562–1575.

These studies showed also the existence of many types of *Saccharomyces* hybrids (*cerevisiae* × *eubayanus*, *cerevisiae* × *uvarum*, *cerevisiae* × *kudriavzevii*) that are telling for yeast classification: many *Saccharomyces* hybrids have been mistaken for as pure species. Yeast taxonomy is enriched with the new field dealing with hybrids, natural or constructed. Whole genome sequencing and comparative genomics has allowed researchers to detect hybrids, broadening our knowledge of genome evolution.

An example in natural hybrids:

Saubin M, Devillers H, Proust L, Brier C, Grondin C, Pradal M, Legras JL, Neuvéglise C. 2020. Investigation of genetic relationships between *Hanseniaspora* species found in grape musts revealed interspecific hybrids with dynamic genome structures. *Front Microbiol* 10:2960.

An example for constructed hybrids:

Szabó A, Antunovic Z, Karanyicz E, Sipiczki M. 2020. Diversity and postzygotic evolution of the mitochondrial genome in hybrids of *Saccharomyces* species isolated by double sterility barrier. *Front Microbiol* 07 May 2020

Lastest review on the genus *Saccharomyces*.

Alsammar H, Delneri D. 2020. An update on the diversity, ecology and biogeography of the *Saccharomyces* genus. *FEMS Yeast Res.* 20(3):foaa013.

The eight *Saccharomyces* species are *S. cerevisiae*, *S. paradoxus*, *S. eubayanus*, *S. uvarum*, *S. kudriavzevii*, *S. arboricola*, *S. mikatae* and *S. jurei* (from Table 1. Common niches and global distribution of the wild *Saccharomyces* populations). For *Saccharomyces* hybrids see Figure 2. Common *Saccharomyces* hybrids and the source of their isolation.

Articles in which *Saccharomyces uvarum* species name was used (15 articles published until November 14<sup>th</sup> 2019):

- 1 Gorter de Vries AR, Pronk JT, Daran JG. Lagerbrewing yeasts in the era of modern genetics. *FEMS Yeast Res* 19:foz063.
- 2 Langdon QK, Peris D, Baker EP, Oplente DA, Nguyen HV, Bond U, Gonçalves P, Sampaio JP, Libkind D, Hittinger CT. 2019. Fermentation innovation through complex hybridization of wild and domesticated yeasts. *Nat Ecol Evol.* 3:1576–1586.
- 3 Gallone B, Steensels J, Mertens S, Dzialo MC, Gordon JL, Wauters R, Theßeling FA, Bellinazzo F, Saels V, Herrera-Malaver B, Prah T, White C, Hutzler M, Meußdoerffer F, Malcorps P, Souffriau B, Daenen L, Baele G, Maere S, Verstrepen KJ. 2019. Interspecific hybridization facilitates niche adaptation in beer yeast. *Nat Ecol Evol.* 3:1562–1575.
- 4 Lancaster SM, Payen C, Smukowski Heil C, Dunham MJ. Fitness benefits of loss of heterozygosity in *Saccharomyces* hybrids. *Genome Res* 29:1685-1692.
- 5 Smukowski Heil CS, Large CRL, Patterson K, Hickey AS, Yeh CC, Dunham MJ. 2019. Temperature preference can bias parental genome retention during hybrid evolution. *PLoS Genet.* 15(9):e1008383.
- 6 Li XC, Fay JC. 2019. Multiple changes underlie allelic divergence of *cup2* between *Saccharomyces* species. *G3 (Bethesda)* 9:3595-3600.
- 7 Melisa González Flores, María Eugenia Rodríguez, Andrea C. Origone, Juan Martín Oteiza, Amparo Querol and Christian Ariel Lopes. 2019. *Saccharomyces uvarum* isolated from patagonian ciders shows excellent fermentative performance for low temperature cidermaking. *Food Research International* 126:108656.
- 8 Su Y, Gamero A, Rodríguez ME, Lopes CA, Querol A, Guillamón JM. 2019 Interspecific hybridisation among diverse *Saccharomyces* species: A combined biotechnological solution for low-temperature and nitrogen-limited wine fermentations. *Int J Food Microbiol.* 310:108331.
- 9 Morgan SC, McCarthy GC, Watters BS, Tantikachornkiat M, Zigg I, Cliff MA, Durall DM. 2019. Effect of sulfite addition and pied de cuve inoculation on the microbial communities and sensory profiles of Chardonnay wines: dominance of indigenous *Saccharomyces uvarum* at a commercial winery. *FEMS Yeast Res.* 19(5):foz049.
- 10 Sanchez MR, Payen C, Cheong F, Hovde BT, Bissonnette S, Arkin AP, Skerker JM, Brem RB, Caudy AA, Dunham MJ. 2019. Transposon insertional mutagenesis in *Saccharomyces uvarum* reveals trans-acting effects influencing species-dependent essential genes. *Genome Res.* 29:396-406.
- 11 Su Y, Origone AC, Rodríguez ME, Querol A, Guillamón JM, Lopes CA. 2019 Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species indifferent nitrogen conditions. *Int J Food Microbiol* 291:111-120.
- 12 Petrizelli M, de Vienne D, Dillmann C. 2019. Decoupling the variances of heterosis and inbreeding effects is evidenced in yeast's life-history and proteomic traits. *Genetics* 211(2):741-756.
- 13 Li XC, Peris D, Hittinger CT, Sia EA, Fay JC. 2019. Mitochondria-encoded genes contribute to evolution of heat and cold tolerance in yeast. *Sci Adv* 5(1):eaav1848.
- 14 Alsammar HF, Naseeb S, Brancia LB, Gilman RT, Wang P, Delneri D. 2019. Targeted metagenomics approach to capture the biodiversity of *Saccharomyces* genus in wild environments. *Environ Microbiol Rep* 11(2):206-214.
- 15 García-Ríos E, Guillén A, de la Cerda R, Pérez-Través L, Querol A, Guillamón JM. 2019. Improving the cryotolerance of wine yeast by interspecific hybridization in the genus *Saccharomyces*. *Front Microbiol* 9:3232.

I am pleased to announce that I have been granted the status of Professor Emeritus, effective July 1<sup>st</sup> 2020. I plan on continuing my scholarly activities, but this is likely to mark the end of my teaching career, which began 47 years ago at Macdonald College with a course in Introductory Microbiology for Diploma in Agriculture students. The highlight was retrieving rumen content from a fistulated cow for microscopy and fermenting cider to be consumed at the graduation party. As a PhD candidate at UC Davis, among other things, I taught a course entitled “The Life of Yeasts”, as Herman Phaff’s sabbatical replacement. At UWO, I was involved in numerous courses, including Microbiology for Nutrition students, Introductory Mycology, a senior course on Yeasts, an introduction to growth kinetics and fermented beverages in an Applied Microbiology course. A graduate course on Yeast Systematics gradually transmuted into a senior course in Biosystematics and Phylogenetics, the last seven lectures of which were offered online this spring

from the comfort of my own home. Yeast systematics is also a topic that I enjoyed discussing as an instructor in five international courses held in Portugal, Mexico, and Brazil. I also taught so-called superclasses (700 students at a time) in genetics as well as ecology and evolution in our first year Introductory Biology course, and, for the longest time running, an introduction to Evolutionary Genetics, complete with laboratories entailing Excel simulations of major evolutionary processes, the development of which I am quite proud. A later graduate course dealt with the public perception of science, with a special focus on evolution and genetics. As of late, I taught (myself and the students both) Bioethics, fulfilling, at least in part, my teenage dream of becoming a card-carrying philosopher.

I have immensely enjoyed sharing knowledge with students, but my greatest satisfaction came when the flow of knowledge was from them to me.

The following paper, whose abstract appeared in the December 2019 issue, is now in print.

- 1 Santos ARO, Lee DK, Ferreira AG, do Carmo MC, Rondelli VM, Barros KO, Hsiang T, Rosa CA, Lachance MA. 2020. The yeast community of *Conotelus* sp. (Coleoptera: Nitidulidae) in Brazilian passionfruit flowers (*Passiflora edulis*) and description of *Metschnikowia amazonensis* sp. nov., a large-spored clade yeast. *Yeast* 37: 253-260.

Recently accepted papers.

- 2 Lee DK, Santos ARO, Hsiang T, Rosa CA, Lachance MA. 2020. Catching speciation in the act—act 2: *Metschnikowia lacustris* sp. nov., a sister species to *Metschnikowia dekortorum*. *Antonie van Leeuwenhoek* <https://doi.org/10.1007/s10482-020-01395-5>

The isolation of a single yeast strain in the clade containing *Metschnikowia dekortorum*, in the Amazon biome of Brazil, incited us to re-examine the species boundaries within the clade. The strain (UFMG-CM-Y6306) was difficult to position relative to neighbouring species using standard barcode sequences (ITS-D1/D2 rRNA gene region). Mating took place freely with a strains of *M. bowlesiae*, *M. dekortorum*, and *M. similis*, but two-spored asci, indicative of a fertile meiotic progeny, were formed abundantly only with certain strains of *M. dekortorum*. Accordingly, we examined mating success among

every phylotype in the clade and constructed a phylogeny based on a concatenation of 100 of the largest orthologous genes annotated in draft genomes. The analyses confirmed membership of the Amazonian isolate in *M. dekortorum*, but also indicated that the species should be subdivided into two. As a result, we retain three original members of *M. dekortorum* in the species, together with the new isolate, and reassign six isolates recovered from Mesoamerican lacustrine habitats to *Metschnikowia lacustris* sp. nov. The type is UWOPS 12-619.2 $\tau$  (isotype CBS 16250 $\tau$ ). MycoBank: MB 833751.

- 3 Morais CG, Sena LMF, Lopesa MR, Santos ARO, Barrosa KO, Alves CR, Uetanabaro APT, Lachance MA, Rosa CA. 2020. Production of ethanol and xylanolytic enzymes by yeasts inhabiting rotting wood isolated in sugarcane bagasse hydrolysates. *Fungal Biology* <https://doi.org/10.1016/j.funbio.2020.03.005>

- 4 Moreira JD, Santos ARO, Oliveira FLC, Cadete RM, Freire AL, Morais PB, Lachance MA, Rosa CA 2020 *Zygotoruspora cariocana* sp. nov., a yeast species isolated from tree bark in Brazil. <https://doi.org/10.1099/ijsem.0.004089>
- 5 Matos TTD, Teixeira JF, Macías LG, Santos ARO, Suh SO, Barrio E, Lachance MA, Rosa CA. 2020. *Kluyveromyces osmophilus* is not a synonym of *Zygosaccharomyces mellis*: reinstatement as *Zygosaccharomyces osmophilus* comb. nov. Int J Syst Evol Microbiol <https://doi.org/10.1099/ijsem.0.004182>
- 6 Adesokan I, Sanni, AI, Lachance MA 2020 Biochemical and molecular characterization of yeasts isolated from Nigerian traditional fermented food products. African Journal of Microbiological Research. In press.
- 7 Lee DK, Hsiang T, Lachance MA, Smith DR. 2020 Do *Metschnikowia* yeasts have the strangest mitochondrial genomes of all fungi? Current Biology (accepted May 2020)

By sequencing 71 mitochondrial DNAs from the yeast genus *Metschnikowia*, we uncovered one of the most extreme examples of mitochondrial intron diversity observed to date. These mitogenomes capture

nearly the entire known gene-size and intron-content range for *cox1* and *cob* across eukaryotic life and show remarkable differences in structure.

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## GMV – Italian Group of Wine Microbiology

**Communicated by Prof. Patrizia Romano, School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale Ateneo Lucano 10, 85100, Potenza (PZ), Italy**  
<[pot2930@gmail.com](mailto:pot2930@gmail.com)>.

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For some years we have formed an Italian group, the Group of Wine Microbiology (GMV), which I coordinate, concerning the microbiology of wine. It consists of 24 Italian research groups (20 Universities and 4 Centers) and is under the patronage of the Italian Academy of Vine and Wine (AIVV) and of the Italian Society of Agricultural, Food and Environmental

Microbiology (SIMTREA). The mission of the GMV is to disseminate research on wine microorganisms and to transfer the results to the world of wine production.

I have collected and communicated recent publications concerning yeasts in the wine sector from colleagues of different Italian institutions.

The following are papers from the partners of GMV published in 2019-2020 or in press.

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**GMV-I 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](mailto:blaiotta@unina.it)>.**

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- 1 De Filippis F, Aponte M, Piombino P, Lisanti M.T, Moio L, Ercolini D, Blaiotta G. 2019. Influence of microbial communities on the chemical and sensory features of Falanghina sweet passito wines. Food Res. Int, 120: 740-747.

Natural (N) as well as starter inoculated (S), inoculated with *Saccharomyces cerevisiae* M3-5; CZS, *Candida zemplinina* T13, *Zygosaccharomyces bailii* NS113 and *Saccharomyces cerevisiae* M3-5 fermentations of Falanghina must from dehydrated grape were monitored. Culture dependent analyses and amplicon-based high-throughput sequencing targeting 18S rRNA and 16S rRNA genes were used to monitor

the fungal and bacterial communities (8 sampling points during 65 days). The resulting wines were subject to both sensory evaluation and volatile organic compounds analysis. Fungal community of uninoculated musts (N) at beginning of the fermentation was mainly represented by *Aureobasidium*, *Cladosporium*, *Sclerotinia*, while *Candida*, *Debaryomyces*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Saccharo-*

*myces* and *Zygosaccharomyces* showed a very low occurrence. The dominance of *Hanseniaspora vineae* and/or *Hanseniaspora uvarum* was clear up to 29th days of fermentation. *S. cerevisiae* occurred in all the phases but become dominant only at the end of the process. The odour profiles as evaluate by Quantitative Descriptive Analysis (QDA) highlighted a significant

impact of the fungal populations on the olfactory profiles of the wines. Raisins, dried fruits, Sherry and liqueur were stronger in both S and CZS, while N was mostly discriminated by solvent/chemical and floral features. Outcomes underpin the impact of microbiota on the chemical and odour traits of Falanghina passito wines.

- 2 Lisanti M.A, Blaiotta G, Nioi C, Moio L. 2019. Alternative methods to SO<sub>2</sub> for microbiological stabilization of wine. *Compr. Rev. Food Sci. F*, 18: 455-479.

The use of sulfur dioxide (SO<sub>2</sub>) as wine additive is able to ensure both antioxidant protection and microbiological stability. In spite of these undeniable advantages, in the last two decades the presence of SO<sub>2</sub> in wine has raised concerns about potential adverse clinical effects in sensitive individuals. The winemaking industry has followed the general trend towards the reduction of SO<sub>2</sub> concentrations in food, by expressing at the same time the need for alternative control methods allowing reduction or even elimination of SO<sub>2</sub>. In the light of this, research has been strongly oriented toward the study of alternatives to the use of SO<sub>2</sub> in wine. Most of the studies have focused on methods able to replace the antimicrobial activity of SO<sub>2</sub>. This review article gives a comprehensive overview of the current state of the

art about the chemical additives and the innovative physical techniques that have been proposed for this purpose. After a focus on the chemistry and properties of SO<sub>2</sub> in wine, as well as on wine spoilage and on the conventional methods used for the microbiological stabilization of wine, recent advances on alternative methods proposed to replace the antimicrobial activity of SO<sub>2</sub> in winemaking are presented and discussed. Even though many of the alternatives to SO<sub>2</sub> showed good efficacy, nowadays no other physical technique or additive can deliver the efficacy and broad spectrum of action as SO<sub>2</sub> (both antioxidant and antimicrobial), therefore the alternative methods should be considered a complement to SO<sub>2</sub> in low sulfite winemaking, rather than being seen as its substitutes.

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**GMV-II Department of Agricultural Science, University of Sassari, Viale Italia 39, 07100 Sassari, Italy. 6th Communicated by Professor Marilena Budroni**  
<[mbudroni@uniss.it](mailto:mbudroni@uniss.it)> <https://www.uniss.it>.

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- 1 Feghali N, Albertin W, Tabet E, Rizk Z, Bianco A, Zara G, Masneuf-Pomarede I, Budroni M. 2019. Genetic and phenotypic characterisation of a *Saccharomyces cerevisiae* population of ‘Merwah’ white wine. *Microorganisms*. 7, 492; doi:10.3390/microorganisms7110492

The study of yeast biodiversity represents an important step in the preservation of the local heritage, and this work in particular has an innovative character since no further studies have investigated ‘Merwah’, one of the main grape varieties used in winemaking in Lebanon. To gain deeper knowledge of the genetic diversity and population structure of native *Saccharomyces cerevisiae* wine strains, 202 isolates were collected during spontaneous alcoholic fermentation of eight must/wine samples of cultivar ‘Merwah’, over two consecutive years (2016, 2017) in a traditional winery in Mount Lebanon (1400 m a.s.l.). The isolates were identified as *S. cerevisiae* on the basis of their morphology and preliminary sequence analysis of their internal transcribed spacer (ITS)

PCR. They were then characterised at the strain level by interdelta PCR and genotyped using multiplex PCR reactions of 12 microsatellite markers. High genetic diversity was observed for the studied population. To select potential yeast starter strains from this population, micro-fermentations were carried out for 22 *S. cerevisiae* strains that were selected as representative of the ‘Merwah’ wine yeast population in order to determine their technological and oenological properties. Three indigenous yeast strains might represent candidates for pilot-scale fermentation in the winery, based on relevant features such as high fermentation vigour, low production of volatile acidity and H<sub>2</sub>S and low residual sugar content at the end of alcoholic fermentation.

- 2 Mannazzu I, Domizio P, Carboni G, Zara S, Zara G, Comitini F, Budroni M, Ciani M. (jump link) 2019. Yeast killer toxins: from ecological significance to application. Crit. Rev, Biotechnol 39(5): 603-617

Killer toxins are proteins that are often glycosylated and bind to specific receptors on the surface of their target microorganism, which is then killed through a target-specific mode of action. The killer phenotype is widespread among yeast and about 100 yeast killer species have been described to date. The spectrum of action of the killer toxins they produce targets spoilage and pathogenic microorganisms. Thus, they have potential as natural antimicrobials in food and for biological control of

plant pathogens, as well as therapeutic agents against animal and human infections. In spite of this wide range of possible applications, their exploitation on the industrial level is still in its infancy. Here, we initially briefly report on the biodiversity of killer toxins and the ecological significance of their production. Their actual and possible applications in the agro-food industry are discussed, together with recent advances in their heterologous production and the manipulation for development of peptide-based therapeutic agents.

- 3 Zara G, van Vuuren H.J.J, Mannazzu I, Zara S, Budroni M. 2019. Transcriptomic response of *Saccharomyces cerevisiae* during fermentation under oleic acid and ergosterol depletion (Article). Fermentation, 5(3)3, 57; doi:10.3390/fermentation5030057

Under anaerobic/hypoxic conditions, *Saccharomyces cerevisiae* relies on external lipid supplements to modulate membrane lipid fraction in response to different stresses. Here, transcriptomic responses of two *S. cerevisiae* wine strains were evaluated during hypoxic fermentation of a synthetic must with/without ergosterol and oleic acid supplementation. In the absence of lipids, the two strains, namely EC1118 and M25, showed different behaviour, with M25 significantly decreasing its fermentation rate from the 72 h after inoculum. At this time point, the whole genome transcriptomic analysis revealed common and strain-specific responses to the lack of lipid supplementation. Common responses included the upregulation of the genes involved in

ergosterol biosynthesis, as well as the seripauperin and the heat shock protein multigene families. In addition, the upregulation of the aerobic isoforms of genes involved in mitochondrial electron transport is compatible with the previously observed accumulation of reactive oxygen species in the two strains during growth in absence of lipids. Considering the strain-specific responses, M25 downregulated the transcription of genes involved in glucose transport, methionine biosynthesis and of those encoding mannoproteins required for adaptation to low temperatures and hypoxia. The identification of these pathways, which are presumably involved in yeast resistance to stresses, will assist industrial strain selection.

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**GMV-III 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](mailto:angela.capece@unibas.it)>.**

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- 1 Guaragnella N, Chiara M, Capece A, Romano P, Pietrafesa R, Siesto G, Manzari C, Pesole G. 2020. Genome sequencing and comparative analysis of three *Hanseniaspora uvarum* Indigenous wine strains reveal remarkable biotechnological potential. Front Microbiol 10:3133. doi: 10.3389/fmicb.2019.03133

A current trend in winemaking has highlighted the beneficial contribution of non-*Saccharomyces* yeasts to wine quality. *Hanseniaspora uvarum* is one of the more represented non-*Saccharomyces* species onto grape berries and plays a critical role in influencing the wine sensory profile, in terms of complexity and organoleptic richness. In this work, we analyzed a group of *H. uvarum* indigenous wine strains as for genetic as for technological traits, such as resistance to SO<sub>2</sub> and β-glucosidase activity. Three strains were

selected for genome sequencing, assembly and comparative genomic analyses at species and genus level. *Hanseniaspora* genomes appeared compact and contained a moderate number of genes, while rarefaction analyses suggested an open accessory genome, reflecting a rather incomplete representation of the *Hanseniaspora* gene pool in the currently available genomes. The analysis of patterns of functional annotation in the three indigenous *H. uvarum* strains showed distinct enrichment for

several PFAM protein domains. In particular, for certain traits, such as flocculation related protein domains, the genetic prediction correlated well with relative flocculation phenotypes at lab-scale. This feature, together with the enrichment for oligo-peptide transport and lipid and amino acid metabolism domains, reveals a promising potential of these indigenous strains to be applied in fermentation

processes and modulation of wine flavor and aroma. This study also contributes to increasing the catalog of publicly available genomes from *H. uvarum* strains isolated from natural grape samples and provides a good roadmap for unraveling the biodiversity and the biotechnological potential of these non-*Saccharomyces* yeasts.

- 2 Capece A, Pietrafesa R, Siesto G, Romano P. 2020. Biotechnological approach based on selected *Saccharomyces cerevisiae* starters for reducing the use of sulphur dioxide in wine. *Microorganism*, 8, 738; doi:10.3390/microorganisms8050738.

Sulphites are considered the main additives in winemaking for their antimicrobial, antioxidant and anti-oxidasic activities. The current concern about potential negative effects of SO<sub>2</sub> on consumer health has focused the interest on replacing or reducing SO<sub>2</sub> use. Our work is aimed to develop a strategy based on the use of selected starter culture, able to perform wine fermentation without SO<sub>2</sub> addition. Four selected *Saccharomyces cerevisiae* indigenous strains were tested as mixed starter cultures in laboratory scale fermentations. The starter culture characterized by similar percentage of dominance of both strains composing the mixed starter and able to produce a wine characterized by the best combination of

chemical and aromatic characteristics was chosen. This mixed culture was tested as starter at pilot scale with and without SO<sub>2</sub> addition, by using a higher inoculum level in the vinification without SO<sub>2</sub>. The selected starter confirmed higher dominance ability in vinification without SO<sub>2</sub> addition than in SO<sub>2</sub>-added fermentation, demonstrating that the sulphite addition is not a guarantee to reach an absolute dominance of starter culture on indigenous microflora. The proposed biotechnological tool allowed to produce good quality wines possessing also “functional properties”, as NO-SO<sub>2</sub> added wines were characterized by high polyphenol content and antioxidant activity.

- 3 Capece A, Pietrafesa R, Siesto G, Romaniello R, Condelli N, Romano P. 2019. Selected indigenous *Saccharomyces cerevisiae* strains as profitable strategy to preserve typical traits of Primitivo wine. *Fermentation*, 5(4), 87; doi:10.3390/fermentation5040087.

Wine production by inoculated fermentation with commercial *Saccharomyces cerevisiae* strains is an ordinary practice in modern winemaking in order to assure the final quality of wine, although this procedure results in the production of highly homogeneous wines. The use of indigenous selected starters represents a useful tool to control alcoholic grape must fermentation, safeguarding the typical sensory characteristics of wine produced from specific regions. In this study, we selected three indigenous *S. cerevisiae* strains among 16 indigenous strains previously isolated from the spontaneous fermentation of Primitivo grapes, which were collected from the vineyards of three different cellars. The three selected

starters (one for each cellar) were tested during fermentations at pilot scale by performing in each cellar two trials: one with an indigenous starter (specific for the winery), and one with the commercial starter AWRI796 (common to all the cellars). Starter dominance ability and influence on aromatic quality of the wine were used as criteria to test the suitability of these indigenous starters to be used at the cellar scale. The results obtained in this study showed that the indigenous strains were characterized by very high dominance ability, and the aromatic quality of wine was strongly influenced both by the inoculated strain and the interaction strain/grape must.

- 1 Oro L, Canonico L, Marinelli V, Ciani M, Comitini F. 2019. Occurrence of *Brettanomyces bruxellensis* on grape berries and in related winemaking cellar. *Front. Microbiol*, 10: 415. doi: 10.3389/fmicb.2019.00415.

The spoilage yeasts belonging to the genus *Dekkera* (anamorph *Brettanomyces*) are associated with the fermentation process and can be responsible for off-flavors in wine. *Brettanomyces bruxellensis* is difficult to isolate from natural environments because of its low diffusion, low presence on the grape surface and low competition capacity, slow growth, and VBNC (viable but not culturable) state, even when selective media are used. In this study, to investigate the origins and occurrence of *B. bruxellensis* in winemaking, a total of 62 samples from grapes, winery environment, and fermenting musts were taken through direct isolation with a selective medium. *B. bruxellensis* was not directly detected in the grape samples but was instead widely isolated from the winery environment samples. However, using a

combination of enrichment and selective media, eight of fifteen grape samples were positive for *B. bruxellensis*. Analysis of the genetic traits of the isolates indicated a strict relationship among the strains from the vineyard and the winery. Isolates from the vineyard and the winery were both part of the more common and dominant biotypes suggesting that the vineyard may be the contamination source of *B. bruxellensis* in the winery environment. For this, grapes may represent the possible primary origin source from which a flow toward the winery environment originates. On the other hand, the wide occurrence of *B. bruxellensis* in winery indicates that this environment can be considered as the favorable ecological niche for colonization and diffusion of these yeast.

- 2 Canonico L, Solomon M, Comitini F, Ciani M, Varela C. 2019. Volatile profile of reduced alcohol wines fermented with selected non-*Saccharomyces* yeasts under different aeration conditions. *Food Microbiol*, 103247 doi: 10.1016/j.fm.2019.103247

Over the last decades there has been an increase in ethanol concentration in wine. High ethanol concentration may impact negatively wine flavor and can be associated with harmful effects on human health. In this study, we investigated a microbiological approach to reduce wine ethanol concentration, using three non-*Saccharomyces* yeast strains (*Metschnikowia pulcherrima*, *Torulasporea delbrueckii* and *Zygosaccharomyces bailii*) in sequential fermentations with *S. cerevisiae* under different aeration conditions. At the same time, we evaluated the volatile profile of the resulting reduced alcohol Chardonnay wines. Results showed that the non-*Saccharomyces* yeasts tested were able to reduce wine ethanol concentration when oxygen was provided. Compared to *S. cerevisiae* wines, ethanol reduction was 1.6% v/v, 0.9% v/v and 1.0% v/v for *M. pulcherrima*, *T. delbrueckii* and *Z. bailii* sequential fermentations, respectively. Under

the conditions evaluated here, aeration did not affect acetic acid production for any of the non-*Saccharomyces* strains tested. Although aeration affected wine volatile profiles, this was depended on yeast strain. Thus, wines produced with *M. pulcherrima* under aeration of 0.05 volume of air per volume of culture per minute (VVM) showed excessive ethyl acetate content, while *Z. bailii* wines produced with 0.05 VVM aeration had increased concentrations of higher alcohols and volatile acids. Increased concentrations of these compounds over their sensory thresholds, are likely to impact negatively on wine sensory profile. Contrarily, all three non-*Saccharomyces* strains under 0.025 VVM aeration conditions produced wines with reduced ethanol concentration and acceptable chemical volatile profiles.

- 1 Englezos V, Pollon M, Rantsiou K, Ortiz-Julien A, Botto R, Río Segade S, Giacosa S, Rolle L, Cocolin L. 2019. *Saccharomyces cerevisiae*-*Starmerella bacillaris* strains interaction modulates chemical and volatile profile in red wine mixed fermentations. *Food Res. Int.*, 122: 392-401. doi: 10.1016/j.foodres.2019.03.072

Mixed fermentations using selected non-*Saccharomyces cerevisiae* strains has been proposed to modulate wine particular characteristics. Among the non-*Saccharomyces* yeasts, *Starmerella bacillaris* have gained a great attention for its ability to reduce ethanol content, increase glycerol amount and total acidity, and improve chromatic characteristics of the wines. However, *Starm. bacillaris* performances are affected by the interactions established with different *S. cerevisiae* strains. Therefore, the aim of this study was to understand the impact of *S. cerevisiae* strain selection on *Starm. bacillaris* in mixed fermentations. With this purpose, ten different commercial *S. cerevisiae* strains were inoculated 48 h after a well-characterized *Starm. bacillaris* strain. Results showed that growth dynamics and fermentation behavior of *Starm. bacillaris* were

influenced in a couple-dependent manner. In general, mixed fermentations showed reduced ethanol, acetic acid and ethyl acetate levels and increased, glycerol amounts, compared to wines produced from *S. cerevisiae* in pure fermentations. Furthermore, in mixed fermentations an increase in volatile compounds when compared to the pure culture fermentations was observed. In particular, it was possible to note a general increase of some specific higher alcohols, acids and esters like isoamyl alcohol, isobutanol, isovaleric acid, hexanol and ethyl hexanoate. This study highlighted that *S. cerevisiae* strain choice plays a key role and affects *Starm. bacillaris* performance during fermentation and, consequently, the final composition of the wines in terms of technological parameters and volatile organic compounds.

- 2 Englezos V, Rantsiou K, Torchio F, Pollon M, Giacosa S, Río Segade S, Gerbi V, Rolle L, Cocolin L. 2019. Efficacy of ozone against different strains of *Brettanomyces bruxellensis* on winegrapes postharvest and impact on wine composition. *Am. J. Enol. Vitic.*, 70(3): 249-258. doi: 10.5344/ajev.2019.18058

*Brettanomyces bruxellensis* is one of the most feared spoilage wine yeasts. For this reason, several preventive actions have been developed to control this unwanted yeast during winemaking. In this study, the efficacy of ozone to control *B. bruxellensis* population was evaluated. With this purpose, a mix of three *B. bruxellensis* strains was inoculated on post-harvest Barbera berries. Subsequently, grapes were ozone-treated either in aqueous (6 or 12 min) or gaseous forms (12 or 24 h) and alcoholic fermentation was performed using a *Saccharomyces cerevisiae* strain. Results demonstrated that all ozone treatments reduced *B. bruxellensis* viability on grapes, but the greatest impact was observed for the longer treatment with gaseous ozone (24 h). Furthermore, *B. bruxellensis* growth during fermentation was discouraged by all

ozone treatments. In particular, wines from grape berries treated with gaseous ozone form for 12 and 24 h had the lowest population density of the spoilage yeast after seven days of fermentation. In all treatments, *B. bruxellensis* population increased when *S. cerevisiae* cells decreased. A total of 480 *B. bruxellensis* colonies were isolated from grape berries and during the fermentation. The analysis of molecular fingerprints showed that all three strains had similar sensitivity to the ozone treatments applied. Chemical analyses of obtained wines demonstrated that the gaseous ozone treatment for 24 h produced wines with the lowest acetic acid content, while in wines produced from grape berries treated with water (6 or 12 min), 4-ethylphenols were detected.

- 3 Englezos V, Castrillo Cachón D, Rantsiou K, Blanco P, Petrozziello M, Pollon M, Giacosa S, Río Segade S, Rolle L, Cocolin L. 2019. Effect of mixed species alcoholic fermentation on growth and malolactic activity of lactic acid bacteria. *Appl. Microbiol. Biotechnol*, 103: 7687-7702. Doi: 10.1007/s00253-019-10064-1.

In this study pure and mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* strains were conducted. Furthermore, two Lactic Acid bacteria (LAB) species (*Oenococcus oeni* and *Lactobacillus plantarum*) were inoculated at the beginning or at the end of the alcoholic fermentation (AF) to conduct malolactic fermentation (MLF). The purpose of this study was to elucidate the effect of the above-mentioned yeast inoculation protocol on the malolactic activity of LAB and on the chemical, chromatic and volatile profiles of Nebbiolo wines. In general, the early inoculation of the LAB reduced by up to 2 weeks the vinification time. In these conditions, *L. plantarum* completed MLF faster than

*O. oeni*. However, this LAB completed MLF only when inoculated at the beginning of the AF. LAB performances, particularly *O. oeni*, were promoted by the presence of *Starm. bacillaris*. The main metabolites of oenological interest were mainly affected by the presence of non-*Saccharomyces* yeast. Divergently, the inoculated LAB species had a greater impact on the volatile and chromatic profile of the wines than the inoculation time. These results help to better control the MLF in mixed fermentation with *Starm. bacillaris* and *S. cerevisiae* thus obtaining wines with higher quality, in terms of chemical, colour and aroma point of view.

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**GMV-VI Laboratory of Food Microbiology, Department of Agronomy, Food, Natural resources, Animals and Environment - DAFNAE, University of Padova (UNIPD), Viale dell'Università, 16 35020, Legnaro (DP), Italy. Communicated by Professor Viviana Corich <[viviana.corich@unipd.it](mailto:viviana.corich@unipd.it)> [www.dafnae.unipd.it](http://www.dafnae.unipd.it)**

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- 1 Lemos Junior W.J.F, Nadai C, Rolle L, da Silva Gulao E, Miguez da Rocha Leão L.H, Giacomini A, Corich V, Vincenzi S. 2020. Influence of the mannoproteins of different strains of *Starmerella bacillaris* used in single and sequential fermentations on foamability, tartaric and protein stabilities of wines. *OENO one*, 54 No. 2; doi.org/10.20870/oenone.2020.54.2.2948.

**Aim:** In this work, seven strains of *Starmerella bacillaris* were analysed for their ability to release polysaccharides during alcoholic fermentation (AF), both in single-strain and in sequential AF together with *Saccharomyces cerevisiae*. **Methods and Results:** A synthetic polysaccharide-free must was used to characterise the mannoproteins (MPs) released. The MPs were quantified, characterised in terms of carbohydrate composition, and tested to assess their ability to reduce protein and tartrate instabilities and their ability to affect the foaming properties of wine.

**Conclusions:** All the tested strains in sequential AF increased the total MPs production. Moreover, the strains affected the MPs properties in different ways regarding tartaric and protein stabilities. The MPs released in sequential AF by some *S. bacillaris* strains showed a significant effect on protein stabilisation and tartaric stability. An effect on the foamability was found for MPs obtained in single-strain AFs of *S. bacillaris*. **Significance and impact of the study:** An improvement in wine stability can be achieved using the sequential AF.

- 2 Nadai C, Vendramin C, Carlot M, Andrighetto C, Giacomini A, Corich V. 2019. Dynamics of *Saccharomyces cerevisiae* strains isolated from Vvine bark in vineyard: influence of plant age and strain presence during grape must spontaneous fermentations. *Fermentation*, 5(39): 62; doi:10.3390/fermentation5030062.

In this study, two vineyards of different age were chosen. During three years, a sampling campaign was performed for isolating vineyard-associated *Saccharomyces cerevisiae* (*S. cerevisiae*) strains. Bark portions and, when present, grape bunches were

regularly collected from the same vine plants during the overall sampling period. Each bark portion was added to a synthetic must, while each grape bunch was manually crushed, and fermentations were run to isolate *S. cerevisiae* strains. All collected yeasts were

identified at different species and strain levels to evaluate the genetic variability of *S. cerevisiae* strains in the two vineyards and strains dynamics. Moreover, bark-associated strains were compared with those isolated from spontaneous fermentations of grapes collected during the two harvests. Regarding the youngest vineyard, no *S. cerevisiae* was identified on bark and grape surface, highlighting the importance of vine age on yeast colonization. Results reported the isolation of *S. cerevisiae* from vine bark of the old

vineyard at all sampling times, regardless of the presence of the grape bunch. Therefore, this environment can be considered an alternative ecological niche that permanently hosts *S. cerevisiae*. Bark-associated strains were not found on grape bunches and during pilot-scale vinifications, indicating no significant strain transfer from vine bark to the grape must. Commercial starters were identified as well both in vineyards and during vinifications.

- 3 Lemos Junior W.J.F, Nadai C, Crepalde L.T, de Oliveira V.S, de Matos A.D, Giacomini A, Corich V. 2019. Potential use of *Starmerella bacillaris* as fermentation starter for the production of low-alcohol beverages obtained from unripe grapes. *Int. J. Food Microbiol*, 303: 1-8  
doi: 10.1016/j.ijfoodmicro.2019.05.006.

To obtain beverages with reduced alcohol content, the use of unripe grapes, with low sugar and high malic acid concentration, was recently explored. Due to the low sugar, ethanol and glycerol production is limited during fermentation affecting important sensory aspects such as the palate fullness of these beverages. The high acidity influences their organoleptic quality, as well. So far, only *S. cerevisiae* starter, used in conventional fermentations, have been tested in this condition, and no selection has been performed to identify alternative yeasts suitable for unripe grape fermentation. *S. bacillaris* is known for the low ethanol tolerance, high glycerol and moderate volatile acidity production. Therefore, this non-*Saccharomyces* yeast have been investigated to improve the quality of low-alcohol beverages. Seven

*S. bacillaris* strains were tested in synthetic musts with different sugar and malic acid levels, mimicking natural ripe and unripe grape musts. In all the tested conditions, *S. bacillaris* produced higher glycerol than *S. cerevisiae*. In single-strain fermentation at low sugar and high malic acid no *S. bacillaris* strains was able to transform all the sugars, although the produced ethanol was lower than that at high sugar condition. Therefore, sequential fermentations with *S. cerevisiae* were evaluated at low sugar and high malic acid. In this condition all the sugars were consumed and a significant glycerol increase was found. These results were confirmed when sequential fermentations were run in natural unripe grape must. Moreover, an increase in malic acid degradation, with respect to EC1118 single-strain fermentation, was observed.

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**GMV-VII DAGRI (Department of Agriculture, Food Environment & Forestry- University of Florence- Italy, Piazzale delle Cascine 18, I-50144 Florence, Italy and FoodMicroTeam s.r.l. Academic Spin Off, Via Santo Spirito 14, I-50125 Florence, Italy Communicated by Professor Lisa Granchi <[lisa.granchi@unifi.it](mailto:lisa.granchi@unifi.it)> [www.foodmicroteam.it](http://www.foodmicroteam.it).**

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- 1 Granchi L, Ganucci D, Buscioni G, Mangani S. Guerrini S. 2019. The biodiversity of *Saccharomyces cerevisiae* in spontaneous wine fermentation: The occurrence and persistence of winery-strains. *Fermentation*, 5: 86; doi: 10.3390/fermentation5040086.

*Saccharomyces cerevisiae* populations occurring in spontaneous wine fermentations display a high polymorphism, although few strains are generally able to dominate the fermentative process. Recent studies have suggested that these indigenous *S. cerevisiae* strains are representative of a specific oenological ecosystem, being associated to a given wine-producing area or a single winery. In contrast, according to other ecological studies, no correlation between genotypic and phenotypic groups of the native *S. cerevisiae*

strains and their origin was found. In this work, several *S. cerevisiae* strains were isolated in consecutive years from spontaneous fermentations carried out in the same wineries located in different oenological areas in Tuscany, and their persistence was assessed by molecular methods. Some predominant *S. cerevisiae* strains persisted in different fermentations in the same winery from one year to another and they seemed to be representative of a single winery rather than of an oenological area. Therefore, data suggested

the idea of the “winery effect” or a microbial terroir at a smaller scale. The use of these typical strains as

starter yeasts could provide wines with the distinctive characteristics of a particular winery or sub-zone.

- 2 Ganucci D, Guerrini S, Mangani S, Vincenzini M, Granchi L. 2018. Quantifying the effects of ethanol and temperature on the fitness advantage of predominant *Saccharomyces cerevisiae* strains occurring in spontaneous wine fermentations. *Front. Microbiol*, 9: 1563. doi: 10.3389/fmicb.2018.01563.

Different *Saccharomyces cerevisiae* strains are simultaneously or in succession involved in spontaneous wine fermentations. In general, few strains occur at percentages higher than 50% of the total yeast isolates (predominant strains), while a variable number of other strains are present at percentages much lower (secondary strains). Since *S. cerevisiae* strains participating in alcoholic fermentations may differently affect the chemical and sensory qualities of resulting wines, it is of great importance to assess whether the predominant strains possess a “dominant character.” Therefore, the aim of this study was to investigate whether the predominance of some *S. cerevisiae* strains results from a better adaptation capability (fitness advantage) to the main stress factors of oenological interest: ethanol and temperature. Predominant and secondary *S. cerevisiae* strains from different wineries were used

to evaluate the individual effect of increasing ethanol concentrations (0-3-5 and 7% v/v) as well as the combined effects of different ethanol concentrations (0-3-5 and 7% v/v) at different temperature (25–30 and 35°C) on yeast growth. For all the assays, the lag phase period, the maximum specific growth rate ( $\mu_{max}$ ) and the maximum cell densities were estimated. In addition, the fitness advantage between the predominant and secondary strains was calculated. The findings pointed out that all the predominant strains showed significantly higher  $\mu_{max}$  and/or lower lag phase values at all tested conditions. Hence, *S. cerevisiae* strains that occur at higher percentages in spontaneous alcoholic fermentations are more competitive, possibly because of their higher capability to fit the progressively changing environmental conditions in terms of ethanol concentrations and temperature.

- 3 Guerrini S, Mangani S, Romboli Y, Luti S, Pazzagli L, Granchi L, 2018. Impact of *Saccharomyces cerevisiae* strains on health-promoting compounds in wine. *Fermentation*, 4: 26; doi:10.3390/fermentation4020026

Moderate wine consumption is associated with human health benefits (reduction of cardiovascular risk and neurodegenerative diseases, decrease of onset of certain cancers) attributed to a series of bioactive compounds, mainly polyphenols, with antioxidant power capable of counteracting the negative action of free radicals. Polyphenols are naturally present in the grapes, but an additional amount originates during winemaking. The aim of this work was to assess the ability of four commercial and two indigenous *Saccharomyces cerevisiae* strains to produce bioactive compounds (tyrosol, hydroxytyrosol, tryptophol, melatonin and glutathione) during alcoholic fermentation. In order to exclude the fraction of antioxidant compounds naturally occurring in grapes, the strains were inoculated in a synthetic must. At the

end of fermentation the bioactive compounds were analysed by High-Performance Liquid Chromatography, while antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Moreover, freeze-dried samples, originating from the experimental wines, were used to perform ex-vivo assays on cultured cells (RAW 264.7 murine macrophages) with the aim to evaluate their antioxidant and anti-inflammatory activities. The results indicated that the production of the considered bioactive compounds is a strain-specific property; therefore, the different yeast strains utilized during fermentation have different capabilities to modify the antioxidant and anti-inflammatory properties of the wine.

- 1 Capozzi V, Berbegal C, Tufariello M, Grieco F, Spano G, Grieco F. 2019. Impact of co-inoculation of *Saccharomyces cerevisiae*, *Hanseniaspora uvarum* and *Oenococcus oeni* autochthonous strains in controlled multi starter grape must fermentations. *LWT-Food Sci. Technol*, 109: 241-249, doi: 10.1016/j.lwt.2019.04.045.

The use of multi-species starter cultures is an approach of increasing significance for winemakers in order to improve the general quality and safety of the final product. As first step of the present study, we isolated and characterize two *Saccharomyces cerevisiae* yeast starter strains, denoted as ITEM 167292 and ITEM 17293, from natural must fermentations of “Negroamaro” grapes. As second step, we studied the interactions during grape must fermentation between these two strains, the *Hanseniaspora uvarum* strain ITEM 8785 and five autochthonous *Oenococcus oeni* strains, by microbial counts and by quantifying L-malic acid and ethanol concentrations. The best performing *O. oeni* strain,

namely OT4, was used to create, with the *H. uvarum* strain ITEM 8785, two mixed starter formulations with the strains ITEM 167292 and ITEM 17293. The three microbial species showed to be compatible and to complete the fermentative processes producing wines denoted by reduced acetic acid concentrations. The performance of the mixed starter formulations were then validated by carrying pilot-scale vinifications. At the best of our knowledge, this report is the first study regarding the utilization of selected *H. uvarum*/*S. cerevisiae*/*O. oeni* autochthonous strains in a simultaneous multi-starter inoculation for the industrial production of regional typical wines.

- 2 Grieco F, Carluccio M.A, Giovinazzo G. 2019. Autochthonous *Saccharomyces cerevisiae* starter cultures enhance polyphenols content, antioxidant activity and anti-inflammatory response of Apulian red wines. *Foods*, 8: 453, doi: 10.3390/foods8100453.

Several biotic and abiotic factors can influence the amount of polyphenols in grape tissues. During the vinification the temperature, the presence of grape seeds and peel, use of enzymes, can influence the extraction of polyphenols. However, little information is still available on the effects of yeast strains used on the polyphenolic composition of wine. With this aim, two selected *Saccharomyces cerevisiae* strains, ITEM 14093 and ITEM 14077 were used to produce wine from two Italian grape cv, Primitivo and Negroamaro. At the end of the alcoholic fermentation, the content of total polyphenols of particular classes of polyphenols

(stilbenes, phenolic acids, flavonols and flavanols), and the antioxidant activity were evaluated and compared in the obtained wines. We also examined the effects of red wine extracts in culture model of vascular inflammation. The results obtained comparing wine extracts obtained by utilizing commercial or autochthonous yeast showed that wines obtained with selected yeast significantly inhibited vascular inflammation. The results are positively biased giving rise the healthy property of wine drinking.

- 3 Russo P, Tufariello M, Renna R, Tristezza M, Taurino M, Palombi L, Capozzi V, Rizzello C.G, Grieco F. 2020. New insights into the oenological significance of *Candida zemplinina*: impact of selected autochthonous strains on the volatile profile of Apulian wines. *Microorganisms*, 8: 628, doi:10.3390/microorganisms8050628

In this investigation, we explored the oenological significance of *Candida zemplinina* (syn *Starmerella bacillaris*) isolates from Apulian grape musts. Moreover, we provide the first evidence of the impact of different *C. zemplinina* strains on the wine aromatic properties tested as monocultures. We described the

diversity of *C. zemplinina* strains isolated from grapes and the variability of ‘volatile’ phenotypes associated with this intraspecific variability. Thirty-three isolates were characterized at strain level by PCR-based approach and, among these, 16 strains were identified and then tested by microfermentation tests carried out

in grape must. Analyzed strains were low producers of acetic acid and hydrogen sulphide, not able to decarboxylate a panel of representative amino acids, whereas they showed fructophilic character and significant glycerol production. Volatile profiles of produced wines were investigated by gas chromatography–mass spectrometry. The Odor Activity Values of all molecules were calculated and

12 compounds showed values above their odor thresholds. Two selected strains (35NC1 and 15PR1) could be considered as possible starter cultures since they were able to positively affect the sensory properties of obtained wine. This report firstly supplies evidence on the strain-specific impact of different *C. zemplinina* strains on the final aroma of produced wines.

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**GMV-IX Centro di Trasferimento Tecnologico. Fondazione Edmund Mach. Via Mach 1 38010 San Michele all'Adige (TN). Communicated by Dr. Raffaele Guzzon**  
<[raffaele.guzzon@fmach.it](mailto:raffaele.guzzon@fmach.it)> [www.fmach.it](http://www.fmach.it).

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- 1 Kinzurik M.I, Deed R.C, Herbst-Johnstone M, Slaghenaufi D, Guzzon R, Gardner R.C, Larcher R, Fedrizzi B. 2020. Addition of volatile sulfur compounds to yeast at the early stages of fermentation reveals distinct biological and chemical pathways for aroma formation. *Food Microbiol*, 89, 103435. DOI: 10.1016/j.fm.2020.103435.

Volatile sulfur compounds (VSCs) greatly influence the sensory properties and quality of wine and arise via both biological and chemical mechanisms. VSCs formed can also act as precursors for further downstream VSCs, thus elucidating the pathways leading to their formation is paramount. Short-term additions of exogenous hydrogen sulfide (H<sub>2</sub>S), ethanethiol (EtSH), S-ethylthio acetate (ETA), methanethiol (MeSH) and S-methylthio acetate (MTA) were made to exponentially growing fermentations of synthetic grape medium. The VSC profiles produced from live yeast cells were compared with those from dead cells and no cells. Interestingly, this experiment allowed the identification of specific biochemical

and/or chemical pathways; e.g. most of the conversion of H<sub>2</sub>S to EtSH, and the further step from EtSH to ETA, required the presence of live yeast cells, as did the conversion of MeSH to MTA. In contrast, the reaction from MTA to MeSH and ETA to EtSH was due primarily to chemical degradation. Ultimately, this research unravelled some of the complex interactions and interconversions between VSCs, pinpointing the key biochemical and chemical nodes. These pathways are highly interconnected and showcase the complexity of both the sulfur pathways in yeast and the reactive chemistry of sulfur-containing compounds.

- 2 Bordiga M, Guzzon R., Larcher R, Travaglia F, Arlorio M, Coisson J.D. 2019. Variation in content of tyramine, histamine, 2-phenylethylamine, tryptamine and their precursor amino acids in a Chardonnay wine by using different commercial active dry yeasts and nitrogen sources. *Int. J. Food Sci. Technol*, 55(2): 559-568. DOI: 10.1111/ijfs.14307.

Wine is a fermented beverage that could be affected by high concentrations of biogenic amines, thus altering organoleptic and health properties. In this work, the capacity of different selected yeast strains regarding to affect the content of tyramine, histamine, 2-phenylethylamine, tryptamine and their precursor amino acids (pAA) during fermentation has been described. Overall, biogenic amines (BAs) and pAA contents appeared significantly related both to the strain and to the nutrient supplementation applied. Among BAs, without and (with) nitrogen source

addition the concentrations covered a range between 0.1 and 9.5 (0.1-10.3) mg L<sup>-1</sup> for tyramine; 0.1 and 4.4 (0.1-4.6) mg L<sup>-1</sup> for histamine; 0.4 and 1.1 (0.4-1.4) mg L<sup>-1</sup> for 2-phenylethylamine; and 0.02 and 0.14 (0.01-0.12) mg L<sup>-1</sup> for tryptamine, respectively. Considering that also wine yeast species are responsible for BAs formation, the evidence from fermentation trials underlined the relevance of a careful choice of ADY strains and nitrogen sources in the management of alcoholic fermentation in wine.

- 1 Nardi T. Microbial resources as a tool for enhancing sustainability in winemaking. *Microorganisms* 2020, 8, 507; doi/10.3390/microorganisms8040507.

Volatile sulfur compounds (VSCs) greatly In agriculture, the wine sector is one of the industries most affected by the sustainability issue. It is responsible for about 0.3% of annual global greenhouse gas emissions from anthropogenic activities. Sustainability in vitiviculture was firstly linked to vineyard management, where the use of fertilizers, pesticides and heavy metals is a major concern. More recently, the contribution of winemaking, from grape harvest to bottling, has also been considered. Several cellar processes could be improved for reducing the environmental impact of the whole chain, including microbe-driven transformations. This paper reviews the potential of microorganisms and interactions thereof as a natural, environmentally friendly tool to improve the

sustainability aspects of winemaking, all along the production chain. The main phases identified as potentially interesting for exploiting microbial activities to lower inputs are: (i) pre-fermentative stages, (ii) alcoholic fermentation, (iii) stage between alcoholic and malolactic fermentation, (iv) malolactic fermentation, (v) stabilization and spoilage risk management, and (vi) by-products and wastewater treatment. The presence of proper yeast or bacterial strains, the management and timing of inoculation of starter cultures, and some appropriate technological modifications that favor selected microbial activities can lead to several positive effects, including (among other) energy savings, reduction of chemical additives such as sulfites, and reuse of certain residues.

- 1 Roudil L, Russo P, Berbegal C, Albertin W, Spano G, Capozzi V. 2020. Non-*Saccharomyces* commercial starter cultures: scientific trends, recent patents and innovation in the wine sector. *Recent Patents on Food, Nutrition & Agriculture* 11:27-39; doi: 10.2174/2212798410666190131103713.

In the field of microbial starter cultures in oenology, non-*Saccharomyces* species/strains have been receiving increasing interest. This trend arises clear if we consider starter cultures portfolio of multinationals and national companies that develop and sell microbial-based biotechnological solutions for the wine sector. While the genotypic and phenotypic diversity of these yeasts have been extensively reviewed, poor consideration was given to the innovative application of non-*Saccharomyces* commercial starter cultures in the scientific and patent literature. The study encompassed 26 different

commercial yeasts starter cultures commercialized in the wine sector, providing a timeline of relevant introductions on the market, together with information on the regulatory environment. Using this non-*Saccharomyces* commercial preparations as queries, we explored the scientific literature, also highlighting the presence of tests on proprieties claimed by producers. Finally, the review proposed a survey on the recent patents about non-*Saccharomyces* application in oenology, viticulture and alcoholic beverages.

- 2 Tufariello M, Capozzi V, Spano G, Cantele G, Venerito P, Mita G, Grieco F. 2020. Effect of co-inoculation of *Candida zemplinina*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* for the industrial production of Negroamaro wine in Apulia (Southern Italy). *Microorganisms*, 8(5), 726; doi:10.3390/microorganisms8050726.

The employment of multi-species starter cultures has growing importance in modern winemaking for

improving the complexity and wine attributes. The assessment of compatibility for selected species/strains

at the industrial-scale is crucial to assure the quality and the safety associated with fermentations. An aspect particularly relevant when the species belong to non-*Saccharomyces*, *Saccharomyces* spp. and malolactic bacteria, three categories with different biological characteristics and oenological significance. To the best of our knowledge, the present report is the first study regarding the utilization of a combined starter culture composed of three strains of non-*Saccharomyces*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* for production of wine at the industrial scale. More in-depth, this work investigated the oenological potential of the autochthonous characterized strains from the Apulian region (Southern Italy), *Candida zemplinina* (syn. *Starmerella bacillaris*) 35NC1, *S. cerevisiae* (NP103), and *L. plantarum* (LP44), in co-inoculation following a complete scale-up scheme. Microbial dynamics, fermentative profiles and production of volatile secondary compounds were assessed in lab-scale micro-vinification tests and then the performances of the mixed starter formulation were further evaluated

by pilot-scale wine production. The above results were finally validated by performing an industrial-scale vinification on 100HL of Negroamaro cultivar grape must. The multi-starter formulation was able to rule the different stages of the fermentation processes effectively, and the different microbial combinations enhanced the organoleptic wine features to different extents. The findings indicated that the simultaneous inoculation of the three species affect the quality and quantity of several volatile compounds, confirming that the complexity of the wine can reflect the complexity of the starter cultures. Moreover, the results underlined that the same mixed culture could differently influence wine quality when tested at the lab-, pilot- and industrial-scale. Finally, we highlighted the significance of employment non-*Saccharomyces* and *L. plantarum*, together with *S. cerevisiae*, autochthonous strains in the design of custom-made starter culture formulation for typical regional wine production with pronounced unique quality.

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**GMV-XII DAFE - Department of Agriculture, Food and Environment, University of Pisa, Via Borghetto 80 56124, Pisa, Italy. Communicated by Dr. Annita Toffanin**  
<[annita.toffanin@unipi.it](mailto:annita.toffanin@unipi.it)> [www.agr.unipi.it/](http://www.agr.unipi.it/)

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- 1 Guzzon R, Malacarne M, Larcher R, Franciosi E, Toffanin A. 2019. The impact of grape processing and carbonic maceration on the microbiota of early stages of winemaking. *J. Appl. Microbiol*, 128; doi:10.1111/jam.14462

The work investigates the impact of grapes processing at the beginning of winemaking on the composition of microbiota during the oenological fermentations and on the composition of obtained wines. The experiments were conducted in a biodynamic winery to exclude interference due to microbial starters. Three winemaking protocols, with different pre-fermentative management of grapes, were followed by plate count and next generation sequencing on 16S for bacteria and internal transcribed spacer sequencing (ITS) for yeast. The grape processing influenced the evolution of microbiota (especially lactic and acetic acid bacteria) and the fermentation rate. The highest biodiversity was observed in the experiment carried out with whole

grapes and carbonic maceration, with the presence of bacterial groups not usually found in winemaking (*Bacteroidales*, *Clostridiales*, *Oscillospira*). The different microbiotas influenced the organic acid profile of wines, the content of biogenic amines and the perception of organoleptic descriptors linked to the vine cultivar (Syrah). The work elucidates the reasons of these difficulties and identified some microbial groups rarely associated with winemaking. The ratio of ethanol accumulation along with physical management of grapes and the supply of oxygen during the early stages of winemaking are powerful instruments of oenological variability, able to offer new possibilities to winemakers in order to defining the quality of red wines.

- 1 Binati R.L, Lemos Junior W.J.F, Luzzini G, Slaghenaufi D, Ugliano M, Torriani S. 2020. Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on *Lachancea thermotolerans*, *Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. *Int. J. Food Microbiol.*, 318:108470; doi: 10.1016/j.ijfoodmicro.2019.108470.

*Saccharomyces cerevisiae* starter cultures are largely used in winemaking to repress the wild microorganisms and achieve more predictable and desired outcomes. Notwithstanding, alternative microbial resources received increasing attention for their potential to produce wines with more distinctive and typical features. Our previous survey revealed a great inter- and intra-species diversity in an extensive collection of non-*Saccharomyces* wine yeasts from multiple regions of Italy. This study aimed to explore the detected biodiversity evaluating the quality of wines obtained by sequential inoculation of specific selected strains of the collection (*Lachancea thermotolerans* or *Metschnikowia* spp. or *Starmerella bacillaris*), and *S. cerevisiae* EC1118. Fermentations of natural grape must at laboratory scale were followed by microbiological, chemical and sensorial analysis of the wines. The results indicated that each yeast species and strain exerted a distinctive impact on the wine, giving final products clearly separated with

Principal Component Analysis. In particular, *L. thermotolerans* contributed producing relevant amounts of lactic acid and had the highest potential to reduce ethanol content; the presence of *S. bacillaris* increased the level of glycerol, and, remarkably, reduced acetaldehyde and total SO<sub>2</sub>; *Metschnikowia* spp. promoted the formation of higher alcohols and esters, and reduced volatile phenols. The sensory analysis based on the orthonasal aroma confirmed the separation between the wines obtained with the sequential fermentations and the control with single inoculation of EC1118, although the three non-*Saccharomyces* species used could not be clearly distinguishable by the panelists. This study indicates that the use of selected native non-*Saccharomyces* strains in conjunction with *S. cerevisiae* positively modulates some relevant chemical parameters, and improves the aromatic intensity of wine, therefore justifying investments in non-conventional yeasts as co-starter cultures.

- 2 Lemos Junior W.J.F, Binati R.L, Felis G.E, Slaghenaufi D, Ugliano M, Torriani S. 2020. Volatile organic compounds from *Starmerella bacillaris* to control gray mold on apples and modulate cider aroma profile. *Food Microbiol.*, 89: 103446; doi: 10.1016/j.fm.2020.103446.

Gray mold caused by *Botrytis cinerea* is a fungal disease that can determine significant economic losses of apple during the storage phase. An alternative to reduce the use of traditional synthetic fungicides is to employ the yeast *Starmerella bacillaris* as biological control agent (BCA), also with positive effect on apple juice fermentation for the production of cider. Thus, we aimed to evaluate the safety of 16 *S. bacillaris* strains and their ability to control *B. cinerea*. In addition, the fermentation performances in apple juice and the volatile organic compounds (VOCs) profile were assessed, both in single-strain and in sequential fermentations with *Saccharomyces cerevisiae*. The *in vitro* assays showed that all *S. bacillaris* strains can be

considered safe from the analyzed virulence factors, and were able to significantly constrain the growth of *B. cinerea*, reducing mycelial growth of 50% in dual-culture and of 90% through VOCs. Moreover, *in vivo* antagonistic assays revealed a visible decrease of gray mold rot symptoms on apples confirming the potential of *S. bacillaris* as BCA. GC-MS analysis of the ciders obtained showed increased concentrations in the sequential fermentation of some higher alcohols and terpenes, positively correlated with the cider aromatic quality, and suggested the involvement of benzyl alcohol, known for its antimicrobial action, in the biocontrol efficacy.

- 3 Binati R.L, Innocente G, Gatto V, Celebrin A, Polo M, Felis G.E, Torriani S. 2019. Exploring the diversity of a collection of native non-*Saccharomyces* yeasts to develop co-starter cultures for winemaking. *Food Res. Internat.*, 122:432-442; doi: 10.1016/j.foodres.2019.04.043.

The inoculation of *Saccharomyces cerevisiae* starter cultures in grape musts is a common practice in wineries worldwide; however, native non-*Saccharomyces* yeast species are increasingly investigated as co-starters to augment the complexity and regionality of wine. In this study, an extensive collection of non-*Saccharomyces* yeasts from high-sugar matrices was created and screened with the aim to discover new strains with potentially positive oenological traits. After mining >400 yeasts from 167 samples collected across multiple Italian regions, the isolates were identified based on RAPD-PCR analysis and ITS sequencing. About one quarter of them, belonging to the genera *Starmerella*, *Lachancea* and *Metschnikowia*, were picked up for an in-depth molecular and physiological characterization, since

these yeasts were well stressed and have a good oenological reputation. Following the genotyping, stress tolerance assays, enzymatic activity trials and single inoculum fermentations, a huge diversity was acknowledged within and between the species. Strains of *S. bacillaris* showed a high tolerance to ethanol and increased glycerol production, *L. thermotolerans* reduced volatile acidity while increasing total acidity with lactic acid, and *Metschnikowia* spp. exhibited remarkable aroma-related enzymatic activities, which are all prized features in winemaking. Since most of the characteristics analyzed were species and strain dependent, the obtained results are valuable for the selection of a new generation of co-starters for attempting mixed fermentation strategies aimed to improve the overall quality of regional wine.

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**GMV-XIV Laboratory of Microbial Biotechnology, Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo (UNITE), via Balzarini 1, 64100 Teramo (TE), Italy. Communicated by Professor Rosanna Tofalo <[rtofalo@unite.it](mailto:rtofalo@unite.it)> [http://www.unite.it/UniTE/Bioscienze\\_e\\_tecnologie\\_agro-alimentari\\_e\\_ambientali](http://www.unite.it/UniTE/Bioscienze_e_tecnologie_agro-alimentari_e_ambientali).**

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- 1 Tofalo R, Perpetuini G, Battistelli N, Tittarelli F, Suzzi G. 2020. Correlation between *IRC7* gene expression and 4-mercapto-4-methylpentan-2-one production in *Saccharomyces cerevisiae* strains. *Yeast* doi.org/10.1002/yea.3468 (In press).

Volatile thiols are not present in must but are synthesized and released by wine yeasts during alcoholic fermentation. In this study, autochthonous and commercial *Saccharomyces cerevisiae* strains were characterized for the expression of the main genes involved in thiols metabolism and their production in wine. New primer sets were developed on the basis of the S288c genome to evaluate the expression of *Cys3*, *Cys4*, *MET17* and *IRC7* gene.

Obtained data revealed the occurrence of some thiols, for example, 4-mercapto-4-methylpentan-2-one (4-MMP) and 3-mercaptohexan-1-ol (3-MH) in Pecorino white wine. All genes were upregulated, but only for *IRC7* was found a correlation with 4-MMP release: strains with the highest production showed the highest transcription level. *IRC7* gene could be proposed as target for the selection of *S. cerevisiae* strains to increase thiols content in wine.

- 2 Perpetuini G, Tittarelli F, Battistelli N, Suzzi G, Tofalo R. 2020. Contribution of *Pichia manshurica* strains to aroma profile of organic wines. *Eur. Food Res. Technol*; doi.org/10.1007/s00217-020-03499-8 (In press).

In this study, the volatile compounds produced by 11 *Pichia manshurica* strains — isolated from spoiled bottled organic wines of Montepulciano d'Abruzzo and filter membranes — were evaluated in must and wine. Moreover, a quick, reliable and inexpensive method to identify this spoilage species was reported. In particular, a species-specific primer pair was developed using 26S rRNA as target gene. Primers were tested on different yeast species and a band of

300 bp was obtained only for *P. manshurica* strains. More than 70 volatile compounds were identified. The differences were quantitative rather than qualitative and the majority of the strains released compounds associated with off-flavours and off-odours in must and wine. The obtained data revealed the ability of *P. manshurica* strains to release compounds which could produce off-flavours and odours.



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



**ICY Commissioners Meeting, Tuesday, October 22, 2019  
35<sup>th</sup> The International Specialised Symposium on Yeasts (ISSY35)  
Sherwood Exclusive Kemer Hotel, Antalya, Turkey**

**Minutes of Meeting**

**Present (35):** Hiroshi Takagi (ICY Chair; Japan), Charles Abbas (ICY Vice-Chair; USA), Huseyin Erten (ISSY35 Chair; Turkey), Tiina Alamäe (Estonia), Jacobus (Koos) Albertyn (South Africa), Feng-Yan Bai (China), Kyria Boundy-Mills (USA), Pietro Buzzini (Italy), Charoen Charoenchai (Thailand), Patrick Fickers (Belgium), Peter Gabor (Hungary), Lisa Granchi (Italy), Anne Gschaedler (Mexico), Thomas W. Jeffries (USA), Lene Jespersen (Denmark), Vladimir Jiranek (Australia), Hyun Ah Kang (South Korea), Anna Maraz (Hungary), Diethard Mattanovich (Austria), Vivien Measday (Canada), Leda Mendonça-Hagler (Brazil), John Morrissey (Ireland), Vladimir Mrša (Croatia), Elena Naumova (Russia), Patricia Lappe Oliveras (Mexico), Volkmar Passoth (Sweden), Patrizia Romano (Italy), Mathabatha Evodia Setati (South Africa), Andriy A. Sibirny (Ukraine), Matthias Sipiczki (Hungary), Nitnipa Soontorngun (Thailand), Hana Sychrova (Czech Republic), Masako Takashima (Japan), Johan Thevelein (Belgium), Teresa Zoladek (Poland)

**Apologies (25):** Javier Carvajal Barriga (Ecuador), Florian Franz Bauer (South Africa), Teun Boekhout (Netherlands), Ee-Sin Chen (Singapore), Heide-Marie Daniel (Belgium), Diego Libkind Frati (Argentina), Dra. Ma Angelica Ganga (Chile), Brigitte Gasser (Austria), Ivan Hapala (Slovakia), Ida J. van der Klei (Netherlands), Sally Ann Meyer (USA), George Miloshev (Bulgaria), Jens Nielsen (Sweden), Liliana Godoy Olivares (Chile), Steve Oliver (UK), Sakkie Pretorius (Austria), Bernard Prior (South Africa), Jack T. Pronk (Netherlands), Alexander Rapoport (Latvia), Peter Raspor (Slovenia), Doris Rauhut (Germany), Ana Clara Schenberg (Brazil), Rosane Freitas Schwan (Brazil), Petrovič Uroš (Slovenia), Andrey Yurkov (Germany)

**Meeting Agenda**

**Chair's Opening Remarks:** Dr. Hiroshi Takagi welcomed 35 delegates to the meeting with awamori

“Hanahana”, which is a traditional distilled alcoholic beverage in Okinawa, Japan, brewed with his yeast strain <<https://www.frontiersin.org/articles/10.3389/fgene.2019.00490/full>>. He thanked Dr. Hüseyin Erten and the Organizing Committees for the excellent job regarding ISSY35. He also presented the meeting agenda and confirmed the minutes of the previous meeting held in Bariloche, Argentina (October 3, 2018).

**Tribute to Dr. Matti Korhola:** Dr. Takagi informed of the sad news that our honorable Commissioner, Dr. Matti Korhola (Finland) passed away in July 20, 2019. He was a very active member of ICY and a solid and reliable person as an excellent yeast scientist, particularly linking science and industry, in both Alkomohr Biotech Ltd. and University of Helsinki. Commissioners mourned his death by paying one minute of silence tribute. As sincerest condolences, Commissioners will dedicate a memorial address to honor his contribution to the community. Dr. Takagi proposed that, if possible, his memorial lecture be included in the program of ICY15 organized by Dr. Diethard Mattanovich. Dr. Takagi also asked Dr. Elena Naumova to write an introductory message, to collect information, and to build the contents by the end of this year. Finally, Dr. Takagi will ask Dr. Rob Samson, the secretary-general of IUMS, to upload the contents to the special site “Gallery of colleagues in yeast research who passed away” [iums.org/index.php/gallery-of-past-yeast-researchers](https://iums.org/index.php/gallery-of-past-yeast-researchers) in our official webpage.

**New Commissioners to ICY:** Dr. Takagi introduced five candidates for ICY membership. Dr. Milan Čertík is Associate Professor in Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia, and is an expert on biotechnological production of biologically active lipophilic metabolites focused on polyunsaturated fatty acids and pigments by yeasts, nominated by Dr. Ivan

Hapala. Dr. Adrianna V. Skoneczna is Associate Professor in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, and is an expert on yeast cellular mechanisms of maintaining genome integrity, DNA repair and cellular response to environmental stresses, nominated by Dr. Teresa Zoladek and Dr. Andriy Sibirny. Dr. Christopher Todd Hittinger is Associate Professor in Genetics, University of Wisconsin-Madison, Madison, USA, and is an expert on genomic and physiological studies of yeast phylogeny including the origins and domestication of brewing yeasts result from the genomes of 1,000 yeast species, nominated by Dr. Thomas W. Jeffries. Dr. Aleksey Kachalkin is Leading Researcher in Faculty of Soil Science and Curator for the Yeast collection of the Soil Biology Department in Lomonosov Moscow State University, Moscow, Russia, and is an expert on yeast biodiversity, ecology and taxonomy, paying particular attention to soil yeasts, nominated by Dr. Elena Naumova. Dr. Francisco Carrau is Professor of Enology and Fermentation Biotechnology, Universidad de la Republica, Uruguay, and is an expert on the development of *Hanseniaspora vineae* yeast as a eukaryotic cell model and as a non-conventional yeast for the production of unique wines in the market, nominated by Dr. Sakkie Pretorius. Each candidate provided his/her CV with list of publications and two Letters of Recommendation from relevant National or International Society and the current members of ICY. These documents were spread electronically among Commissioners, who could express their attitude to candidates. All proposed candidates got full support from 33 Commissioners with very few negative opinions. Commissioners unanimously elected all of them as new members of ICY. In addition, Dr. Takagi received the necessary documents regarding the nomination of two new UK Commissioner candidates (Profs. Daniela Delneri, Manchester, and Tom Ellis, Imperial college London) from Dr. Steve Oliver just before ISSY35, as replacements for the UK Commissioners, Drs. Steve Oliver and Ian Roberts, but due to the lack of time for distributing the documents to all the Commissioners, we could not reached the conclusion. Dr. Takagi will circulate the documents for voting (Pros/Cons) to all the Commissioners as soon as possible. If we are agreed, Drs. Delneri and Ellis will be able to attend the Commissioners meeting in Vienna as the UK representatives.

**Updates for Future ISSY/ICY Meetings:** Each organizer provided a progress report on meeting preparation. Dr. Diethard Mattanovich presented the

update for ICY15 that will be held in Vienna, Austria (August 23-27, 2020). Dr. Vivien Measday also presented the update for ISSY36 that will be held in Vancouver, Canada (July 6-9, 2021).

**Suggestions for Future ISSY:** Dr. Vladimir Jiranek officially proposed ISSY37 (2022) including the potential date and venue. After discussion, Commissioners accepted his proposal to organize ISSY37 held in Adelaide, Australia, in July, 2022. Drs. Jiranek and Sakkie Pretorius will start the preparation of ISSY37 including its subtitle with the local/domestic/international organizing committee. For ISSY38 (2023), Regarding ISSY38 (2023), Dr. Takagi proposed that Northern Europe is the best candidate region, particularly Denmark and Sweden, as a host country, in terms of the geographical balance and the presence of active yeast community. After discussion, Dr. Takagi will ask Drs. Jens Nielsen, Volkmar Passoth and Lene Jespersen to discuss the host country and chair of ISSY38. It was also discussed that there may be other countries who wish to host ISSY38 and ICY is open for receiving additional or alternative proposals from any nation's Commissioners, ideally in advance of ICY15 (2020) held in Vienna. As a schedule, we will decide the potential venue, date and chair for ISSY38 at the ICY Commissioners meeting held in Vienna, Austria, 2020. As for ICY16 (2024), which is a general congress every 4 years after ICY15 (2020), Dr. Takagi introduced South Africa as the best country for hosting ICY16, in terms of the geographical balance and the presence of active yeast community. Also, before ISSY36, Dr. Takagi asked Dr. Florian Franz Bauer, who is a S. African Commissioner, to consider this issue with colleagues in S. Africa in terms of the availability, willingness and motivation for hosting ICY16. He kindly accepted it, but unfortunately he could not come to ISSY35. In this meeting, instead of Dr. Bauer, Dr. Mathabatha Evodia Setati (S. African Commissioner) briefly presented the outline of ICY16 including the venue (Cape Town) with an advertising brochure. After some questions, comments and opinions among the Commissioners, these proposals for ICY16 (2024) (host country and chair) were unanimously accepted by attending Commissioners. Dr. Takagi will request Dr. Bauer to give a final presentation at the Commissioners meeting in Vienna next year (ICY15).

Miscellaneous information: 1) "The Yeasts" project by Dr. Teun Boekhout <[www.theyeasts.org](http://www.theyeasts.org)>. Dr. Boekhout, who missed this meeting, will give the Commissioners an update soon. 2) "Yeasts in the production of wine" published by Drs. Patrizia

Romano and Graham Fleet. Dr. Romano has successfully completed and published this book from Springer Nature to be dedicated to Dr. Fleet. 3) SIMB Annual Meeting Session organized by Drs. Takagi and Scott Baker from Pacific Northwest National Laboratory, Richland, WA, USA. They will propose one session entitled “yeast metabolic engineering with the aid of systems biology and synthetic biology”, at San Francisco, CA, USA, August 9-12, 2020.

Chair Closing Remarks: On behalf of ICY, Dr. Hiroshi Takagi expressed gratitude once again to Dr. Hüseyin Erten and his staff for the excellent meeting, well balanced and organized scientific and cultural program. The ICY meeting was closed.

December 23, 2019

Minutes presented by:



Dr. Hiroshi Takagi  
ICY Chair



Dr. Charles Abbas  
ICY Vice-Chair

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## Future Meetings

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### 47th Annual Conference on Yeasts (ACY), Smolenice Castle, Slovakia, May 11-14, 2021

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The 47th Annual Conference on Yeasts (ACY) will be held on May 11-14, 2021 in Smolenice Castle (Slovakia). Organizers from the Commission on Yeasts of the Czechoslovak Society of Microbiology and Institute of Chemistry, the Slovak Academy of Sciences in collaboration with Faculty of Chemical and Food Technology, Slovak University of Technology, the Faculty of Natural Sciences, Comenius University, the Institute of Animal Biochemistry and Genetics, and the Slovak Academy of Sciences will be there to welcome you.

The major themes for the ACY are the Molecular and Cell Biology of Yeasts, Biotechnology of Yeasts, Yeast Diversity and Interactions, and Community Resources.

Please find more information about the conference on the webpage: <https://yeastconference.sk/home/>

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### 2<sup>nd</sup> International Workshop on Brewing Yeasts (IWOBY2020) Bariloche, Patagonia, Argentina

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This year we were planning the organization of the 2<sup>nd</sup> International Workshop on Brewing Yeasts (IWOBY2020) to be held again in Bariloche, Patagonia, Argentina. The planned date is November 21-22. Despite the present situation related to COVID-19 we are still optimistic. We will be updating the status of the meetings as things develop. Please stay up to date at <https://iwoby.com.ar/>

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

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We are all facing a serious situation concerning the corona virus and hope that you and your beloved ones are safe and healthy. After careful consideration of the near future and intense consultation within the ICY society, we have to announce that we will postpone ICY15 to August 2021. We are really looking forward to welcome you in Vienna from August 22-26, 2021!

With kind regards,

Diethard Mattanovich and the ICY15 organizing team

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**15th International Conference on Culture Collections - ICC15**  
**Universidad de La Frontera, Campus Pucón, Región de la Araucanía, Chile**

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The 15<sup>th</sup> International Conference on Culture Collections, to be held in Pucón, Chile, was planned for November 16-20, 2020. A new date will be announced in due course. For updates, see <http://www.iccc15.ufro.cl/index.php>.

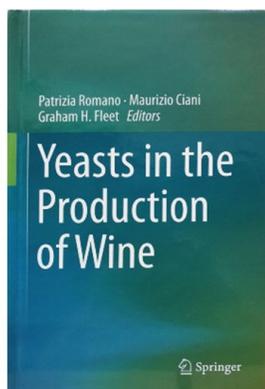
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**New Book**

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**Romano P, Ciani M, Fleet GH (eds). 2019. Yeasts in the Production of Wine. Springer**

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It is well established that certain strains of yeasts are suitable for transforming grape sugars into alcohol, while other yeast strains are not suitable for grape fermentations. Recent progress has clearly demonstrated that the sensory profile of a wine is characteristic of each vine cultivated, and the quality and technological characteristics of

the final product varies considerably due to the strains which have performed and/or dominated the fermentation process. Because of their technological properties, wine yeast strains differ significantly in

their fermentation performance and in their contribution to the final bouquet and quality of wine, such as useful enzymatic activities and production of secondary compounds related both to wine organoleptic quality and human health. The wine industry is greatly interested in wine yeast strains with a range of specialized properties, but as the expression of these properties differs with the type and style of wine to be made, the actual trend is in the use of selected strains, which are more appropriate to optimize grape quality. Additionally, wine quality can be influenced by the potential growth and activity of undesirable yeast species, considered spoilage yeasts, which cause sluggish and stuck fermentation and detrimental taste and aroma in the wine.

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<https://link.springer.com/book/10.1007/978-1-4939-9782-4>

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## Brief News Item

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### Potentially invalid taxonomic descriptions of yeasts

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Dear Colleagues:

In the previous issue of the Yeast Newsletter we announced a joint Consensus Statement on potentially invalid taxonomic descriptions of yeasts. Discussions during the ISSY34 meeting brought us to the decision to write a text to comment on the problem of formally invalid yeast species. The wording used in descriptions of many yeast species was not always conform with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). Incorrect designation of the holotype and its deposition in a culture collection made approximately 300 names of yeasts formally invalid.

With this statement we want to express our

concerns to the International Commission on Taxonomy of Fungi (ICTF) to find a workable solution to the problem. We aim to find a stable approach to cite type strains of yeasts in publications, to protect retroactively many yeast names and reduce name changes due to incorrect typification to a minimum.

We have already received input and feedback from more than 20 yeast researchers and a few taxonomic experts from the ICTF. Please feel free to contact us if you wish to contribute to the discussion. We will send you the text for review and commenting. We will carefully collect all comments and incorporate suggestions.

With kind regards,

Andrey Yurkov

Teun Boekhout

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## Fifty Years Ago

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### Y E A S T

A News Letter for Persons Interested in Yeast

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

June 1970

Volume XIX, Number 1

Editor

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

Associate Editor

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

Associate Editor

F. M. Clark, University of Illinois, Urbana, Illinois

Associate Editor

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

\*\*\*\*\*

Ajinomoto Co., Inc., Central Research Laboratories, Suzuki-cho, Kawasaki, Japan. Communicated by T. Nakase.

2. Now we have finished the analysis of the DNA base composition of the yeast genera Pichia, Debaryomyces, Torulopsis, Cryptococcus and Rhodotorula, and are preparing the manuscripts for publication.

Microbiological Laboratory of the Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, Dubravska cesta, CSSR. Communicated by Dr. A. Kocková-Kratochvílová.

The previous taxonomies of yeasts build their systems on predominantly morphological and biochemical characters. In this study, apart from these two, also evaluation of other aspects such as serology, genetics and technology was introduced, which resulted in formation of a special set of characters. On the basis of this complex attitude the numerical evaluation resulted in the formation of completely natural groups of these strains or intermediary groups. In a study of 88 strains, originally determined as S. carlsbergensis, S. uvarum, S. logos and S. monacensis, S. mandshuricus etc., two groups were formed, one of which includes the original strains of S. carlsbergensis in sensu stricto, the other S. uvarum.

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Institute of Fermentation, Yamanashi University, 1 - Kitashin-machi, Kofu, Japan. Communicated by Shoji Goto.

The yeast flora in core samples from two stratigraphic drillings in central Japan consisted of asporogenous yeasts alone, viz., Candida, Trichosporon and Rhodotorula. Particularly, C. guilliermondii var. japonica was characteristic of the yeast flora in core samples. Their abilities to grow under extreme environmental conditions was demonstrated. An analysis of the data obtained suggests that yeasts may have survived in the deep lithosphere. Yeasts were detected in core samples taken from depths of about 800 to 2.500 meters. The deepest sample from which C. guilliermondii var. japonica was isolated was obtained at a depth of about 2.500 meters.

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Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201. Communicated by M. A. Gordon.

Certain strains of C. neoformans of capsule type C, including some of the above variants and their parent cultures, developed, in infected mouse tissues, very large, spherical, thick-walled, heavily-encapsulated cells, some of which, upon transfer to agar media, produced as many as eight internal spores. These often appeared to arise through free-cell formation, leaving an epiplasmic residue in many cases, but failed to take ordinary ascospore stains. There was some evidence of conjugation preceding sporulation.

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Microbiology Department, Macdonald College of McGill University, Quebec, Canada. Communicated by Ronald E. Simard.

The persistence of Rhodotorula glutinis in polluted water and its distribution pattern correlated with various pollution indices suggest that this organism could be used as a pollution indicator.

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland. Communicated by Prof. Heikki Suomalainen.

T. Nurminen, E. Oura and H. Suomalainen, The enzymic composition of the isolated cell wall and plasma membrane of baker's yeast. Biochem. J. 116 (1970) 61-69.

A study was made of the enzyme content of the isolated cell walls and of a plasma-membrane preparation obtained by centrifugation after enzymic digestion of the cell walls of baker's yeast. The isolated cell walls showed no hexokinase, alkaline phosphatase, esterase or NADH oxidase activity. It was concluded that these enzymes exist only in the interior of the cell. Further, only a negligible activity of deamidase was detectable in the cell walls. Noticeable amounts of saccharase, phosphatases hydrolysing p-nitrophenyl phosphate, ATP, ADP, thiamin pyrophosphate and  $PP_1$ , with optimum activity at pH 3-4, and an activity of  $Mg^{2+}$ -dependent adenosine triphosphatase at neutral pH, were found in the isolated cell walls. During enzymic digestion, the other activities appearing in the cell walls were mostly released into the medium, but the bulk of the  $Mg^{2+}$ -dependent adenosine triphosphatase remained in the plasma-membrane preparation. Accordingly, it may be assumed that the enzymes released into the medium during digestion are located in the cell wall outside the plasma membrane, whereas the  $Mg^{2+}$ -dependent adenosine triphosphatase is an enzyme of the plasma membrane. This enzyme differs from the phosphatases with pH optima in the range pH 3-4 with regard to location, pH optimum, substrate specificity and different requirement of activators.

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Instituto Nacional de Investigaciones Agronómicas. Sección de Bioquímica, Madrid, Spain. Communicated by J. Santa María.

"Significación taxonómica de las propiedades fisiológicas de las especies incluidas en el género Kluyveromyces" ("Taxonomic significance of the physiological properties of the species included in the genus Kluyveromyces"). J. Santa María, Consuelo Sánchez.

A comparative study of the different physiological properties of Sacch. dobzhanskii, Sacch. lactis, Sacch. drosophilum, Sacch. fragilis, Sacch. phaseolusporus and Sacch. wickerhamii, shows: 1) There is a close affinity amongst these 6 yeast species. 2) These six species are clearly differentiated from other Saccharomyces. 3) There is very little relationship between these six species and Kluyveromyces polysporus (typical species of the genus Kluyveromyces).

Therefore, it seems justified to include Sacch. dobzhanskii, Sacch. lactis, Sacch. drosophilum, Sacch. fragilis, Sacch. phaseleosporus and Sacch. wickerhamii in a genus distinct from both Saccharomyces and Kluyveromyces, which must be Dekkeromyces.

Department of Food Science and Technology, University of California, Davis, Calif. 95616. Communicated by H. J. Phaff.

We are continuing our work on the base composition and homology of yeast DNA. Coworkers in this project are Dr. A. Martini, Miss Sally A. Meyer and Mr. Chester W. Price. Dr. Martini has worked out a much improved method for extraction of highly polymerized DNA from yeasts which previously yielded little or no spoolable DNA. Full details will be given in the fall News Letter. Publications:

J. W. Fell, A. C. Hatzell, I. L. Hunter and H. J. Phaff. Leucosporidium, gen. n., the heterobasidiomycetous stage of several yeasts of the genus Candida. Antonie van Leeuwenhoek 35, 433-462, 1969.

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Groupe Euratom de L'Université de Louvain, Laboratoire D'Enzymologie, de Croylaan 46, 3030 Heverlee, Belgium. Communicated by A. Goffeau.

Segregational respiratory-deficient mutants of a petite-negative yeast Schizosaccharomyces pombe 972h<sup>-</sup>.

H. HESLOT, C. LOUIS and A. GOFFEAU

No viable respiratory-deficient mutants of Schizosaccharomyces pombe 972h<sup>-</sup> could be obtained by acriflavine and ethidium bromide treatments. These mutagens induce 15 to 70% of microcolonies that, after a growth-lag of a few days, further develop into normal respiratory-competent colonies. These results suggest that lethal, unstable vegetative petites were induced.

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Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia. Communicated by E. Minárik.

The following manuscript has been submitted for publication to Biologické práce SAV (Biological Works SAV) in Bratislava: E. Minárik - P. Rágala: "Influence of fungicides on the yeast flora of the vine", Difolatan and some others) show a strong inhibition of yeast activity; the natural composition of the yeast flora of grapes is significantly changed. Saccharomyces species are most affected, the development of some less active species in fermentation, e.g. Torulopsis bacillaris, as well as the fungus Hyalodendron sp. being stimulated. Thiuram fungicides (e.g. Polyram, Polyram-Combi, Basfungin, Kupfer-Polyram) do not slow down the development of yeasts. TMTD, on the other hand, inhibited sporogenous yeast species similarly to some phtalimid fungicides. This preparation stimulates the development of Torulopsis bacillaris in the yeast flora of the fermenting grape juice.