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

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

Associate Editors

Patrizia Romano
Universitas Mercatorum
Piazza Mattei 10
00186 Rome
Italy

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

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Editorial

Arnold L. Demain (1927-2020)

Friend, colleague, mentor, and long-time subscriber to the Yeast Newsletter Arny Demain died in April this year, from complications due to Covid-19. At 92 years old, he left a long career of excellence in the area of fermentation biology. I had the privilege of enjoying Arny's company on several occasions. He always struck me as a warm, caring, and unassuming person, oblivious to his outstanding accomplishments. He was generous with his gratitude to all those who interacted with him. We were last together in 2011 at the congress of the International Union of Microbiological Societies in Sapporo. The congress was held shortly after the Tōhoku earthquake and the resulting Fukushima nuclear accident. In recognition of their attendance in spite of perceived risk, delegates were rewarded with a late afternoon visit by Emperor Akihito, himself a fish systematist. I was delighted to be on the list of people invited to attend a dinner reception attended by the Emperor. A much shorter list of VIPs, Arny among them, were selected to converse with Akihito. This did not stop Arny from doing all he could to make me feel at ease in the prestigious crowd, introducing me to some of the world's top microbiologists. Readers can find out more about Arny's career by reading an editorial by Sergio Sánchez that appeared in a special issue of the Journal of Antibiotics (2010; 63:412–414) dedicated to Arny: <https://www.nature.com/articles/ja201069>. His MIT colleague, Raleigh McElvery, allowed me to reproduce an obituary from the university's News Website.

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

M.A. Lachance, Editor

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>.

I started translating the book “The Rise of Yeast: how the sugar fungus shaped civilization”, from Nicholas Money, Oxford University Press, into Portuguese. An agreement has been established between the publishing house of the University of Campinas (<https://editoraunicamp.com.br/>) and OUP. Publication is envisaged for March 2021.

I recently joined the COST Action Yeast4Bio (Non-Conventional Yeasts for the Production of Bioproducts, <https://yeast4bio.eu/>), #CA18229. We had a virtual meeting between Nov 11 and 12, 2020. Several presentations were made by different research groups and I talked about “The potential use of *Kluyveromyces marxianus* in sugarcane- and whey-based biorefineries”.

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>.

The following papers have been published.

- 1 Agnieszka Wilkowska, Joanna Berłowska, Adriana Nowak, Ilona Motyl, Aneta Antczak-Chrobot, Maciej Wojtczak, Alina Kunicka-Styczynska, Michał Binczarski, Piotr Dziugan. 2020. Combined yeast cultivation and pectin hydrolysis as an effective method of producing prebiotic animal feed from sugar beet pulp. *Biomolecules* 10:724 - doi:10.3390/biom10050724

An effective and ecological method for liberation of pectin-derived oligosaccharides (POS) from sugar beet pulp (SBP) was developed using enzymatic and microorganism-mediated biomass conversion. The POS may be applied in the production of prebiotic feed additives. Various yeast strains were screened for their capacity for protein synthesis and mono-saccharide assimilation. Combined yeast cultivation and pectin hydrolysis were found to be an effective method of producing prebiotics. Separate enzymatic hydrolysis and fermentation of SBP resulted in the release of 3.6 g of POS per 100 g d.w., whereas the yield of POS acquired after the combined process was 17.9% higher, giving 4.2 g of POS per 100 g d.w.

Introducing the yeast into the process improved hydrolysis performance due to lower enzyme inhibition by mono- and disaccharides. The prebiotic effect of the POS was assessed by in vitro fermentation using individual cultures of gastrointestinal bacteria. The POS in the SBP hydrolysate effectively promoted the growth of lactobacilli and bifidobacteria. A large increase in adherence to Caco-2 cells in the presence of POS was noted for beneficial *Lactobacillus brevis* strains, whereas pathogenic bacteria and yeast (*C. albicans*, *C. lusitaniae*, *C. pelliculosa*), responsible for infections in breeding animals, showed much weaker adhesion.

- 2 Piotr Patelski, Joanna Berłowska, Maria Balcerek, Urszula Dziekonska-Kubczak, Katarzyna Pielech-Przybylska, Dawid Dygas, Jakub Jedrasik. 2020. Conversion of potato industry waste into fodder yeast biomass. *Processes* 8:453 - doi:10.3390/pr8040453

In this study, we evaluate potato pulp waste as a potential raw material for obtaining yeast biomass. A portion of the carbohydrates in the potato pulp waste can thereby be converted into more valuable protein. The potato pulp waste was analyzed in terms of protein and ash content, dry mass, simple sugars, and starch content. Two kinds of hydrolysis were performed (thermo-acidic and enzymatic) to produce media for cultivating *Candida guilliermondii* and

Pichia stipitis. The hydrolysates and post-cultivation leachates were analyzed by High Performance Liquid Chromatography (HPLC). The highest biomass yield after 48 h (39.3%) was noted for *Candida guilliermondii* yeast grown on enzymatic hydrolysate-based medium. Our results prove that potato waste pulp is a promising raw material for the production of yeast single-cell protein (SCP).

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

Recent publication.

- 1 Golubev WI. 2020. Sensitivity of *Yarrowia lipolytica* to *Wickerhamomyces* mycocins. *Problems Med Mycology* 22(3):26-28.

All strains of *Yarrowia lipolytica* are sensitive or weakly sensitive to mycocins of three species *Wickerhamomyces*. *W. cifferi* and some strains of *W. anomalus* exhibit the most fungicidal activity.

Interrelation was not revealed between the extent of strain sensitivity to mycocins and their origin and mating type.

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>.

Recent publications.

- 1 James TY, Stajich JE, Hittinger CT, Rokas A. 2020. Toward a fully resolved fungal tree of life. *Annu Rev Microbiol* 74:291-313 - DOI: 10.1146/annurev-micro-022020-051835

In this review, we discuss the current status and future challenges for fully elucidating the fungal tree of life. In the last 15 years, advances in genomic technologies have revolutionized fungal systematics, ushering the field into the phylogenomic era. This has made the unthinkable possible, namely access to the entire genetic record of all known extant taxa. We first review the current status of the fungal tree and highlight areas where additional effort will be required. We then review the analytical challenges

imposed by the volume of data and discuss methods to recover the most accurate species tree given the sea of gene trees. Highly resolved and deeply sampled trees are being leveraged in novel ways to study fungal radiations, species delimitation, and metabolic evolution. Finally, we discuss the critical issue of incorporating the unnamed and uncultured dark matter taxa that represent the vast majority of fungal diversity.

- 2 Libkind D, Čadež N, Ofulente DA, Langdon QK, Rosa CA, Sampaio JP, Gonçalves P, Hittinger CT, Lachance MA. 2020. Towards yeast taxogenomics: lessons from novel species descriptions based on complete genome sequences. *FEMS Yeast Res* 20:foaa042 - DOI: 10.1093/femsyr/foaa042.

In recent years, ‘multi-omic’ sciences have affected all aspects of fundamental and applied biological research. Yeast taxonomists, though somewhat timidly, have begun to incorporate complete genomic sequences into the description of novel taxa, taking advantage of these powerful data to calculate more reliable genetic distances, construct more robust phylogenies, correlate genotype with phenotype and

even reveal cryptic sexual behaviors. However, the use of genomic data in formal yeast species descriptions is far from widespread. The present review examines published examples of genome-based species descriptions of yeasts, highlights relevant bioinformatic approaches, provides recommendations for new users and discusses some of the challenges facing the genome-based systematics of yeasts.

- 3 Stoneman HR, Wrobel RL, Place M, Graham M, Krause DJ, De Chiara M, Liti G, Schacherer J, Landick R, Gasch AP, Sato TK, Hittinger CT. 2020. CRISpy-Pop: a web tool for designing CRISPR/Cas9-driven genetic modifications in diverse populations. *G3 (Bethesda)* 10:4287-4294 - DOI: 10.1534/g3.120.401498.

CRISPR/Cas9 is a powerful tool for editing genomes, but design decisions are generally made with respect to a single reference genome. With population genomic data becoming available for an increasing number of model organisms, researchers are interested in manipulating multiple strains and lines. CRISpy-pop is a web application that generates and filters guide RNA sequences for CRISPR/Cas9 genome editing for diverse yeast and bacterial strains. The current implementation designs and predicts the activity of guide RNAs against more than 1000 *Saccharomyces cerevisiae* genomes, including 167 strains frequently used in bioenergy research.

- 4 Shen XX, Steenwyk JL, LaBella AL, Oplente D, Zhou X, Kominek J, Li Y, Groenewald M, Hittinger CT, Rokas A. 2020. Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota. *Sci Adv* 6:eabd0079 - DOI: 10.1126/sciadv.abd0079.

Ascomycota, the largest and most well-studied phylum of fungi, contains three subphyla: Saccharomycotina (budding yeasts), Pezizomycotina (filamentous fungi), and Taphrinomycotina (fission yeasts). Despite its importance, we lack a comprehensive genome-scale phylogeny or understanding of the similarities and differences in the mode of genome evolution within this phylum. By examining 1107 genomes from Saccharomycotina (332), Pezizomycotina (761), and Taphrinomycotina (14) species, we inferred a robust genome-wide phylogeny that resolves several contentious relationships and estimated that the Ascomycota last

- 5 Haase MAB, Kominek J, Oplente DA, Shen XX, LaBella AL, Zhou X, DeVirgilio J, Hulfachor AB, Kurtzman CP, Rokas A, Hittinger CT. In press. Repeated horizontal gene transfer of GALactose metabolism genes violates Dollo's law of irreversible loss. *Genetics bioRxiv* DOI: 10.1101/2020.07.22.216101.

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

Zymomonas mobilis, an increasingly popular bacterial bioenergy research model, is also supported. CRISpy-pop is available as a web application (<https://CRISpy-pop.glbrc.org/>) with an intuitive graphical user interface. CRISpy-pop also cross-references the human genome to allow users to avoid the selection of guide RNAs with potential biosafety concerns. Additionally, CRISpy-pop predicts the strain coverage of each guide RNA within the supported strain sets, which aids in functional population genetic studies. Finally, we validate how CRISpy-pop can accurately predict the activity of guide RNAs across strains using population genomic data.

common ancestor likely originated in the Ediacaran period. Comparisons of genomic properties revealed that Saccharomycotina and Pezizomycotina differ greatly in their genome properties and enabled inference of the direction of evolutionary change. The Saccharomycotina typically have smaller genomes, lower guanine-cytosine contents, lower numbers of genes, and higher rates of molecular sequence evolution compared with Pezizomycotina. These results provide a robust evolutionary framework for understanding the diversity and ecological lifestyles of the largest fungal phylum.

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

- 6 LaBella AL, Opulente DA, Steenwyk J, Hittinger CT, Rokas A. Signatures of optimal codon usage predict metabolic ecology in budding yeasts. *bioRxiv* DOI: 10.1101/2020.07.22.214635.

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

yeasts of the CUG-Ser1 clade and with dairy-associated ecological niches in the family Saccharomycetaceae. For example, optimal codon usage of *GAL* genes is greater than 85% of all genes in the major human pathogen *Candida albicans* (CUG-Ser1 clade) and greater than 75% of genes in the dairy yeast *Kluyveromyces lactis* (family Saccharomycetaceae). We further find a correlation between optimization in the thiamine biosynthesis and *GAL* pathways. As a result, optimal codon usage in thiamine biosynthesis genes is also associated with dairy ecological niches in Saccharomycetaceae, which may reflect competition with co-occurring microbes for extracellular thiamine. This work highlights the potential of codon optimization as a tool for gaining insights into the metabolic ecology of microbial eukaryotes. Doing so may be especially illuminating for studying fungal dark matter—species that have yet to be cultured in the lab or have only been identified by genomic material.

- 7 Li Y, Steenwyk JL, Chang Y, Wang Y, James TY, Stajich JE, Spatafora JW, Groenewald M, Dunn CW, Hittinger CT, Shen XX, Rokas A. A genome-scale phylogeny of Fungi; insights into early evolution, radiations, and the relationship between taxonomy and phylogeny. *bioRxiv* DOI: 10.1101/2020.08.23.262857

Phylogenomic studies based on genome-scale amounts of data have greatly improved understanding of the tree of life. Despite their diversity, ecological significance, and biomedical and industrial importance, large-scale phylogenomic studies of Fungi are lacking. Furthermore, several evolutionary relationships among major fungal lineages remain controversial, especially those at the base of the fungal phylogeny. To begin filling these gaps and assess progress toward a genome-scale phylogeny of the entire fungal kingdom, we compiled a phylogenomic data matrix of 290 genes from the genomes of 1,644 fungal species that includes representatives from most major fungal lineages; we also compiled 11 additional data matrices by subsampling genes or taxa based on filtering criteria previously shown to improve phylogenomic inference. Analyses of these 12 data matrices using concatenation- and coalescent-based approaches yielded a robust phylogeny of the kingdom

in which ~85% of internal branches were congruent across data matrices and approaches used. We found support for several relationships that have been historically contentious (e.g., for the placement of Wallemiomycotina (Basidiomycota), as sister to Agaricomycotina), as well as evidence for polytomies likely stemming from episodes of ancient diversification (e.g., at the base of Basidiomycota). By examining the relative evolutionary divergence of taxonomic groups of equivalent rank, we found that fungal taxonomy is broadly aligned with genome sequence divergence, but also identified lineages, such as the subphylum Saccharomycotina, where current taxonomic circumscription does not fully account for their high levels of evolutionary divergence. Our results provide a robust phylogenomic framework to explore the tempo and mode of fungal evolution and directions for future fungal phylogenetic and taxonomic studies.

Recent publications.

- 1 Flores MG, Rodríguez ME, Peris D, Barrio E, Querol A, Lopes CA. 2020. Human-associated migration of Holarctic *Saccharomyces uvarum* strains to Patagonia. *Fungal Ecology* 48:100990

The domestication of *Saccharomyces cerevisiae*, as the result of its adaptation to human-manipulated processes, has been well analyzed by both phenotypic and genomic approaches. However, in other yeast species with industrial applications, such as *Saccharomyces uvarum*, these studies are very limited. To deepen knowledge about possible domestication in *S. uvarum*, an analysis of the genetic diversity of a series of *S. uvarum* strains isolated from different habitats was performed. Our results show that the greatest *S. uvarum* population diversity worldwide is observed in Patagonia, where strains of this species can be isolated from industrial and traditional fermentations as well as from natural environments. This greater Patagonian diversity is due to the presence of strains belonging to two genetically

differentiated populations, South America B (SA-B), and Holarctic/South America A (H/SA-A). The H/SA-A population of Patagonia is directly related to apple fermentation environments, mainly from cider fermentations but also, to a lesser extent, from traditional apple chicha. Our data suggest that strains from the Holarctic population colonized Patagonia. This is possibly associated with the introduction of apple trees by European immigrants, since the Spanish colonization of Chile in the 16th century and the introduction of new apple tree cultivars in the upper valley of the Negro River, Argentina, during the 19th century. During this process of colonization, Holarctic strains hybridized with SA-B yeasts, generating a series of admixed strains, mainly present in the traditional apple chichi fermentations.

- 2 Sorribes-Daudéna R, Peris D, Martínez-Pastor MT, Puig S. 2020. Structure and function of the vacuolar Ccc1/VIT1 family of iron transporters and its regulation in fungi. *Comput Struct Biotechnol* 18:3712-3722

Iron is an essential micronutrient for most living beings since it participates as a redox active cofactor in many biological processes including cellular respiration, lipid biosynthesis, DNA replication and repair, and ribosome biogenesis and recycling. However, when present in excess, iron can participate in Fenton reactions and generate reactive oxygen species that damage cells at the level of proteins, lipids and nucleic acids. Organisms have developed different molecular strategies to protect themselves against the harmful effects of high concentrations of iron. In the case of fungi and plants, detoxification mainly occurs by importing cytosolic iron into the vacuole through the Ccc1/VIT1 iron transporter. New sequenced genomes and bioinformatic tools are facilitating the functional characterization, evolution and ecological relevance of metabolic pathways and homeostatic networks across the Tree of Life. A thorough sequence analysis shows that Ccc1/VIT1 homologs are widely

distributed among organisms with the exception of animals. The recent elucidation of the crystal structure of a Ccc1/VIT1 plant ortholog has enabled the identification of both conserved and species-specific motifs required for its metal transport mechanism. Moreover, recent studies in the yeast *Saccharomyces cerevisiae* have also revealed that multiple transcription factors including Yap5 and Msn2/Msn4 contribute to the expression of *CCC1* in high-iron conditions. Interestingly, Malaysian *S. cerevisiae* strains express a partially functional Ccc1 protein that renders them sensitive to iron. Different regulatory mechanisms have been described for non-Saccharomycetaceae Ccc1 homologs. The characterization of the structure, function and regulation of Ccc1/VIT1 proteins is of high interest in the development of biofortified crops and in the protection against microbial-derived diseases.

1 More articles on *Saccharomyces* hybrids

In my last last communication (YNL June 2020) I missed the following article:

Lairón-Peris M, Pérez-Través L, Muñiz-Calvo S, Guillamón JM, Heras JM, Barrio E, Querol A. 2000. Differential contribution of the parental genomes to a *S. cerevisiae* × *S. uvarum* hybrid, inferred by phenomic, genomic, and transcriptomic analyses, at different industrial stress conditions. *Front Bioeng Biotechnol* 8:129. doi: 10.3389/fbioe.2020.00129.

The team led by Dr. A. Querol pioneered the description of *Saccharomyces* hybrids. In the special Issue “Yeast Interspecies Hybrids” Dr. Querol has published a review on the *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids:

Peris D, Pérez-Torrado R, Hittinger CT, Barrio E, Querol A. 2018. On the origins and industrial applications of *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids. *Yeast* 35(1):51-69. doi: 10.1002/yea.3283.

In that review the authors commented on the Biological Species Concept (BSC): “... the application of the BSC in yeast in general,¹ and in the genus *Saccharomyces* in particular, is fuzzy. As an example, the two closest relatives, *S. eubayanus* and *S. uvarum*, can interbreed and generate hybrids with levels of spore viability between 7.3% and 18%.”

The most recent article on *Saccharomyces* hybrids published with Dr. Querol as coauthor:

Morard M, Ibáñez C, Adam AC, Querol A, Barrio E, Toft C. 2020. Genomic instability in an interspecific hybrid of the genus *Saccharomyces*: a matter of adaptability. *Microb Genom* 6(10):mgen000448. doi: 10.1099/mgen.0.000448.

2 Biological Species Concept (BSC): the case of *S. cariocanus*

In the June issue of the YNL, readers might note a difference concerning the eight current *Saccharomyces* species listed in the following two papers:

¹ Editor’s note:

But see Lee et al. 2018 *Antonie van Leeuwenhoek* 111:1935–1953 and Lachance et al. 2020 in this issue of the YNL for an example of 36 BSC-delimited yeast species.

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

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

The two main differences were the status of *S. bayanus* (hybrid versus pure species) and the species *Saccharomyces cariocanus*. In Liti et al (2006), the authors wrote, in the abstract, “Using the biological species definition, yeasts of the genus *Saccharomyces* sensu stricto comprise six species and one natural hybrid [...] Furthermore, we show that one species of *Saccharomyces* –*S. cariocanus*– differs from a population of *S. paradoxus* by four translocations, but not by sequence. There is molecular evidence of recent introgression from *S. cerevisiae* into the European population of *S. paradoxus*, supporting the idea that in nature the boundary between these species is fuzzy”.

Thus *S. cariocanus* is not a separate species but a particular strain of *S. paradoxus*.

Liti G, Barton DB, Louis EJ. 2006 Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. *Genetics*. 2006 Oct;174(2):839-50. doi: 10.1534/genetics.106.062166.

3 Genomes of strain CBS 380 (former type strain of the obsolete *Saccharomyces bayanus*, Saccardo, 1895)

a. In the following article the composition of the hybrid genome of strain CBS 380 has been defined three times because the genomes of this strain have been obtained by three independent laboratories:

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.

These genomes have been assembled by Q Langdon from the SRA deposited at the time that they were sequenced. The assemblies are available at NCBI under the following Accessions numbers alongside with two genomes of strains NBRC1948 and NCAIM676, which are identified as *S. eubayanus* × *S. uvarum* hybrids (named *S. bayanus*).

TPA_asm: *Saccharomyces bayanus* strain CBS 380, whole genome shotgun sequencing project.
 ACCESSION DUXY00000000
 - TPA_asm: *Saccharomyces bayanus* strain CBS 380, whole genome shotgun
 ACCESSION DUXZ00000000
 - *Saccharomyces eubayanus* x *Saccharomyces uvarum* strain FM473, whole genome shotgun sequencing project.
 (Note :FM47=NRRL Y-12624=CBS380)
 ACCESSION VZXL00000000
 - TPA_asm: *Saccharomyces bayanus* NBRC1948, whole genome shotgun sequencing project.
 ACCESSION DUXM00000000
 -TPA_asm: *Saccharomyces bayanus* strain FM1309 (=monosporic-derivative of NBRC 1948), whole genome shotgun sequencing project.
 ACCESSION DUXN00000000
 - TPA_asm: *Saccharomyces bayanus* strain NCAIM 676, whole genome shotgun sequencing project.
 ACCESSION DUXK00000000

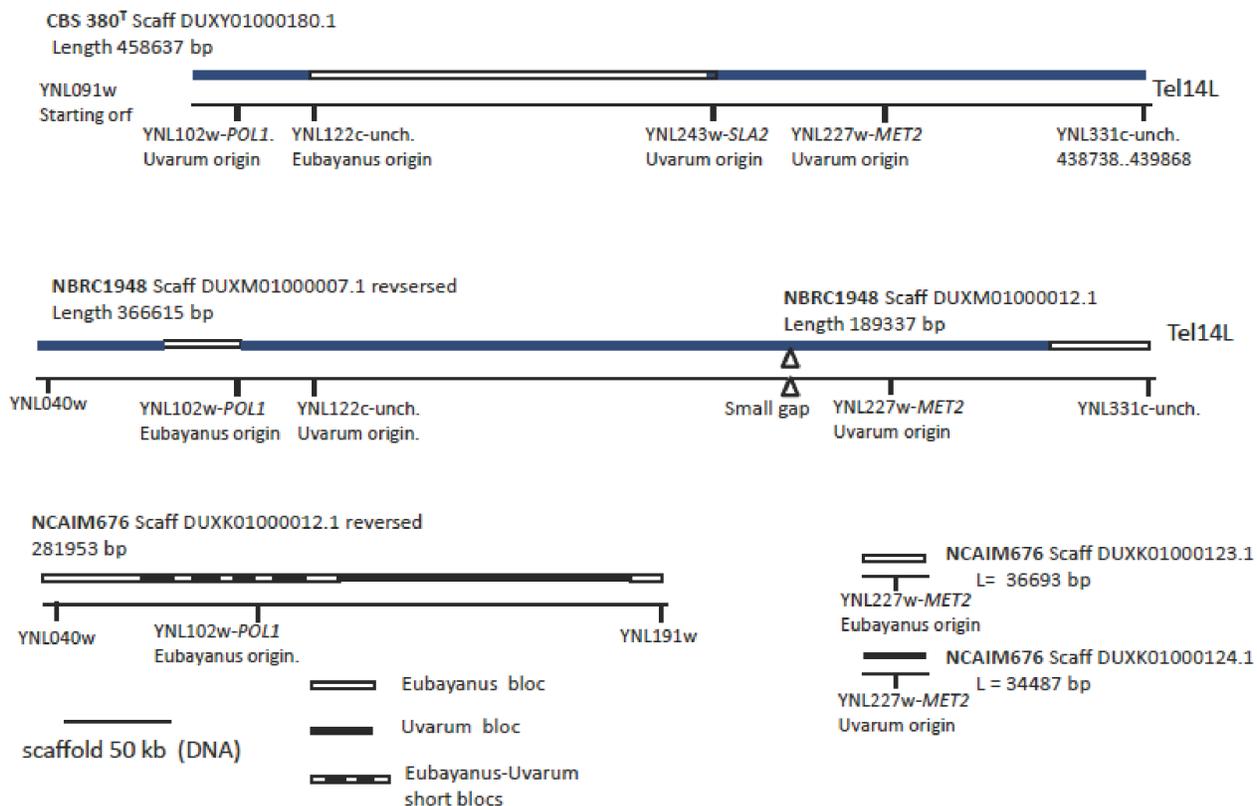
b. Erratum

The genome of strain CBS 1503 has been deposited under the name *S. eubayanus* which is an error, as this strain should have been named *S. pastorianus* (formerly *S. monacensis*).

- TPA_asm: *Saccharomyces eubayanus* strain CBS 1503, flattened_line_172, whole genome shotgun sequence.
 ACCESSION DUYH01000087.

c. Analysis of the a scaffold corresponding to the left arm of chromosome 14 in strain CBS 380 showing the chimeric nature of at least one chromosome in a *S. eubayanus* x *S. uvarum* hybrid. Comparison of this chromosome segment with that of two other hybrids NBRC1948 and NCAIM676. Sequences from database were manually annotated and ORFs or DNA regions of around 100kb were identified by BlastN on NCBI (Nucleotides collection (nr/nt)/Organism: *Saccharomyces eubayanus* (taxid:1080349) or BlastN on SGD Fungal Blast against *S. bayanus* and *S. pastorianus*).

Chimeric chromosome 14 (partial) in different *S. eubayanus* x *S. uvarum* hybrids (formerly *Saccharomyces bayanus*)



The above figure (almost to scale) illustrates the chimeric chromosome N at left arm but with varying composition in three different hybrids.

d. Beware of commercial *S. bayanus* strains

The name *S. bayanus* has been misused in many articles found in the past and even recently.

On Pubmed, a search for *Saccharomyces bayanus* (<https://pubmed.ncbi.nlm.nih.gov/?term=Saccharomyces+bayanus&sort=date>) yielded many articles in which strains were commercialized by companies in France or Germany under different brand names, such as EC1118 and LalvinQA23. These two strains appear on SGD as *S. cerevisiae* as follows:

On <https://www.yeastgenome.org/blast-sgd>

OTHER *S. cerevisiae* strains: GENOMIC SEQUENCE

(In alphabetical order)

AWRI1631 (AWRI) [...] EC1118 (Genoscope) [...]

LALVIN QA23 (AWRI)

OTHER *S. cerevisiae* strains: PROTEIN ENCODING

(DNA or Protein)

(Same number of strains as above)

In 2016 we published a work demonstrating that the galactose-negative wine yeast *S. cerevisiae* was mis-named as *S. bayanus*.

Dulermo R, Legras JL, Brunel F, Devillers H, Sarilar V, Neuvéglise C, Nguyen HV. 2016. Truncation of Gal4p explains the inactivation of the GAL/MEL regulon in both *Saccharomyces bayanus* and some

Saccharomyces cerevisiae wine strains. FEMS Yeast Res. 16(6):fow070. doi: 10.1093/femsyr/fow070.

The abstract states: “In the past, the galactose-negative (Gal(-)) phenotype was a key physiological character used to distinguish *Saccharomyces bayanus* from *S. cerevisiae*. In this work, we investigated the inactivation of GAL gene networks in *S. bayanus* [...] and in *S. cerevisiae* wine strains erroneously labelled '*S. bayanus*' [...] Similarly, two different truncated *GAL4* alleles were found in *S. cerevisiae* wine strains EC1118 and LalvinQA23. The lack of *GAL4* activity in these strains was corrected by introducing a full-length copy of *S. cerevisiae GAL4* on a CEN4/ARS plasmid.”

To avoid such errors, authors should re-identify commercial strains by determining the D1/D2 sequence and querying it against the NCBI nucleotides database.

I found one case of strains commercialized as “*S. bayanus*” where the authors determined the D1/D2 sequence but omitted to subject them to a Blast search, such that the sequences (Blasted by me) clearly matches that of *S. cerevisiae*, although the name *S. bayanus* was used in the article:

Aslankoochi et al. 2016. Non-conventional yeast strains increase the aroma complexity of bread. PLoS One 11(10):e0165126
doi: 10.1371/journal.pone.0165126.

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

Recent publications from our department.

- 1 Yurkov AM, Sannino C, Turchetti B. 2019. *Mrakia fibulata* sp. nov., a psychrotolerant yeast from temperate and cold habitats. Antonie van Leeuwenhoek 113:499-510.

Tree fluxes are sugar-rich, sometimes ephemeral, substrates occurring on sites where tree sap (xylem or phloem) is leaking through damages of tree bark. Tree sap infested with microorganisms has been the source of isolation of many species, including the biotechnologically relevant carotenoid yeast *Phaffia rhodozyma*. Tree fluxes recently sampled in Germany yielded 19 species, including several psychrophilic yeasts of the genus *Mrakia*. Four strains from tree fluxes represented a potential novel *Mrakia* species previously known from two isolates from superficial glacial melting water of Calderone Glacier (Italy). The Italian isolates, originally identified as *Mrakia*

aquatica, and two strains from Germany did not show any sexual structures. But another culture collected in Germany produced clamped hyphae with teliospores. A detailed examination of the five isolates (three from Germany and two from Italy) proved them to be a novel yeast species, which is described in this manuscript as *Mrakia fibulata* sp. nov. (MB 830398), holotype DSM 103931 and isotype DBVPG 8059. In contrast to other sexually reproducing *Mrakia* species, *M. fibulata* produces true hyphae with clamp connections. Also, this is the first psychrotolerant *Mrakia* species which grows above 20 °C. Spring tree fluxes are widespread and can be recognized and

sampled by amateurs in a Citizen Science project. This substrate is a prominent source of yeasts, and may harbor unknown species, as demonstrated in the present work. The description of *Mrakia fibulata* is

- 2 Kachalkin AV, Turchetti B, Inácio J, Carvalho C, Mašínová T, Pontes A, Röhl O, Glushakova AM, Akulov A, Baldrian P, Begerow D, Buzzini P, Sampaio JP, Yurkov A. 2019. Rare and undersampled dimorphic basidiomycetes. *Mycol Progr* 18:945–971.

The diversity of yeasts has grown rapidly as the discovery of new species has benefited from intensified sampling and largely improved identification techniques. An environmental study typically reports the isolation of yeast species, some of which are new to science. Rare species represented by a few isolates often do not result in a taxonomic description. Nucleic acid sequences from these undescribed yeasts remain in public sequence databases, often without a proper taxonomic placement. This study presents a constrained phylogenetic analysis for many rare yeasts from unpublished but publicly available DNA sequences and from studies previously conducted by the authors of this work. We demonstrate that single isolates are an important source of taxonomic findings such as including new genera and species. Independent surveys performed during the last 20 years on a large geographic scale yielded a number of single strains, which were proved to be conspecific in the phylogenetic analyses presented here. The following new species were resolved and described: *Vustinia terrea* Kachalkin, Turchetti & Yurkov gen. nov. et sp. nov.; *Udeniomyces caspiensis* Kachalkin sp. nov.; *Udeniomyces orazovii* Kachalkin sp. nov.; *Tausonia rosea* Kachalkin sp. nov.; *Itersonilia diksonensis* Kachalkin sp. nov.; *Krasilnikozyma fibulata* Glushakova & Kachalkin, *Kwoniella*

dedicated to our volunteer helpers and amateurs, like Anna Yurkova (9-years-old daughter of Andrey Yurkov), who collected the sample which yielded the type strain of this species.

fici Turchetti sp. nov.; *Heterocephalacria fruticeti* f.a. Carvalho, Roehl, Yurkov & Sampaio sp. nov.; *Heterocephalacria gelida* f.a. Turchetti & Kachalkin sp. nov.; *Heterocephalacria hypogea* f.a. Carvalho, Roehl, Yurkov & Sampaio sp. nov.; *Heterocephalacria lusitanica* f.a. Inacio, Carvalho, Roehl, Yurkov & Sampaio sp. nov.; *Piskurozoma arborea* Yurkov, Kachalkin, Mašínová & Baldrian sp. nov.; *Piskurozoma silvicultrix* Turchetti, Mašínová, Baldrian & Yurkov sp. nov.; *Piskurozoma stramentorum* Yurkov, Mašínová & Baldrian sp. nov.; *Naganishia nivalis* Turchetti sp. nov.; and *Yurkovia nerthusi* Yurkov & Begerow, sp. nov. In addition, two new combinations were proposed *Krasilnikozyma curviuscula* (Babeva, Lisichkina, Reshetova & Danilevich) Yurkov, Kachalkin & Sampaio comb. nov. and *Hannaella taiwanensis* (F.L. Lee & C.H. Huang) Yurkov comb. nov. The order Cyphobasidiales T. Spribille & H. Mayrhofer is rejected in favor of the older name Erythrobasidiales R. Bauer, Begerow, J.P. Sampaio, M. Weiss & Oberwinkler. Other potential novel species identified in this paper await future description. Phylogenetic placement of yet unpublished sequences is believed to facilitate species descriptions and improve classification of yeasts from environmental sequence libraries.

- 3 Tasselli G, Filippucci S, D'Antonio S, Cavalaglio G, Turchetti B, Cotana F, Buzzini P. 2019. Optimization of enzymatic hydrolysis of cellulosic fraction obtained from stranded driftwood feedstocks for lipid production by *Solicoccozyma terricola*. *Biotechnol Rep* 24:e00367.

Stranded driftwood feedstocks may represent, after pretreatment with steam explosion and enzymatic hydrolysis, a cheap C-source for producing biochemicals and biofuels using oleaginous yeasts. The hydrolysis was optimized using a response surface methodology (RSM). The solid loading (SL) and the dosage of enzyme cocktail (ED) were varied following a central composite design (CCD) aimed at optimizing the conversion of carbohydrates into lipids (Y_L) by the yeast *Solicoccozyma terricola* DBVPG

5870. A second-order polynomial equation was computed for describing the effect of ED and SL on Y_L . The best combination (ED = 3.10%; SL = 22.07%) for releasing the optimal concentration of carbohydrates which gave the highest predicted Y_L (27.32%) was then validated by a new hydrolysis. The resulting value of Y_L (25.26%) was close to the theoretical maximum value. Interestingly, fatty acid profile achieved under the optimized conditions was similar to that reported for palm oil.

- 4 De Vero L, Boniotti MB, Budroni M, Buzzini P, Cassanelli S, Comunian R, Gullo M, Logrieco AF, Mannazzu I, Musumeci R, Perugini I, Perrone G, Pulvirenti A, Romano P, Turchetti B, Varese C. 2019. Preservation, characterization and exploitation of microbial biodiversity: the perspective of the Italian network of culture collections. *Microorganisms* 7:685.

Microorganisms represent most of the biodiversity of living organisms in every ecological habitat. They have profound effects on the functioning of any ecosystem, and therefore on the health of our planet and of human beings. Moreover, microorganisms are the main protagonists in food, medical and biotech industries, and have several environmental applications. Accordingly, the characterization and preservation of microbial biodiversity are essential not only for the maintenance of natural ecosystems but also for research purposes and biotechnological exploitation. In this context, culture collections (CCs) and microbial biological resource centers (mBRCs) are

crucial for the safeguarding and circulation of biological resources, as well as for the progress of life sciences. This review deals with the expertise and services of CCs, in particular concerning preservation and characterization of microbial resources, by pointing to the advanced approaches applied to investigate a huge reservoir of microorganisms. Data sharing and web services as well as the tight interconnection between CCs and the biotechnological industry are highlighted. In addition, guidelines and regulations related to quality management systems (QMSs), biosafety and biosecurity issues are discussed according to the perspectives of CCs and mBRCs.

- 5 Troilo A, De Francesco G, Marconi O, Sileoni V, Turchetti B, Perretti G. 2019. Low carbohydrate beers produced by a selected yeast strain from an alternative source. *J Am Soc Brew Chem* 78:80.

Recently, the brewing industry has invested significant resources into the development of new specialty beers, in response to increased consumer demand. Low carbohydrate and novel flavored beers have resulted and these are particularly attractive for consumers, even if technologically challenging to produce. The aim of this work was to produce a low carbohydrate and novel flavored beer in a traditional manner. A *Saccharomyces cerevisiae* strain isolated from an alternative source (i.e., yeast strain DBVPG 6580 was used in a Brazilian bioethanol production facility) was selected because of its starch degrading character and this yeast was employed in a traditional brewery plant to achieve this goal. The strain DBVPG

6580 was used to ferment worts of different densities (12 and 20°P) and at different fermentation temperatures (18 and 25 °C), to explore possible technological applications. The results demonstrated that yeast DBVPG 6580 was able to degrade dextrans to fermentable sugars, giving an apparent attenuation above 100% and allowing for the production of a low carbohydrate beer by traditional methods. Moreover, strain DBVPG 6580 produced novel flavored beers, rich in fruity, floral, banana and pear flavors. This yeast strain demonstrated that under optimal conditions, it possessed the ability to ferment a high-density wort, which could lead to important economic advantages for the producer.

- 6 Sannino C, Mezzasoma A, Buzzini P, Turchetti B. 2019. Non-conventional yeasts for producing alternative beers. In: Sibirny A. (ed) *Non-conventional yeasts: from basic research to applications*. Springer, Heidelberg, pp. 361-388.

Since immemorial time beer is the product of wort fermentation catalyzed by the cells of two main yeast species, namely, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, with hop addition. However, in recent years the beer market changed completely and novel types of beers, such as low-alcohol and low-calorie beers and spontaneously fermented or flavored beers, have become very popular. These different beers vary for the nature and amounts of water, cereals, hops, and other additives, techniques used for production, storage, and consumption, and also yeasts used as starter. Indeed, some non-conventional yeasts have been recently

proposed as starter cultures for brewing. This chapter provides a comprehensive review of the most recent papers describing yeast species diversity used in brewing industry or found in spontaneous fermented beers. Many species were described for their fermentative aptitude as a single or co-starter, either at lab or pilot scale and, rarely, at the industrial scale. Ascomycetous species are generally used but a member of Basidiomycota (*Mrakia* sp.) was also considered. A particular focus in the chapter is dedicated to flavor production and to spontaneous fermentation.

- 7 Li AH, Yuan FX, Groenewald M, Bensch K, Yurkov AM, Li K, Han PJ, Guo LD, Aime MC, Sampaio JP, Jindamorakot S, Turchetti B, Inacio J, Fungsin B, Wang QM, Bai FY. 2019. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: proposal of two new orders, three new families, eight new genera and one hundred and seven new species. *Stud. Mycol.*96: 17-140.

Nearly 500 basidiomycetous yeast species were accepted in the latest edition of *The Yeasts: A Taxonomic Study* published in 2011. However, this number presents only the tip of the iceberg of yeast species diversity in nature. Possibly more than 99 % of yeast species, as is true for many groups of fungi, are yet unknown and await discovery. Over the past two decades nearly 200 unidentified isolates were obtained during a series of environmental surveys of yeasts in phyllosphere and soils, mainly from China. Among these isolates, 107 new species were identified based on the phylogenetic analyses of nuclear ribosomal DNA (rDNA) [D1/D2 domains of the large subunit (LSU), the small subunit (SSU), and the internal transcribed spacer region including the 5.8S rDNA

(ITS)] and protein-coding genes [both subunits of DNA polymerase II (RPB1 and RPB2), the translation elongation factor 1- α (TEF1) and the mitochondrial gene cytochrome b (CYTB)], and physiological comparisons. Forty-six of these belong to 16 genera in the *Tremellomycetes* (*Agaricomycotina*). The other 61 are distributed in 26 genera in the *Pucciniomycotina*. Here we circumscribe eight new genera, three new families and two new orders based on the multi-locus phylogenetic analyses combined with the clustering optimisation analysis and the predicted similarity thresholds for yeasts and filamentous fungal delimitation at genus and higher ranks. Additionally, as a result of these analyses, three new combinations are proposed and 66 taxa are validated.

- 8 Verkley G, Perrone G, Pina M, Scholz AH, Overmann J, Zuzuarregui A, Perugini I, Turchetti B, Hendrickx M, Stacey G, Law S, Russell J, Smith D, Lima N. 2019. New ECCO model documents for material deposit and transfer agreements in compliance with the Nagoya Protocol. *FEMS Microbiol. Lett.*, 367: fnaa044.

The European Culture Collections Organisation presents two new model documents for Material Deposit Agreement (MDA) and Material Transfer Agreement (MTA) designed to enable microbial culture collection leaders to draft appropriate agreement documents for, respectively, deposit and supply of materials from a public collection. These tools provide guidance to collections seeking to draft an MDA and MTA, and are available in open access to be used, modified, and shared. The MDA model consists of a set of core fields typically included in a 'deposit form' to collect relevant information to facilitate assessment of the status of the material under

access and benefit sharing (ABS) legislation. It also includes a set of exemplary clauses to be included in 'terms and conditions of use' for culture collection management and third parties. The MTA model addresses key issues including intellectual property rights, quality, safety, security and traceability. Reference is made to other important tools such as best practices and code of conduct related to ABS issues. Besides public collections, the MDA and MTA model documents can also be useful for individual researchers and microbial laboratories that collect or receive microbial cultures, keep a working collection, and wish to share their material with others.

- 9 Sannino C, Borruso L, Smiraglia C, Bani A, Mezzasoma A, Brusetti L, Turchetti B, Buzzini P. 2019. Dynamics of *in situ* growth and taxonomic structure of fungal communities in Alpine supra glacial debris. *Fungal Ecol.* 44: 100891.

Supraglacial debris of Miage Glacier (Mont Blanc, Italy) was used as an *in situ* model for monitoring growth and modification of the taxonomic structure of fungal populations using an in-growth mesh bag approach over three consecutive years. Sterilized debris was placed in mesh bags (MB) and buried in the debris layer. Pristine debris (D) covering the surface of the glacier was also studied for comparative purposes. Quantitative PCR revealed that after a time of between one and two years the fungal

abundance in the sterilized supraglacial debris contained in MB increased to reach a level comparable with those found in D samples. Likewise, the analysis of alpha-diversity exhibited similar dynamics during the three years of study. On the contrary, beta-diversity and Linear discriminant analysis Effect Size (LEfSe), showed apparently fluctuating dynamics from the first to the third year of study and a reduction of the number of fungal taxa discriminating MB and D samples. These observations confirm the hypothesis

that fungal cells deposited on MB either by the surrounding debris or continuously brought via wind or atmospheric precipitations, can be subjected to an *in-situ* growth which leads the abundance of the

fungal communities occurring in MB to levels found in pristine debris (D) but showing fluctuating taxonomic structures.

- 10 Turchetti B, Marconi G, Sannino C, Buzzini P, Albertini E. 2019. DNA methylation changes induced by cold in psychrophilic and psychrotolerant *Naganishia* yeast species. *Microorganisms* 8:296.

The involvement of DNA methylation in the response to cold stress of two different yeast species (*Naganishia antarctica*, psychrophilic, and *Naganishia albida*, psychrotolerant), exhibiting different temperature aptitudes, has been studied. Consecutive incubations at respective optimum temperatures, at 4 °C (cold stress) and at optimum temperatures again, were performed. After Methylation Sensitive Amplified Polymorphism (MSAP) fingerprints a total of 550 and 423 clear and reproducible fragments were amplified from *N. antarctica* and *N. albida* strains, respectively. The two *Naganishia* strains showed a different response in terms of level of DNA methylation during cold stress and recovery from cold stress. The percentage of total methylated fragments in

psychrophilic *N. antarctica* did not show any significant change. On the contrary, the methylation of psychrotolerant *N. albida* exhibited a nonsignificant increase during the incubation at 4 °C and continued during the recovery step, showing a significant difference if compared with control condition, resembling an uncontrolled response to cold stress. A total of 12 polymorphic fragments were selected, cloned, and sequenced. Four fragments were associated to genes encoding for elongation factor G and for chitin synthase export chaperon. To the best of our knowledge, this is the first study on DNA methylation in the response to cold stress carried out by comparing a psychrophilic and a psychrotolerant yeast species.

- 11 Filippucci S, Tasselli G, Labbani FZK, Turchetti B, Cramarossa MR, Buzzini P, Forti L. 2019. Non-conventional yeasts as source of ene-reductases for the bioreduction of chalcones. *Fermentation* 6:29.

Thirteen Non-Conventional Yeasts (NCYs) have been investigated for their ability to reduce activated C=C bonds of chalcones to obtain the corresponding dihydrochalcones. A possible correlation between bioreducing capacity of the NCYs and the substrate structure was estimated. Generally, whole-cells of the NCYs were able to hydrogenate the C=C double bond occurring in (*E*)-1,3-diphenylprop-2-en-1-one, while worthy bioconversion yields were obtained when the

substrate exhibited the presence of a deactivating electron-withdrawing Cl substituent on the B-ring. On the contrary, no conversion was generally found, with a few exceptions, in the presence of an activating electron-donating substituent OH. The bioreduction aptitude of the NCYs was apparently correlated to the logP value: compounds characterized by a higher logP exhibited a superior aptitude to be reduced by the NCYs than compounds with a lower logP value.

- 12 Sannino C, Borruso L, Mezzasoma A, Battistel D, Zucconi L, Selbmann L, Azzaro M, Onofri S, Turchetti B, Buzzini P, Guglielmin M. 2019. Intra- and inter-cores fungal diversity suggests interconnection of different habitats in an Antarctic frozen lake (Boulder Clay, Northern Victoria Land). *Environ Microbiol* 22:3463-3477.

A perennially frozen lake at Boulder Clay site (Victoria Land, Antarctica), characterized by the presence of frost mounds, have been selected as an *in-situ* model for ecological studies. Different samples of permafrost, glacier ice and brines have been studied as a unique habitat system. An additional sample of brines (collected in another frozen lake close to the previous one) was also considered. Alpha- and beta-diversity of fungal communities showed both intra- and inter-cores significant ($p < 0.05$) differences, which suggest the presence of interconnection among the habitats. Therefore, the layers of frost mound and the deep glacier could be interconnected while the

brines could probably be considered as an open habitat system not interconnected with each other. Moreover, the absence of similarity between the lake ice and the underlying permafrost suggested that the lake is perennially frozen based. The predominance of positive significant ($p < 0.05$) co-occurrences among some fungal taxa allowed to postulate the existence of an ecological equilibrium in the habitats systems. The positive significant ($p < 0.05$) correlation between salt concentration, total organic carbon and pH, and some fungal taxa suggests that a few abiotic parameters could drive fungal diversity inside these ecological niches.

- 13 Coleine C, Pombubpa N, Zucconi L, Onofri S, Turchetti B, Buzzini P, Stajich JE, Selbmann L. 2019. Uncover microbial diversity in Antarctic cryptoendolithic communities sampling three representative locations of the Victoria Land. *Microorganisms* 8:942 2020.

The endolithic niche represents an ultimate refuge to microorganisms in the Mars-like environment of the Antarctic desert. In an era of rapid global change and desertification, the interest in these border ecosystems is increasing due to speculation on how they maintain balance and functionality at the dry limits of life. To assure a reliable estimation of microbial diversity, proper sampling must be planned in order to avoid the necessity of re-sampling as reaching these remote locations is risky and requires tremendous logistical and economical efforts. In this study, we seek to determine the minimum number of samples for uncovering comprehensive bacterial and fungal diversity, comparing communities in strict

vicinity to each other. We selected three different locations of the Victoria Land (Continental Antarctica) at different altitudes and showing sandstone outcrops of a diverse nature and origin—Battleship promontory (834 m above sea level (a.s.l.), Southern VL), Trio Nunatak (1,470 m a.s.l., Northern VL) and Mt New Zealand (3,100 m a.s.l., Northern VL). Overall, we found that a wider sampling would be required to capture the whole amplitude of microbial diversity, particularly in Northern VL. We concluded that the inhomogeneity of the rock matrix and the stronger environmental pressure at higher altitudes may force the communities to a higher local diversification.

- 14 Turchetti B, Sannino C, Mezzasoma A, Zucconi L, Onofri S, Buzzini P. 2019. *Mrakia stelviica* sp. nov. and *Mrakia montana* sp. nov., two novel basidiomycetous yeast species isolated from cold environments. *Int J Syst Evol Microbiol* 70:4704-4713.

Five yeast strains were isolated from soil and sediments collected from Alps and Apennines glaciers during sampling campaigns carried out in summer 2007 and 2017, respectively. Based on morphological and physiological tests and on phylogenetic analyses reconstructed with ITS and D1/D2 sequences, the five strains were considered to belong to two related but hitherto unknown species within the genus *Mrakia*, in an intermediate position between *Mrakia*

cryoconiti and *Mrakia arctica*. The names *Mrakia stelviica* (holotype DBVPG 10734^T) and *Mrakia montana* (holotype DBVPG 10736^T) are proposed for the two novel species and a detailed description of their morphological, physiological and phylogenetic features are presented. Both species fermented glucose, sucrose and trehalose, which is an uncommon feature in basidiomycetous yeasts, and showed septate hyphae with teliospore formation.

- 15 Franzetti A, Pittino F, Gandolfi I, Azzoni R, Diolaiuti G, Smiraglia C, Pelfini M, Compostella C, Turchetti B, Buzzini P, Ambrosini R. 2019. Early ecological succession patterns of bacterial, fungal and plant communities along a chronosequence in a recently deglaciated area of Italian Alps. *FEMS Microbiol Ecol* 96:fiaa165.

In this study, the early ecological succession patterns of Forni Glacier (Ortles-Cevedale group, Italian Alps) forefield along an 18-year long chronosequence (with a temporal resolution of 1 year) has been reported. Bacterial and fungal community structures were inferred by high-throughput sequencing of 16S rRNA gene and ITS, respectively. In addition, the occurrence of both herbaceous and arboreous plants was also recorded at each plot. A significant decrease of alpha-diversity in more recently deglaciated areas was observed for both bacteria and plants. Time since deglaciation and pH affected the structure of both fungal and bacterial communities. Pioneer plants could be a major source of colonization

for both bacterial and fungal communities. Consistently, some of the most abundant bacterial taxa and some of those significantly varying with pH along the chronosequence (*Polaromonas*, *Granulicella*, *Thiobacillus*, *Acidiferrobacter*) are known to be actively involved in rock-weathering processes due to their chemolithotrophic metabolism, thus suggesting that the early phase of the chronosequence could be mainly shaped by the biologically controlled bioavailability of metals and inorganic compounds. Fungal communities were dominated by ascomycetous filamentous fungi and basidiomycetous yeasts. Their role as cold-adapted organic matter decomposers, due to their heterotrophic metabolism, was suggested.

Recent publications.

- 1 Pulvirenti A, De Vero L, Blaiotta G, Sidari R, Iosca G, Gullo M, Caridi A. 2020. Selection of wine *Saccharomyces cerevisiae* strains and their screening for the adsorption activity of pigments, phenolics and ochratoxin A. *Fermentation* 6(3):80. <https://doi.org/10.3390/fermentation6030080>

Ochratoxin A is a dangerous mycotoxin present in wines and is considered the principal safety hazard in the winemaking process. Several authors have investigated the ochratoxin A adsorption ability of *Saccharomyces cerevisiae* yeasts, and specifically selected strains for this desired trait. In the present work, a huge selection of wine yeasts was done starting from Portuguese, Spanish and Italian fermenting musts of different cultivars. Firstly, 150 isolates were collected, and 99 non-redundant *S. cerevisiae* strains were identified. Then, the strains were screened following a multi-step approach in order to select those having primary oenological traits, mainly (a) good fermentation performance, (b) low production of H₂S and

(c) low production of acetic acid. The preselected strains were further investigated for their adsorption activity of pigments, phenolic compounds and ochratoxin A. Finally, 10 strains showed the desired features. The goal of this work was to select the strains capable of absorbing ochratoxin A but not pigments and phenolic compounds in order to improve and valorise both the quality and safety of red wines. The selected strains are considered good candidates for wine starters, moreover, they can be exploited to obtain a further enhancement of the specific adsorption/non-adsorption activity by applying a yeast breeding approach.

- 2 Caridi A. 2020. Petri dish method to select yeasts able to produce more pigmented table olives. *Acta Scientiarum. Technology* 42(1), e48132. <https://doi.org/10.4025/actascitechnol.v42i1.48132>

The study of pigment adsorption of yeasts used for table olive fermentation may allow the protection of olive colour, by excluding those strains adsorbing phenolic compounds responsible for the colour. Fifty-one table olive yeasts were grown on Petri dishes using two olive-based screening media - ‘olive pulp agar’ and ‘olive seed agar’; the red, green, and blue colour components of the yeast’s biomass were measured. Wide and significant differences among the yeasts were observed. Based on the statistical analysis,

ten yeasts were selected, excluding all the strains exhibiting a too high pigment adsorption. The research proposes a simple analytical method to characterize yeasts for their pigment adsorption, thus allowing the enhancement of the table olive colour. The two media may be prepared using any olive cultivar, thus allowing a specific screening of the yeasts. The selection of those yeasts unable to adsorb olive pigments may allow the production of more pigmented table olives.

IX Comparative Genomics Group, Barcelona Supercomputing Centre (BSC-CNS), and Institute for Research in Biomedicine (IRB), Jordi Girona 29, 08034 Barcelona, Spain. Communicated by Toni Gabaldón - <http://cgenomics.org/> <toni.gabaldon.bcn@gmail.com>.

Recent publications.

- 1 Gabaldón T 2020 Hybridization and the origin of new yeast lineages. *FEMS Yeast Res* 20(5):foaa040
 - 2 Mixão V, Gabaldón T. 2020. Genomic evidence for a hybrid origin of the yeast opportunistic pathogen *Candida albicans*. *BMC Biol* 18(1):48 - doi: 10.1186/s12915-020-00776-6.
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X Department of Soil Biology, Faculty of Soil Science, Lomonosov Moscow State University, 119234, Leninskie gory, 1/12, Moscow, Russia; All-Russian Collection of Microorganisms (VKM), G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS, 142290, pr. Nauki 5, Pushchino, Russia. Communicated by Aleksey.V. Kachalkin <kachalkin_a@mail.ru>.

Recent publications.

- 1 Glushakova A, Maksimova I, Morozova A, Kachalkin A. 2019. Distribution features of yeasts in soils of South Vietnam (case study of the biogeocenoses of the National Park Cốt Tỉn). IOP Conference Series: Earth and Environmental Science. 368:012012. DOI: 10.1088/1755-1315/368/1/012012

The yeast communities of tropical soils of two biotopes in monsoon tropical forest in the specially protected natural territories of the National Park Cốt Tỉn were studied. It was possible to establish that the distribution of yeasts in the profile of studied soils

differed markedly. Of particular interest was the isolation from the tropical soils of Vietnam the pedobiont yeast species *Saitozyma podzolica*, which is regularly found in soils of temperate latitudes of Russia and other regions.

- 2 Maksimova IA, Kachalkin AV, Yakovleva EYu, Krivosheina MG, Markov AV. 2020. Yeast communities associated with Diptera of the White Sea littoral. Microbiology 89(2):212-218.

Relationship between symbiotic yeast and *Diptera* insects are studied mainly on fruit flies (*Drosophilidae*). Evolutionary experiments showed that changes in the composition of the yeast microbiota vectored by flies in their gut and on the body surface contributes to the adaptation of laboratory *Drosophila* populations to the high-salt growth medium. Although saline substrates are not commonly used by *Drosophilidae* in nature, species adapted to such substrates are known in other families of *Diptera*. Yeast communities associated with these species are studied insufficiently. This is the first report on the yeast communities associated with two *Diptera* species living near the White Sea, *Paracoenia fumosa* (Stenhammar, 1844) (*Ephydriidae*) and *Fucellia fucorum* (Fallén, 1819) (*Anthomyiidae*). Their larvae develop in the coastal saline lagoons and in algae driven ashore. The *P. fumosa* yeast microbiota includes opportunistic species *Pichia kluyveri* and

P. kudriavzevii and is characterized by its highly variable abundance (the number of colony-forming units varies widely in different individuals). *F. fucorum* is characterized by a more stable yeast community, including species common in White Sea algae and coastal waters (*Debaryomyces hansenii*, *Metschnikowia zobellii*, and *M. bicuspidata*). These two species of littoral flies shared no common yeast species. Moreover, none of the yeast species found in the littoral flies was detected in the evolutionary experiment on the adaptation of *Drosophila* to the high-salt growth medium (the similarity is limited only to the *Pichia* genus level). Contrasting differences in the yeast microbiome of two ecologically similar littoral species of *Diptera* may indicate, on the one hand, specificity, and, on the other, evolutionary plasticity of the relationship between salt-tolerant flies and yeast.

- 3 Abdullabekova DA, Magomedova ES, Aliverdieva DA, Kachalkin AV. 2020. Yeast communities of vineyards in Dagestan: ecological, taxonomic, and genetic characteristics. Biology Bulletin 47(4):344–351.

The ecological and taxonomic study of yeast communities of vineyards in Dagestan has demonstrated significant differentiation of species diversity depending on the type of substrate and plant ontogenesis. Thirty-three out of 44 yeast species were isolated from grape berries during the long-term study of this biotope. The seasonal occurrence of yeasts in agrocoenoses is characterized by the maximum species

richness during ripening of grapes in late summer and in autumn. Each stage of plant development is characterized by a specific type of yeast population, which determines the possibility of isolating yeast species from plants and the underlying soils. The DNA fingerprinting method used for *Saccharomyces cerevisiae* strains isolated from the vineyards of Dagestan showed their high genetic heterogeneity.

- 4 Maksimova IA, Glushakova AM, Thanh VN, Kachalkin AV. 2020. *Yamadazyma cocois* f.a, sp. nov, an ascomycetous yeast isolated from coconuts. *Int J Syst Evol Microbiol* 70(5):3491-3496.

During studies on the endophytic yeast communities associated with fruits from Vietnam, three fermenting yeast strains were isolated from fruits of the coconut palm (*Cocos nucifera*). Phylogenetic analysis based on the sequences of the ITS regions and D1/D2 domains of the large subunit rRNA gene showed that these strains represented a single species of the *Yamadazyma* clade that was distinct from the other related species. The new species represented a

basal branch of the clade formed by the *Yamadazyma* species i.e. *Y. insecticola* and *Y. takamatsuzukensis*. Based on the phylogenetic analysis and phenotypic characteristics, the studied strains were assigned to a novel species of the genus *Yamadazyma*, for which the name *Yamadazyma cocois* f.a, sp. nov. is proposed. The holotype is VCIM 4241, with the ex-type cultures VTCC 920004=VKM Y-3049=KBP Y-6091 code 17-68. The MycoBank number is MB 834435.

- 5 Crous PW, Wingfield MJ, Chooi Y-H, Gilchrist CLM, Lacey E, Pitt JI, Roets F, Swart WJ, Cano-Lira JF, Valenzuela-Lopez N, Hubka V, Shivas RG, Stchigel AM, Holdom DG, Jurjević Ž, Kachalkin AV et al. 2020. *Fungal Planet* description sheets: 1042–1111 *Persoonia* 44:301–459.

Novel species of yeasts described in this study:

Candida pellucida AM Glushakova, MA Tomashevskaya & Kachalkin, sp. nov. from *Exomias pellucidus*, and as endophyte from almond seeds.

Heterocephalacria septentrionalis Kachalkin, MA

Tomashevskaya & TA Pankratov, sp. nov. from lichen *Cladonia rangiferina*, and from *Empetrum nigrum*.

Vishniacozyma phoenicis Kachalkin, AS Venzhik & MA Tomashevskaya, sp. nov. from dates fruits.

- 6 Akhapkina IG, Glushakova AM, Rodionova EN, Kachalkin AV. 2020. The effectiveness of antifungal agents against yeasts of *Candida* genus isolated in Moscow region. *Antibiot Chemother* 65(3-4):16–22.

Analysis of changes in the antimicrobial effectiveness against *Candida* isolated from clinical and natural materials during 2014–2019 in Moscow. Antibiotic sensitivity of 186 isolates (75 clinical, 128 natural) of *Candida* species: *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei* (*Pichia kudriavzevii*), *C. intermedia*, *C. tropicalis*, *C. lusitaniae* (*Clavispora*

lusitaniae), *C. guilliermondii* (*Meyerozyma guilliermondii*), was determined by disc-diffusion method. During five years of observation, the antimicrobial sensitivity decreased by 19.51–53.52% for clinical isolates of *Candida* genus and by 4.16–44.39% for natural isolates.

- 7 Glushakova AM, Kachalkin AV, Umarova AB, Kokoreva AA, Ivanova AE, Bolotov AG, Dunaeva EA, Maksimova IA. 2020. Yeast complexes in urban soils of some southern cities of Russia (Krasnodar, Maykop, Simferopol, and Sochi). *Microbiology* 89(5):603–608.

Yeast number and species diversity in urban soils characterized by various intensities of anthropogenic impact in the southern cities of Russia (Krasnodar, Maykop, Simferopol, and Sochi) was investigated. The soils of botanical gardens and the reference soils in the vicinities of cities were used for comparison to reveal the degree of anthropogenic impact. The maximum yeast number was found in a layer of 0–20 cm in the urban soils of Sochi and Simferopol, where it was 5.7 ± 0.2 and 5.4 ± 0.01 log (CFU/g), respectively. The minimum number of yeasts, 2.0 ± 0.1 log (CFU/g), was characteristic of a 60–80-cm layer in the soils in the vicinities of all cities. The number of yeasts was shown to depend

primarily on the depth and type of soil and to a lesser extent on location. A total of 20 yeast species were isolated from the studied soils: 10 ascomycetes and 10 basidiomycetes. All basidiomycete yeasts isolated from urban soils and control soils were typical representatives of pedobiont and epiphytic yeast complexes. In the soils of the major tourist cities of Sochi and Simferopol, a high relative abundance of ascomycete species *Candida sake* and *Meyerozyma guilliermondii* was found, which fundamentally distinguished the studied soils from the ones of Krasnodar and Maykop. The cities of Sochi and Simferopol are characterized by a higher anthropogenic load compared to Krasnodar and

Maykop, which is associated not only with their high population, but also with a significant tourist load. Detection in urban soils of *C. sake* and *M. guilliermondii*

which, according to some data, are clinically significant, was therefore consequential.

8 Glushakova AM, Rodionova EN, Kachalkin AV. 2020. Yeasts in feces of pigeons (*Columba livia*) in the city of Moscow. *Cur Microbiol* - DOI: 10.1007/s00284-020-02251-5

The yeast number and species diversity in feces of pigeons (*Columba livia*) were studied in various locations of the city of Moscow: parks, playgrounds and school grounds. The total number and species composition of yeasts in feces depended on the isolation temperature. The average yeast number at a cultivation temperature of 25 °C was 3.2×10^5 CFU/g, at 37 °C – 2.5×10^6 CFU/g. At 37 °C, the number of yeasts was higher due to a more abundant growth of pathogenic and opportunistic yeast species. In total, 13 species of yeasts were isolated from feces:

eight ascomycetes and five basidiomycetes. The study revealed high values of relative abundance and frequency of occurrence of pathogenic species *Candida albicans* and opportunistic yeasts, i.e. *Diutina catenulata*, *Millerozyma farinosa*, *Pichia kudriavzevii*, and *Trichosporon asahii*. Pigeon feces were found to present a constant source of “infection” of the urban environment with yeast species that are hazardous to human health, especially for immunosuppressive individuals.

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

List of recent publications.

- 1 Sipiczki M, Antunovics Z, Szabo A. 2020. *MAT* heterozygosity and the second sterility barrier in the reproductive isolation of *Saccharomyces* species. *Curr Genet* 66:957-969.
 - 2 Sipiczki M. 2020. *Metschnikowia pulcherrima* and related pulcherrimin-producing yeasts: Fuzzy species boundaries and complex antimicrobial antagonism. *Microorganisms* 8:1029.
 - 3 Szabó A, Antunovics 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* 11:838.
 - 4 Čadež N, Drumonde-Neves J, Sipiczki M, Dlačny D, Lima T, Pais C, Schuller D, Franco-Duarte R, Lachance MA, Péter G. 2020. *Starmerella vitis* f.a. sp. nov, a yeast species isolated from flowers and grapes. *Antonie Van Leeuwenhoek*. 113:1289-1298.
 - 5 Sipiczki M.: *Mycosarcoma aegyptiacum* sp. nov, an antagonistic polymorphic basidiomycetous yeast related to smut fungi. *Int. J. System. Evol. Microbiol.* 70:1086-1092, 2020.
 - 6 Horváth E, Sipiczki M, Csoma H, Miklós I. 2020. Assaying the effect of yeasts on growth of fungi associated with disease. *BMC Microbiol* 20:320.
 - 7 Sipiczki M. 2019. Yeasts in botrytized wine making. In “Yeasts in the production of wine (eds. Romano, P, Ciani, M, Fleet, G. H.)” Springer, New York, pp. 229-261.
 - 8 Nagy Z, Medgyes-Horvath A, Szalay C, Sipiczki M, Sveiczler A. 2019. Phylogenetic analyses of proteins coordinating G2 size control in fission yeast. *Period Polytech Chem Eng* 63:555-568.
 - 9 Kallai Z, Pfliegler WP, Mitercsak J, Szendei G, Sipiczki M. 2019. Preservation of diversity and oenological properties of wine yeasts during long-term laboratory maintenance: A study of strains of a century-old Tokaj wine yeast collection. *LWT - Food Sci Technol* 101:789–798.
 - 10 Sipiczki M. 2019. Yeast two- and three-species hybrids and high-sugar fermentation. *Microbial Biotechnol* 12:1101-1108.
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XII Laboratory of Yeast Systematics, Research Institute for Agricultural and Life Sciences, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan. Communicated by Masako Takashima <mt207623@nodai.ac.jp>.

In March 2019, I retired from JCM (Japan Collection of Microorganisms, RIKEN BioResource Center) and continued my work with Prof. Takashi Sugita at Meiji Pharmaceutical University. I have now joined the Tokyo University of Agriculture as Professor and will continue my research on yeast systematics, combining traditional methods based on phenotypic characteristics with the latest information including genome information and contributing to the continued update of the classification of yeasts.

Recent publications.

- 1 Takashima M, Kurakado S, Cho O, Kikuchi K, Sugiyama J, Sugita S. 2020. Description of four *Apiotrichum* and two *Cutaneotrichosporon* species isolated from guano samples from bat-inhabited caves in Japan. *Int J Syst Evol Microbiol*. 70:4458-4469 - doi: 10.1099/ijsem.0.004277.
- 2 Takashima M, Manabe RI, Nishimura Y, Endoh R, Ohkuma M, Sriswasdi S, Sugita T, Iwasaki W. 2019. Recognition and delineation of yeast genera based on genomic data: Lessons from Trichosporonales. *Fungal Genet Biol*. 130:31-42. doi: 10.1016/j.fgb.2019.04.013.
- 3 Sriswasdi S, Takashima M, Manabe RI, Ohkuma M, Iwasaki W. 2019. Genome and transcriptome evolve separately in recently hybridized *Trichosporon* fungi. *Communications Biology* 2:263 - doi: 10.1038/s42003-019-0515-2.
- 4 Takashima M, Manabe R, Ohkuma M. 2019. Draft genome sequences of basidiomycetous epiphytic phylloplane yeast type strains *Dioszegia crocea* JCM 2961 and *Dioszegia aurantiaca* JCM 2956. *Microbiol Resour Announc* 8: e01727-18. e01727-18. doi: 10.1128/MRA.01727-18.
- 5 Takashima M, Suh SO, Bai FY, Sugita T. 2019. Takashi Nakase's last tweet: What is the current direction of microbial taxonomy research? *FEMS Yeast Res* 19:foz066. doi: 10.1093/femsyr/foz066.

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

Recent papers and research news.

- 1 Exploiting yeast biodiversity for whisky fermentations. The PhD research of Martina Daute, in collaboration with the Scotch Whisky Research Institute was featured in a recent article of "Unfiltered" published by the Scotch Malt Whisky Society - <https://unfiltered.smws.com/10-2020/think-tank/>
- 2 Eliodorio KP, de Gois e Cunha GC, Muller C, Lucaroni AC, Giudici R, Walker GM, Alves Jr SL and Basso TO. 2019. Advances in yeast alcoholic fermentations for the production of bioethanol, beer and wine. *Adv Appl Microbiol* 109:61-119 - <https://doi.org/10.1016/bs.aamb.2019.10.002>
- 3 Leinhardt T, Black K, Saget S, Porto Costa M, Chadwick D, Rees RM, Williams M, Spillane C, Iannetta PPM, Walker G, Styles D. 2019. Data for life cycle assessment of legume biorefining for alcohol. Data in Brief, Manuscript Ref. No.: DIB-D-19-01361 doi: 10.1016/j.dib.2019.104242
- 4 Black K, Tziboula-Clarke A, White PJ, Iannetta PPM, Walker G. 2020. Optimised processing of faba bean (*Vicia faba* L.) kernels as a brewing adjunct. *J Inst Brew* - in press.
- 5 Walker GM, Basso TO. 2020. Mitigating stress in industrial yeasts. *Fungal Biol* 124:387-397 - 10.1016/j.funbio.2019.10.010

- 6 Black K, Daute M, Tziboula-Clarke A, White PJ, Iannetta P, Walker G. 2020. Utilization of low nitrogen barley for production of distilling quality malt. *J Am Soc Brew Chem* <https://doi.org/10.1080/03610470.2020.1796090>
- 7 Basso TO, Walker RSK, Basso LC, Walker GM. 2019. The future of bioethanol. In: *Ethanol as a Green Alternative Fuel: Insight and Perspectives*. Edited by Helen Treichel, Sérgio Luiz Alves Jr, Gislaïne Fongaro and Caroline Müller. Nova Science Publishers, Inc. Hauppauge, NY, USA. <https://novapublishers.com/shop/ethanol-as-a-green-alternative-fuel-insight-and-perspectives/>
- 8 Nasidi M, Agu R, Walker GM, Deeni Y. 2019. Sweet sorghum: agronomic practice for food, animal feed and fuel production in Sub-Saharan Africa. In: *Sweet Sorghum: Characteristics, Cultivation and Uses*. Nova Science Publishers, Inc. ISBN: 978-1-53615-386-6

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

Recently published papers.

- 1 Yurkov AM, Sannino C, Turchetti B. 2020. *Mrakia fibulata* sp. nov., a psychrotolerant yeast from temperate and cold habitats. *Antonie van Leeuwenhoek* 113:499-510.
 Tree fluxes are sugar-rich, sometimes ephemeral, substrates occurring on sites where tree sap (xylem or phloem) is leaking through damages of tree bark. Tree sap infested with microorganisms has been the source of isolation of many species, including the biotechnologically relevant carotenoid yeast *Phaffia rhodozyma*. Tree fluxes recently sampled in Germany yielded 19 species, including several psychrophilic yeasts of the genus *Mrakia*. Four strains from tree fluxes represented a potential novel *Mrakia* species previously known from two isolates from superficial glacial melting water of Calderone Glacier (Italy). The Italian isolates, originally identified as *Mrakia aquatica*, and two strains from Germany did not show any sexual structures. But another culture collected in Germany produced clamped hyphae with teliospores. A detailed examination of the five isolates (three from Germany and two from Italy) proved them to be a novel yeast species, which is described in this manuscript as *Mrakia fibulata* sp. nov. (MB 830398), holotype DSM 103931 and isotype DBVPG 8059. In contrast to other sexually reproducing *Mrakia* species, *M. fibulata* produces true hyphae with clamp connections. Also, this is the first psychrotolerant *Mrakia* species which grows above 20 °C. Spring tree fluxes are widespread and can be recognized and sampled by amateurs in a Citizen Science project. This substrate is a prominent source of yeasts, and may harbor unknown species, as demonstrated in the present work. The description of *Mrakia fibulata* is dedicated to our volunteer helpers and amateurs, like Anna Yurkova (9-years-old daughter of Andrey Yurkov), who collected the sample which yielded the type strain of this species.
- 2 Hajihosseinali M, Nasr S, Amoozegar MA, Yurkov A. 2020. *Saccharomycopsis oxydans* sp. nov, a new non-fermentative member in the genus *Saccharomycopsis* isolated from a traditional dairy product of Iran. *Int J Syst Evol Microbiol* 70:1059-1063.
 A total of 21 yeast isolates were recovered as part of a research project on biodiversity of yeasts in traditional dairy products in Alborz province, Iran. Standard protocols were used to carry out phenotypic, biochemical, physiological characterization and the phylogenetic analysis of combined the D1/D2 domain of the large ribosomal subunit (26S or LSU) and ITS region sequences. Five strains represented a potential new ascomycetous yeast species. Ascospore formation was not observed in these strains, and they did not ferment the examined carbon sources. Phylogenetic analysis placed these isolates in a well-supported subclade in the genus *Saccharomycopsis*. Here, we describe this novel yeast as *Saccharomycopsis oxydans* sp. nov.
- 3 Li A-H, Yuan F-X, Groenewald M, Bensch K, Yurkov AM et al. 2020. Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: Proposal of two new orders, three new families, eight new genera and one hundred and seven new species. *Stud Mycol* 96:17-140.

Nearly 500 basidiomycetous yeast species were accepted in the latest edition of *The Yeasts: A Taxonomic Study* published in 2011. However, this number presents only the tip of the iceberg of yeast species diversity in nature. Possibly more than 99 % of yeast species, as is true for many groups of fungi, are yet unknown and await discovery. Over the past two decades nearly 200 unidentified isolates were obtained during a series of environmental surveys of yeasts in phyllosphere and soils, mainly from China. Among these isolates, 107 new species were identified based on the phylogenetic analyses of nuclear ribosomal DNA (rDNA) [D1/D2 domains of the large subunit (LSU), the small subunit (SSU), and the internal transcribed spacer region including the 5.8S rDNA

(ITS)] and protein-coding genes [both subunits of DNA polymerase II (RPB1 and RPB2), the translation elongation factor 1- α (TEF1) and the mitochondrial gene cytochrome b (CYTB)], and physiological comparisons. Forty-six of these belong to 16 genera in the *Tremellomycetes* (*Agaricomycotina*). The other 61 are distributed in 26 genera in the *Pucciniomycotina*. Here we circumscribe eight new genera, three new families and two new orders based on the multi-locus phylogenetic analyses combined with the clustering optimisation analysis and the predicted similarity thresholds for yeasts and filamentous fungal delimitation at genus and higher ranks. Additionally, as a result of these analyses, three new combinations are proposed and 66 taxa are validated.

Community papers providing an outline of the classification of the kingdom *Fungi*.

- 4 He MQ, Zhao RL, Hyde K, Begerow D, Kemler M et al. 2019. Notes, outline and divergence times of *Basidiomycota*. *Fungal Diversity*: 105-367.

The *Basidiomycota* constitutes a major phylum of the kingdom *Fungi* and is second in species numbers to the *Ascomycota*. The present work provides an overview of all validly published, currently used basidiomycete genera to date in a single document. An outline of all genera of *Basidiomycota* is provided, which includes 1928 currently used genera names, with 1263 synonyms, which are distributed in 241 families, 68 orders, 18 classes and four subphyla. We provide brief notes for each accepted genus including information on classification, number of accepted species, type species, life mode, habitat, distribution, and sequence information. Furthermore, three phylogenetic analyses with combined LSU, SSU, 5.8s, rpb1, rpb2, and efl datasets for the subphyla *Agaricomycotina*, *Puccinio-mycotina* and

Ustilaginomycotina are conducted, respectively. Divergence time estimates are provided to the family level with 632 species from 62 orders, 168 families and 605 genera. Our study indicates that the divergence times of the subphyla in *Basidiomycota* are 406–430 Mya, classes are 211–383 Mya, and orders are 99–323 Mya, which are largely consistent with previous studies. In this study, all phylogenetically supported families were dated, with the families of *Agaricomycotina* diverging from 27–178 Mya, *Pucciniomycotina* from 85–222 Mya, and *Ustilaginomycotina* from 79–177 Mya. Divergence times as additional criterion in ranking provide additional evidence to resolve taxonomic problems in the *Basidiomycota* taxonomic system, and also provide a better understanding of their phylogeny and evolution.

- 5 Wijayawardene NN, Hyde KD, Al-Ani, LKT, Tedersoo L, Haelewaters D et al. 2020. Outline of *Fungi* and fungus-like taxa. *Mycosphere* 11:1060-1456.

Papers from the International Commission on the Taxonomy of *Fungi* (ICTF).

- 6 Lücking, R, Aime, MC, Robbertse B, Miller AN, Ariyawansa HA et al. 2020. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus* 11:14.

True fungi (*Fungi*) and fungus-like organisms (e.g. *Mycetozoa*, *Oomycota*) constitute the second largest group of organisms based on global richness estimates, with around 3 million predicted species. Compared to plants and animals, fungi have simple body plans with often morphologically and ecologically obscure structures. This poses challenges for accurate and precise identifications. Here we provide a conceptual framework for the identification

of fungi, encouraging the approach of integrative (polyphasic) taxonomy for species delimitation, i.e. the combination of genealogy (phylogeny), phenotype (including autecology), and reproductive biology (when feasible). This allows objective evaluation of diagnostic characters, either phenotypic or molecular or both. Verification of identifications is crucial but often neglected. Because of clade-specific evolutionary histories, there is currently no single tool

for the identification of fungi, although DNA barcoding using the internal transcribed spacer (ITS) remains a first diagnosis, particularly in metabarcoding studies. Secondary DNA barcodes are increasingly implemented for groups where ITS does not provide sufficient precision. Issues of pairwise sequence similarity-based identifications and OTU clustering are discussed, and multiple sequence alignment-based phylogenetic approaches with subsequent verification are recommended as more accurate alternatives. In metabarcoding approaches, the trade-off between speed and accuracy and precision of molecular identifications must be carefully considered. Intra-genomic variation of the ITS and other barcoding markers should be properly documented, as phylotype diversity is not necessarily a proxy of species richness.

Important strategies to improve molecular identification of fungi are: (1) broadly document intraspecific and intragenomic variation of barcoding markers; (2) substantially expand sequence repositories, focusing on undersampled clades and missing taxa; (3) improve curation of sequence labels in primary repositories and substantially increase the number of sequences based on verified material; (4) link sequence data to digital information of voucher specimens including imagery. In parallel, technological improvements to genome sequencing offer promising alternatives to DNA barcoding in the future. Despite the prevalence of DNA-based fungal taxonomy, phenotype-based approaches remain an important strategy to catalog the global diversity of fungi and establish initial species hypotheses.

- 7 Thines M, Aoki T, Crous PW, Hyde KD, Lücking R et al. 2020. Setting scientific names at all taxonomic ranks in italics facilitates their quick recognition in scientific papers. *IMA Fungus* 11:25.

It is common practice in scientific journals to print genus and species names in italics. This is not only historical as species names were traditionally derived from Greek or Latin. Importantly, it also facilitates the rapid recognition of genus and species names when skimming through manuscripts. However, names above the genus level are not always italicized, except in some journals which have adopted this practice for all scientific names. Since scientific names treated under the various Codes of nomenclature are without exception treated as Latin, there is no reason why names above genus level should be handled

differently, particularly as higher taxon names are becoming increasingly relevant in systematic and evolutionary studies and their italicization would aid the unambiguous recognition of formal scientific names distinguishing them from colloquial names. Several leading mycological and botanical journals have already adopted italics for names of all taxa regardless of rank over recent decades, as is the practice in the *International Code of Nomenclature for algae, fungi, and plants*, and we hereby recommend that this practice be taken up broadly in scientific journals and textbooks.

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

Editorial.

- 1 Lachance MA 2020 Guidelines for the publication of novel yeast species descriptions in *Yeast*. *Yeast* 37: 251-252.

Research papers.

- 2 Libkind D, Cadez N, Ofulante D, Langdon Q, Rosa C, Sampaio J, Goncalves P, Hittinger C, Lachance MA. 2020. Towards yeast taxogenomics: lessons from novel species descriptions based on complete genome sequences. *FEMS Yeast Res* 20:foaa042.

See abstract under Dr. Hittinger's entry.

- 3 Čadež N, Drumonde-Neves J, Sipiczki M, Dlačny D, Lima T, Pais C, Schuller D, Franco-Duarte R, Lachance MA, Péter G. 2020. *Starmerella vitis* f.a., sp. nov., a yeast species isolated from flowers and grapes and flowers. *Antonie van Leeuwenhoek* 113:1289–1298.

A novel yeast species of *Starmerella vitis* f.a. sp. nov. is proposed to accommodate five strains isolated from flowers, grapes and an insect in the Azores,

Canada, Hungary, Palau and Taiwan. As the strains were genetically distinct, we used parsimony network analysis based on ITS-D1/D2 sequences to delineate

the species in a statistically objective manner. According to sequence comparisons and phylogenetic analysis, the novel species is most closely related to *Starmerella lactis-condensi*. The two species cannot be

distinguished by conventional physiological tests. The type strain of *Starmerella vitis* f.a., sp. nov. is CBS 16418T; Mycobank number MB 835251.

- 4 Lachance MA, Lee DK, Hsiang T. 2020. Delineating yeast species with genome average nucleotide identity: a calibration of ANI with haplontic, heterothallic *Metschnikowia* species. *Antonie van Leeuwenhoek* 113:2097-2106.

We determined pairwise average nucleotide identity (ANI) values for the genomes of 71 strains assigned to 36 *Metschnikowia* species, 28 of which were represented by multiple isolates selected to represent the range of genetic diversity of the species, and most of which were defined on the basis of reproductive isolation. Similar to what has been proposed for prokaryote species delineation, an ANI value of 95% emerged as a good guideline for the delineation of yeast species, although some overlap exists, whereby members of a reproductive community could have slightly lower values (e.g., 94.3% for *M. kamakouana*), and representatives of distinct sister species could give slightly higher values (e.g., 95.2%

for the sister species *M. drakensbergensis* and *M. proteae*). Unlike what is observed in prokaryotes, a sizeable gap between intraspecific and interspecific ANI values was not encountered. Given the ease with which yeast draft genomes can now be obtained, ANI values are poised to become the new standard upon which yeast species may be delineated on genetic distance. As borderline cases exist, however, the delineation of yeast species will continue to require careful evaluation of all available data. We also explore the often-neglected distinction between phylogenetic relatedness and sequence identity through the analysis of a tree constructed from ANI' (100 - ANI) values.

- 5 Freitas LFD, Batista TM, Santos ARO, Hilário HO, Moreira RG, Franco GR, Morais PB, Lachance MA, Rosa CA. 2020. Yeast communities associated with cacti in Brazil, and the description of *Kluyveromyces starmeri* sp. nov. based on phylogenomic analyses. *Yeast* <https://doi.org/10.1002/yea.3528>

Yeast communities associated with cacti were studied in three ecosystems of Southeast, Central and North Brazil. A total of 473 yeast strains belonging to 72 species were isolated from 190 samples collected. Cactophilic yeast species were prevalent in necrotic tissues, flowers, fruits and insects of cacti collected in Southeast and North Brazil. *Pichia cactophila*, *Candida sonorensis* and species of the *Sporopachydermia* complex were the most prevalent cactophilic species in Southeast and Central regions. *Kodamaea nitidularum*, *Candida restingae* and *Wickerhamiella cacticola* were frequently associated with cactus flowers and fruits. The diversity of yeasts associated with the substrates studied was high. Twenty one novel species were found. One is described here as *Kluyveromyces starmeri* sp. nov. based on 21 isolates obtained from necrotic tissues,

flowers, fruits and associated insects of the columnar cacti *Cereus saddianus*, *Micranthocereus dolichospermaticus* and *Pilosocereus arrabidae* in two different ecosystems in Brazil. Phylogenetic analyses of sequences encoding the gene of the small subunit (SSU) rRNA gene, the internal transcribed spacer, the 5.8S rRNA gene and the D1/D2 domains of the large subunit (LSU) rRNA showed that the species is related to *Kluyveromyces dobzhanskii*, *Kluyveromyces lactis* and *Kluyveromyces marxianus*. Phylogenomic analyses based on 1264 conserved genes shared among the new species and 19 other members of the Saccharomycetaceae confirmed this phylogenetic relationship. The holotype is *K. starmeri* sp. nov. CBS 16103T (=UFMG CM Y3682T). The Mycobank number is MB 836817.

GMV – Italian Group of Wine Microbiology

Communicated by Prof. Patrizia Romano, “Gastronomy, Hospitality and Territories” degree course, Universitas Mercatorum, Piazza Mattei 10, 00186 Roma, Italy.
<pot2930@gmail.com> <patrizia.romano@unimerceatorum.it>.

The following recent papers are from the partners of GMV for topics on food yeasts.

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>.

- 1 Aponte M., Romano R., Villano C., Blaiotta G. 2020. Dominance of *S. cerevisiae* commercial starter strains during Greco di Tufo and Aglianico wine fermentations and evaluation of oenological performances of some indigenous/residential strains. *Foods*, 9(11):1549
doi.org/10.3390/foods9111549.

In order to evaluate dominance/implantation of starter cultures for wine fermentation, both commercial starters and wild strains were monitored during the fermentation of Greco di Tufo (GR) and Aglianico of Taurasi (AGL) musts. Preliminary characterization of commercial strains was carried out by several molecular markers. Five fermentations—four starter-inoculated and one spontaneous—were carried out in duplicates by using grapes from GR and AGL. Trials were monitored, and yeast cultures were isolated within the dominant microflora. Comparison of interdelta patterns allowed to assess the real occurrence of both starters and indigenous strains. A high genetic diversity within *S. cerevisiae* strains was detected. In starter-led fermentations (except for few cases), in addition to the starter strains, indigenous *S. cerevisiae* biotypes were found, as well. Native strains isolated from replicates of the same fermentation showed

different genetic profiles. Spontaneous fermentations were conducted, during the first 5 days, by non-Saccharomyces yeasts and, afterwards, by a high number (16 in the AGL and 20 in the GR) of *S. cerevisiae* biotypes. Indigenous biotypes isolated by GR revealed a high variability in oenological features and, in several cases, showed better performances than those recorded for commercial strains. The study further highlighted the low dominance of some commercial starter cultures. Moreover, autochthonous yeast strains proved to be sometimes more aggressive in terms of fermentation vigor in GR must, likely because better adapted to ecological and technological conditions occurring during winemaking. Finally, the use of such strains for production of autochthonous “pied de cuve” may be a useful strategy for lowering production cost of winemaking.

- 2 Pulvirenti A, de Vero L, Blaiotta G, Sidari R, Iosca G, Gullo M, Caridi A. 2020. Selection of wine *Saccharomyces cerevisiae* strains and their screening for the adsorption activity of pigments, phenolics and ochratoxin A. *Fermentation*, 6(3):80; doi.org/10.3390/fermentation6030080.

Ochratoxin A is a dangerous mycotoxin present in wines and is considered the principal safety hazard in the winemaking process. Several authors have investigated the ochratoxin A adsorption ability of *Saccharomyces cerevisiae* yeasts, and specifically selected strains for this desired trait. In the present work, a huge selection of wine yeasts was done starting from Portuguese, Spanish and Italian fermenting musts of different cultivars. Firstly, 150 isolates were collected, and 99 non-redundant *S. cerevisiae* strains were identified. Then, the strains were screened following a multi-step approach in order to select those having primary oenological traits, mainly (a) good fermentation performance, (b) low

production of H₂S and (c) low production of acetic acid. The preselected strains were further investigated for their adsorption activity of pigments, phenolic compounds and ochratoxin A. Finally, 10 strains showed the desired features. The goal of this work was to select the strains capable of absorbing ochratoxin A but not pigments and phenolic compounds in order to improve and valorise both the quality and safety of red wines. The selected strains are considered good candidates for wine starters, moreover, they can be exploited to obtain a further enhancement of the specific adsorption/non-adsorption activity by applying a yeast breeding approach.

- 3 Murgia MA, Marongiu A, Aponte M, Blaiotta G, Deiana P, Mangia N.P. 2019. Impact of a selected *Debaryomyces hansenii* strain's inoculation on the quality of Sardinian fermented sausages. Food Research International, 121:144-150; doi.org/10.1016/j.foodres.2019.03.042

Dominant yeast species in Salsiccia Sarda, a traditional fermented sausage produced in Sardinia (Italy), were evaluated through the monitoring three typical production processes. Six different species were identified by molecular techniques, but *Debaryomyces (D.) hansenii* proved to be dominant. A *D. hansenii* strain was selected according to its technological features and used in three experimental sausage productions at farm scale with the aim to evaluate its antifungal effect. In all cases, two batches were inoculated with a previously selected autochthonous starter cultures (*Lactobacillus plantarum* and *Staphylococcus xylosum*), whereas two batches were left to spontaneous fermentation.

D. hansenii was inoculated on the sausages surface by brushing after the sausages drying, by immersion in a yeast suspension after the stuffing, or, alternatively, casings were dipped in a yeast suspension before the dough stuffing. Microbial counts in the sausages core did not appear to be affected by *D. hansenii* application, while outcomes obtained for casings appeared soundly diversified. Brushing on the sausages surface at the onset of fermentation proved to be the best approach to treat sausages. Yeast inoculation exerted a noteworthy anti-mould effect, independently of the mode of application and, on the other hand, did not affect the overall quality and typical features of the product.

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

- 1 Carboni G, Fancello F, Zara G, Zara S, Ruiu L, Marova I, Pinna G, Budroni M, Mannazzu I. 2020. Production of a lyophilized ready-to-use yeast killer toxin with possible applications in the wine and food industries. International Journal of Food Microbiology. 16; 335:108883.

Kpkt is a yeast killer toxin, naturally produced by *Tetrapisipora phaffii*, with possible applications in winemaking due to its antimicrobial activity on wine-related yeasts including *Kloeckera/Hanseniaspora*, *Saccharomyces* and *Zygosaccharomyces*. Here, Kpkt coding gene was expressed in *Komagataella phaffii* (formerly *Pichia pastoris*) and the bioreactor production of the recombinant toxin (rKpkt) was obtained. Moreover, to produce a ready-to-use preparation of rKpkt, the cell-free supernatant of the *K. phaffii* recombinant killer clone was 80-fold concentrated and lyophilized. The resulting preparation could be easily solubilized in sterile distilled water and maintained its killer activity for up to six months at 4°C. When applied to grape must, it exerted an extensive killer activity on wild wine-

related yeasts while proving compatible with the fermentative activity of actively growing *Saccharomyces cerevisiae* starter strains. Moreover, it displayed a strong microbicidal effect on a variety of bacterial species including lactic acid bacteria and food-borne pathogens. On the contrary it showed no lethal effect on filamentous fungi and on *Ceratitiscapitata* and *Musca domestica*, two insect species that may serve as non-mammalian model for biomedical research. Based on these results, bioreactor production and lyophilization represent an interesting option for the exploitation of this killer toxin that, due to its spectrum of action, may find application in the control of microbial contaminations in the wine and food industries. © 2020 Elsevier B.V.

- 2 Bianco A, Budroni M, Zara S, Mannazzu I, Fancello F, Zara G. 2020. The role of microorganisms on biotransformation of brewers' spent grain. Appl Microbiol Biotechnol 104, 8661–8678; doi.org/10.1007/s00253-020-10843-1

Brewers' spent grain (BSG) is the most abundant by-product of brewing. Due to its microbiological instability and high perishability, fresh BSG is currently disposed of as low-cost cattle feed. However, BSG is an appealing source of nutrients to obtain products with high added value through

microbial-based transformation. As such, BSG could become a potential source of income for the brewery itself. While recent studies have covered the relevance of BSG chemical composition in detail, this review aims to underline the importance of microorganisms from the stabilization/contamination of fresh BSG to

its biotechnological exploitation. Indeed, the evaluation of BSG-associated microorganisms, which include yeast, fungi, and bacteria, can allow their safe use and the best methods for their exploitation. This bibliographical examination is particularly focused on the role of microorganisms in BSG exploitation to (1) produce enzymes and metabolites of industrial interest, (2) supplement human and animal diets, and (3)

- 3 Zara G, Budroni M, Mannazzu I, Fancello F, Zara S. 2020. Yeast biofilm in food realms: occurrence and control. *World J Microbiol Biotechnol* 36: 134; doi.org/10.1007/s11274-020-

In natural environments, microorganisms form microbial aggregates called biofilms able to adhere to a multitude of different surfaces. Yeasts make no exception to this rule, being able to form biofilms in a plethora of environmental niches. In food realms, yeast biofilms may cause major problems due to their alterative activities. In addition, yeast biofilms are tenacious structures difficult to eradicate or treat with the current arsenal of antifungal agents. Thus, much effort is being made to develop novel approaches to

improve soil fertility. Emerging safety issues in the use of BSG as a food and feed additive is also considered, particularly considering the presence of mycotoxins. Key points • Microorganisms are used to enhance brewers' spent grain nutritional value. • Knowledge of brewers' spent grain microbiota allows the reduction of health risks.

prevent and disrupt yeast biofilms, for example through the use of natural antimicrobials or small molecules with both inhibiting and dispersing properties. The aim of this review is to provide a synopsis of the most recent literature on yeast biofilms regarding: (i) biofilm formation mechanisms; (ii) occurrence in food and in food-related environments; and (iii) inhibition and dispersal using natural compounds, in particular.

- 4 Feghali N, Bianco A, Zara G, Tabet E, Ghanem C, Budroni M. 2020. Selection of *Saccharomyces cerevisiae* starter strain for Merwah wine. *Fermentation*, 6: 43; doi:10.3390/fermentation6020043

In order to select *Saccharomyces cerevisiae* starter strains for "Merwah" wine production, three strains (M.6.16, M.10.16, and M.4.17) previously isolated from "Merwah" must and characterized at the lab scale were tested in pilot-scale fermentation in a Lebanese winery during the 2019 vintage. The three inoculated musts were compared to that obtained with a spontaneous fermentation. During the fermentations, must samples were taken to evaluate the dominance of the inoculated strains, and at the end of fermentation,

the obtained wines were subjected to chemical and sensorial characterization. Molecular monitoring by interdelta analysis revealed that only M.4.17 was able to complete the fermentation and dominate over the wild yeasts. Based on the analysis of principal technological parameters (i.e, residual sugar, fermentative vigor, sulfur production, and acetic acid) and sensorial analysis of the wines obtained, M.4.17 was selected as an adequate starter for the production of typical "Merwah" wine.

- 5 Romani C, Lencioni L, Bartolini A.B, Mannazzu I, Domizio P. 2020. Pilot scale fermentations of Sangiovese: An overview on the impact of Fermentations *Saccharomyces* and non-*Saccharomyces* wine yeasts. *Fermentation*, 6(3), 63; doi.org/10.3390/fermentation6030063

The production of wines with peculiar analytical and sensorial profiles, together with the microbiological control of the winemaking process, has always been one of the main objectives of the wine industry. In this perspective, the use of oenological starters containing non-*Saccharomyces* yeasts can represent a valid tool for achieving these objectives. Here we present the results of seven pilot scale fermentations, each of which was inoculated with a different non-*Saccharomyces* yeast strain and after three days with a commercial *Saccharomyces cerevisiae* starter. The fermentations were carried out in double on 70 L of Sangiovese grape must, the most widely planted red

grape variety in Italy and particularly in Tuscany, where it is utilized for the production of more than 80% of red wines. Fermentations were monitored by assessing both the development of the microbial population and the consumption of sugars at the different sampling times. The impact of the different starters was assessed after stabilization through the evaluation of the standard analytical composition of the resulting wines, also taking into account polysaccharides and volatile compounds. Moreover, quantitative descriptive sensory analyses were carried out. Compared to the control wines obtained by inoculating the *S. cerevisiae* starter strain, those

inoculated with non-*Saccharomyces/Saccharomyces* mixed starters presented a significant differentiation in the chemical-analytical composition. Moreover,

sensory analysis revealed differences among wines mainly for intensity of color, astringency, and dryness mouthfeel perception.

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>

- 1 Pietrafesa A, Capece A, Pietrafesa R, Bely M, Romano P. 2020. *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* mixed starter cultures: Influence of microbial/physical interactions on wine characteristics. *Yeast*, 37: 609–621; doi.org/10.1002/yea.3506.

The growing trend in the wine industry is the reevaluation of the role of non *Saccharomyces* yeasts, promoting the use of these yeasts in association with *Saccharomyces cerevisiae*. Non *Saccharomyces* yeasts contribute to improve wine complexity and organoleptic composition. However, the use of mixed starters needs to better understand the effect of the interaction between these species during alcoholic fermentation. The aim of this study is to evaluate the influence of mixed starter cultures, composed by combination of different *S. cerevisiae* and *Hanseniaspora uvarum* strains, on wine characteristics and to investigate the role of cell to cell contact on the metabolites produced during alcoholic fermentation. In the first step, three *H. uvarum* and two *S. cerevisiae* strains, previously selected, were tested during mixed

fermentations in natural red grape must in order to evaluate yeast population dynamics during inoculated fermentation and influence of mixed starter cultures on wine quality. One selected mixed starter was tested in a double compartment fermentor in order to compare mixed inoculations of *S. cerevisiae/H. uvarum* with and without physical separation. Our results revealed that physical contact between *S. cerevisiae* and *H. uvarum* affected the viability of *H. uvarum* strain, influencing also the metabolic behaviour of the strains. Although different researches are available on the role of cell to cell contact mediated interactions on cell viability of the strains included in the mixed starter, to our knowledge, very few studies have evaluated the influence of cell to cell contact on the chemical characteristics of wine.

- 2 Ozmen Togay S, Capece A, Siesto G, Aksu H, Sandikci Altunatmaz S, Yilmaz Aksu F, Romano P, Karagul Yuceer Y. 2020. Molecular characterization of yeasts isolated from traditional Turkish cheeses. *Food Science and Technology*; doi.org/10.1590/fst.24319.

Thirty-two yeast strains were identified by means of molecular methods isolated from traditional Turkish cheeses (Tulum, Kashkaval, Mihalic, Orgu, White, Sepet, and Goat). *Debaryomyces hansenii* and *Torulasporea delbrueckii* were found as predominant species in cheese samples. Other species which were identified were *Kluyveromyces lactis*, *Candida parapsilosis*, *Clavispora lusitaniae*, *Saccharomyces*

cerevisiae, *K. marxianus*, *Rhodotorula mucilaginosa*, *Meyerozyma guilliermondii* (formerly *Pichia guilliermondii*), *C. zeylanoides* and *Candida albicans*. *Rhodotorula mucilaginosa* and *D. hansenii* strains, from Kashkaval cheese, showed antilisterial activity, whereas only one *K. lactis* strain from Orgu cheese exhibited proteolytic activity.

- 3 Capece A, Romano P, 2019. Yeasts and their metabolic impact on wine flavour. In “Yeasts in the Production of Wine”, P. Romano, M. Ciani, G.H.Fleet (eds), Springer New York pp 43-80; doi.org/10.1007/978-1-4939-9782-4_1.

Organoleptic characteristics of wine, aroma and flavour, are the most important characteristics that define the differences among the vast array of products throughout the world. Yeasts have a prominent role in determining the chemical composition of wine by several mechanisms: by producing enzymes that transform neutral grape compounds into flavour active

compounds (pre-fermentative aroma), secondly by producing many hundreds of flavour active, secondary metabolites (fermentative and post-fermentative aroma) and lastly by extracting flavour components from grape solids and by autolytic degradation of dead yeast cells. These reactions vary with the yeast species and strains contributing to the fermentation. This

review aims to present an overview on major achievements of yeast role in the formation of wine flavour. Firstly we illustrate the yeast metabolic activities involved in wine aroma production in function of wine styles as well as the main factors

affecting flavour quality of wine. Furthermore, the influence of starter cultures (single or mixed) on wine flavour is discussed. Finally, novel methodologies to select wine yeasts in function of their influence on wine aroma are also summarized.

- 4 Capece A, Romaniello R, Pietrafesa A, Siesto G, Pietrafesa R, Zambuto M, Romano P. 2018. Use of *Saccharomyces cerevisiae* var. *boulardii* in co-fermentations with *S. cerevisiae* for the production of craft beers with potential healthy value added. *International Journal of Food Microbiology*, 284: 22–30; doi.org/10.1016/j.ijfoodmicro.2018.06.028.

A high amount of dietary antioxidant intake can be supplied by beverages widely consumed, such as wine, coffee, beer. Recently, an increase in the consumer interest was observed for beer, in consequence of the high phenolic antioxidants and low ethanol content present in this beverage. Among all beer types, in recent years, consumption of craft beers has gained popularity. Being an unpasteurized and unfiltered, craft beer is potentially a new vehicle for delivering health effects. While health benefits of lactic acid bacteria as probiotics are well known, few data are available on probiotic yeasts in fermented food. Therefore, this study was aimed to analyse the effect of integrating the well-known probiotic yeast strain of *S. cerevisiae* var. *boulardii* (S.b) in mixed cultures with *S. cerevisiae* strains for production of beers with increased healthy benefits. The probiotic strain of S.b was tested in mixed cultures with selected *S. cerevisiae* strains, during wort fermentation. As the viability during processing operations is one of the criteria for selecting suitable strains of probiotic

microorganisms, the survival of probiotic yeast during the fermentation and the presence of highly viable cells at the end of fermentations were evaluated. In almost all the mixed fermentations, at the end of the process the probiotic yeast was predominant on *S. cerevisiae* strain, and the experimental beers contained a high number of viable cells of S.b strain (ranging between 8×10^6 and 7.0×10^7 /mL). The analysis of experimental beers for the content of main volatile compounds showed that the inclusion of S.b strain in mixed starter did not affect negatively beer aroma. Moreover, the inclusion of S.b strain in mixed starters determined an increase in the antioxidant activity and polyphenols content, in comparison to beers from single starter fermentations, indicating the influence of S.b strain on these parameters. Some mixed starter cultures tested in this study resulted a very promising tool to increase the healthy quality of the product, such as the improve the antioxidant activity and polyphenols content of beer.

- 5 Capece A, Romaniello R, Siesto G, Romano P. 2018. Conventional and non-conventional yeasts in beer production. *Fermentation*, 4(2): 38; doi.org/10.3390/fermentation4020038.

The quality of beer relies on the activity of fermenting yeasts, not only for their good fermentation yield-efficiency, but also for their influence on beer aroma, since most of the aromatic compounds are intermediate metabolites and by-products of yeast metabolism. Beer production is a traditional process, in which *Saccharomyces* is the sole microbial component, and any deviation is considered a flaw. However, nowadays the brewing sector is faced with an increasing demand for innovative products, and it is diffusing the use of uncharacterized autochthonous starter cultures, spontaneous fermentation, or non-*Saccharomyces* starters, which leads to the production of distinctive and unusual products. Attempts to obtain products with more complex sensory

characteristics have led one to prospect for non-conventional yeasts, i.e, non-*Saccharomyces* yeasts. These generally are characterized by low fermentation yields and are more sensitive to ethanol stress, but they provide a distinctive aroma and flavor. Furthermore, non-conventional yeasts can be used for the production of low-alcohol/non-alcoholic and light beers. This review aims to present the main findings about the role of traditional and non-conventional yeasts in brewing, demonstrating the wide choice of available yeasts, which represents a new biotechnological approach with which to target the characteristics of beer and to produce different or even totally new beer styles.

- 1 Roscini L, Conti A, Casagrande Pierantoni D, Robert V, Corte L, Cardinali G. 2020. Do metabolomics and taxonomic barcode markers tell the same story about the evolution of *Saccharomyces sensu stricto* complex in fermentative environments? *Microorganisms*, 8:1242
doi.org/10.3390/microorganisms8081242.

Yeast taxonomy was introduced based on the idea that physiological properties would help discriminate species, thus assuming a strong link between physiology and taxonomy. However, the instability of physiological characteristics within species configured them as not ideal markers for species delimitation, shading the importance of physiology and paving the way to the DNA-based taxonomy. The hypothesis of reconnecting taxonomy with specific traits from phylogenies has been successfully explored for Bacteria and Archaea, suggesting that a similar route can be traveled for yeasts. In this framework, thirteen single copy loci were used to investigate the predictability of complex Fourier Transform InfaRed spectroscopy (FTIR) and High-performance Liquid

Chromatography–Mass Spectrometry (LC-MS) profiles of the four historical species of the *Saccharomyces sensu stricto* group, both on resting cells and under short-term ethanol stress. Our data show a significant connection between the taxonomy and physiology of these strains. Eight markers out of the thirteen tested displayed high correlation values with LC-MS profiles of cells in resting condition, confirming the low e_cacy of FTIR in the identification of strains of closely related species. Conversely, most genetic markers displayed increasing trends of correlation with FTIR profiles as the ethanol concentration increased, according to their role in the cellular response to different types of stress.

- 2 Corte L, Roscini L, Casagrande Pierantoni D.C, Pellegrino R.M, Emiliani C, Basaglia M. Favaro L. Casella S, Cardinali G. 2020. Delta-integration of single gene shapes the whole metabolomic short-term response to ethanol of recombinant *Saccharomyces cerevisiae* strains. *Metabolites* 10: 40; doi: [10.3390/metabo10040140](https://doi.org/10.3390/metabo10040140)

In yeast engineering, metabolic burden is often linked to the reprogramming of resources from regular cellular activities to guarantee recombinant protein(s) production. Therefore, growth parameters can be significantly influenced. Two recombinant strains, previously developed by the multiple delta-integration of a glucoamylase in the industrial *Saccharomyces cerevisiae* 27P, did not display any detectable metabolic burden. In this study, a Fourier Transform InfraRed Spectroscopy (FTIR)-based assay was employed to investigate the effect of delta-integration on yeast strains' tolerance to the increasing ethanol levels typical of the starch-to-ethanol industry. FTIR fingerprint, indeed, offers a holistic view of the metabolome and is a well-established method to assess the stress response of microorganisms. Cell viability and metabolomic fingerprints have been considered as

parameters to detecting any physiological and/or metabolomic perturbations. Quite surprisingly, the three strains did not show any difference in cell viability but metabolomic profiles were significantly altered and different when the strains were incubated both with and without ethanol. A LC/MS untargeted workflow was applied to assess the metabolites and pathways mostly involved in these strain-specific ethanol responses, further confirming the FTIR fingerprinting of the parental and recombinant strains. These results indicated that the multiple-integration prompted huge metabolomic changes in response to short-term ethanol exposure, calling for deeper metabolomic and genomic insights to understand how and, to what extent, genetic engineering could affect the yeast metabolome.

- 3 Bagheri B, Bauer FF, Cardinali G, Setati ME. 2020. Ecological interactions are a primary driver of population dynamics in wine yeast microbiota during fermentation. *Scientific Reports*, 10;:4911; doi.org/10.1038/s41598-020-61690-z

Spontaneous wine fermentation is characterized by yeast population evolution, modulated by complex

physical and metabolic interactions amongst various species. The contribution of any given species to the

final wine character and aroma will depend on its numerical persistence during the fermentation process. Studies have primarily evaluated the effect of physical and chemical factors such as osmotic pressure, pH, temperature and nutrient availability on mono- or mixed-cultures comprising 2-3 species, but information about how interspecies ecological interactions in the wine fermentation ecosystem contribute to population dynamics remains scant. Therefore, in the current study, the effect of temperature and sulphur dioxide (SO₂) on the dynamics of a multi-species yeast consortium was evaluated in three different matrices including synthetic grape juice, Chenin blanc and Grechetto bianco. The population dynamics were affected by temperature and SO₂, reflecting differences in stress

resistance and habitat preferences of the different species and influencing the production of most volatile aroma compounds. Evidently at 15 degrees C and in the absence of SO₂ non-*Saccharomyces* species were dominant, whereas at 25 degrees C and when 30 mg/L SO₂ was added *S. cerevisiae* dominated. Population growth followed similar patterns in the three matrices independently of the conditions. The data show that fermentation stresses lead to an individual response of each species, but that this response is strongly influenced by the interactions between species within the ecosystem. Thus, our data suggest that ecological interactions, and not only physico-chemical conditions, are a dominant factor in determining the contribution of individual species to the outcome of the fermentation.

GMV-V Department of Life and Environmental Science Polytechnic University of Marche, Italy.
Communicated by Professor Dr. Maurizio Ciani <m.ciani@univpm.it>.

- 1 Agarbati A, Canonico L, Comitini F, Ciani M. 2020. Improved *Saccharomyces cerevisiae* strain in pure and sequential fermentation with *Torulaspora delbrueckii* for the production of Verdicchio wine with reduced sulfites. *Applied Sciences*, 10(19): 1-14, 6722; doi.org/10.3390/app10196722.

The application of yeast strains that are low producers of sulfur compounds is actually required by winemakers for the production of organic wine. This purpose could be satisfied using a native *Saccharomyces cerevisiae* strain improved for oenological aptitudes. Moreover, to improve the aromatic complexity of wines, sequential fermentations carried out with *S. cerevisiae*/non-*Saccharomyces* yeast is widely used. For these reasons, in the present work an improved native *S. cerevisiae* low producer of sulfite and sulfide compounds was evaluated in pure and in sequential fermentation with a selected *Torulaspora delbrueckii*. Additionally, the influence of grape juices coming from three different vintages under

winery conditions was evaluated. In pure fermentation, improved native *S. cerevisiae* strain exhibited a behavior related to vintage, highlighting that the composition of grape juice affects the fermentation process. In particular, an increase in ethyl octanoate (vintage 2017) and phenyl ethyl acetate (vintage 2018) was detected. Moreover, isoamyl acetate was highly consistent and could be a distinctive aroma of the strain. The sequential fermentation *T. delbrueckii*/*S. cerevisiae* determined an increase in aroma compounds such as phenyl ethyl acetate and ethyl hexanoate. In this way, it was possible to produce Verdicchio wine with reduced sulfites and characterized by a peculiar aromatic taste.

- 2 Canonico L, Ciani E, Galli E, Comitini F, Ciani M. 2020. Evolution of aromatic profile of *Torulaspora delbrueckii* mixed fermentation at microbrewery plant. *Fermentation*, 6(1): 7 doi.org/10.3390/fermentation6010007.

Nowadays, consumers require quality beer with peculiar organoleptic characteristics and fermentation management has a fundamental role in the production of aromatic compounds and in the overall beer quality. A strategy to achieve this goal is the use of non-conventional yeasts. In this context, the use of *Torulaspora delbrueckii* was proposed in the brewing process as a suitable strain to obtain a product with a distinctive aromatic taste. In the present work, *Saccharomyces cerevisiae*/*T. delbrueckii* mixed

fermentation was investigated at a microbrewery plant monitoring the evolution of the main aromatic compounds. The results indicated a suitable behavior of this non-conventional yeast in a production plant. Indeed, the duration of the process was very closed to that exhibited by *S. cerevisiae* pure fermentation. Moreover, mixed fermentation showed an increase of some aromatic compounds as ethyl hexanoate, α -terpineol, and β -phenyl ethanol. The enhancement of aromatic compounds was confirmed by the sensory

evaluation carried out by trained testers. Indeed, the beers produced by mixed fermentation showed an

emphasized note of fruity/citric and fruity/esters notes and did not show aroma defects.

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

- 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.

GMV-VII (1) Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark.

(2) Department of Agricultural, Forestry and Food Sciences, University of Turin, Largo Braccini 2, 10095 Grugliasco, Italy.

(3) Jotis S.A, Food Industry, Athens, Greece.

Communicated by Professor Dr. Nadja Larsen <nf@food.ku.dk>.

- 1 ¹Geronikou A, ¹Srimahaeak T, ²Rantsiou K, ³Triantafillidis G, ¹Larsen N, ¹Jespersen L. 2020. Occurrence of yeasts in white-brined cheeses: methodologies for identification, spoilage potential and good manufacturing practices. *Frontiers in Microbiology*. 11: 582778. doi:10.3389/fmicb.2020.582778.

White-brined cheeses are globally produced in a variety of brands. The aim of this review is to get an insight to the technologies used for the detection and the identification of contaminant yeasts in these cheeses. Furthermore, an overview about the occurrence of yeasts, their spoilage potential and their interactions with other microorganisms, as well as guidelines used by dairies to prevent contamination are summarized. Yeasts are recognized as the main spoilage microorganisms of white-brined cheeses.

Today, traditional yeast identification based on morphological and physiological characteristics are supplanted by DNA sequencing, various genotyping techniques and advanced phenotypic identification technologies. However, these biotechnological methodologies are not widespread in the dairy industry. The most found yeasts species causing quality defects in white-brined cheeses are *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces marxianus*, *Kluyveromyces lactis* and

Yarrowia lipolytica. These species may release off-flavors, produce gas, or cause discoloration and textural changes, mainly for their enzymatic activities (proteolytic and lipolytic) and for the metabolism of fermentable carbohydrates and amino acids (biogenic ammine production). An optimized production of white-brined cheese with enhanced quality could be achieved by an implementation of Good

Manufacturing Practice (GMP) and of principles of HACCP. Furthermore, bio-protective cultures offer a promising alternative for the control of spoilage microorganisms in cheese. However, scientific knowledge on the inhibition (antifungal peptides production) and interaction mechanisms in white brined cheeses is still missing.

GMV-VIII (1) Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini 1, 64100 Teramo, Italy
(2) Grupo de Investigación Biotecnología, Facultad de Ingeniería, Universidad de San Buenaventura Cali, Carrera 122 # 6-65, 76001 Cali, Colombia
(3) Department of Agricultural, Forestry and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Torino, Italy; valentina.alessandria@unito.it.
Communicated by Professors Kalliopi Rantsiou <kalliopi.rantsiou@unito.it> and Clemencia Chaves-López <cchaveslopez@unite.it>.

1 ^{1,2}Delgado-Ospina J, ¹Triboletti S, ³Alessandria V, ¹Serio A, ¹Sergi M, ¹Paparella A, ³Rantsiou K, ¹Chaves-López C. 2020. Functional biodiversity of yeasts isolated from Colombian fermented and dry cocoa beans. *Microorganisms*, 8(7):1086. doi:10.3390/microorganisms8071086.

In this study pure and mixed fermentations with *Starmarella 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.

GMV-IX Unimore Microbial Culture Collection (UMCC), Department of Life Sciences, University of Modena and Reggio Emilia. Via Amendola 2 (BESTA building), 42122 Reggio Emilia, Italy. Communicated by Dr. PhD Luciana De Vero <luciana.devero@unimore.it> www.umcc.unimore.it

1 De Vero L, Boniotti M.B, Budroni M, Buzzini P, Cassanelli S, Comunian R, Gullo M, Logrieco A.F, Mannazzu I, Musumeci R, Perugini I, Perrone G, Pulvirenti A, Romano P. Turchetti B, Varese G.C. 2019. Preservation, characterization and exploitation of microbial biodiversity: the perspective of the Italian network of culture collections. *Microorganisms* 7(12): 685 doi.org/10.3390/microorganisms7120685.

Microorganisms represent most of the biodiversity of living organisms in every ecological habitat. They have profound effects on the functioning of any ecosystem, and therefore on the health of our planet

and of human beings. Moreover, microorganisms are the main protagonists in food, medical and biotech industries, and have several environmental applications. Accordingly, the characterization and

preservation of microbial biodiversity are essential not only for the maintenance of natural ecosystems but also for research purposes and biotechnological exploitation. In this context, culture collections (CCs) and microbial biological, resource centres (mBRCs) are crucial for the safeguarding and circulation of biological resources as well as for the progress of life sciences. This review deals with the expertise and services of CCs, in particular concerning preservation

and characterization of microbial resources, by pointing to the advanced approaches applied to investigate a huge reservoir of microorganisms. Data sharing and web services as well as the tight interconnection between CCs and the biotechnological industry are highlighted. In addition, guidelines and regulations related to quality management systems (QMSs), biosafety and biosecurity issues are discussed according to the perspectives of CCs and mBRCs.

- 2 Iosca G, De Vero L, Gullo M, Licciardello F, Quartieri A, Pulvirenti A. 2020. Exploring the microbial community of traditional sourdoughs to select yeasts and lactic acid bacteria. Proceedings. Presented at 1st International Electronic Conference on Microbiology

<https://sciforum.net/paper/view/conference/7126>

Sourdoughs represent an awesome example of ecosystem in which yeasts and lactic acid bacteria (LAB) interact with each other, defining the characteristics of the final product in terms of composition, texture, taste and flavor. Therefore, the identification of dominant yeasts and LAB involved in the fermentation process can lead to the selection of starters with suitable fermentation aptitude and capable of producing desired aromas and/or aromatic precursors. In this work, two sourdoughs samples (A and B) for Panettone production were collected from an artisan bakery. Yeasts and bacteria were isolated at different fermentation steps on selective agar media. A total of 120 isolates were obtained and characterized by conventional microbiology methods as well as

molecular techniques. Non-redundant yeasts and LAB strains were identified by sequencing the D1/D2 domain of the 26S rRNA and the 16S rRNA genes, respectively. The results highlighted the occurrence of *Kazachstania humilis* and *Fructilactobacillus sanfranciscensis* in both sourdoughs. Among LAB also some other strains belonging to *Lactobacillus* genus were found. Moreover, *Saccharomyces cerevisiae* and *Staphylococcus* spp. strains were detected in sample B. The exploration of these two sourdoughs microbiota showed the presence of the main microorganisms normally colonizing this environment and can allow the selection of yeasts and LAB to be used as new starters.

GMV-X DeFENS Department of Food, Environmental and Nutritional Sciences, Università degli studi di Milano, Italy Communicated by Professor Roberto Foschino
<roberto.foschino@unimi.it> <http://eng.defens.unimi.it/ecm/home>

- 1 Valdetara F, Škalič M, Fracassetti D, Louw M, Compagno C, du Toit M, Foschino R, Petrovič U, Divol B, Vigentini I. 2020. Transcriptomics unravels the adaptive molecular mechanisms of *Brettanomyces bruxellensis* under SO₂ stress in wine condition. Food Microbiology, 90: 103483; doi.org/10.1016/j.fm.2020.103483.

Sulfur dioxide is generally used as an antimicrobial in wine to counteract the activity of spoilage yeasts, including *Brettanomyces bruxellensis*. However, this chemical does not exert the same effectiveness on different *B. bruxellensis* yeasts since some strains can proliferate in the final product leading to a negative sensory profile due to 4-ethylguaiacol and 4-ethylphenol. Thus, the capability of deciphering the general molecular mechanisms characterizing this yeast species' response in presence of SO₂ stress could be considered strategic for a better management of SO₂ in winemaking. A RNA-Seq

approach was used to investigate the gene expression of two strains of *B. bruxellensis*, AWRI 1499 and CBS 2499 having different genetic backgrounds, when exposed to a SO₂ pulse. Results revealed that sulphites affected yeast culturability and metabolism, but not volatile phenol production suggesting that a phenotypical heterogeneity could be involved for the SO₂ cell adaptation. The transcriptomics variation in response to SO₂ stress confirmed the strain-related response in *B. bruxellensis* and the GO analysis of common differentially expressed genes showed that the detoxification process carried out by SSU1 gene

can be considered as the principal specific adaptive response to counteract the SO₂ presence. However, nonspecific mechanisms can be exploited by cells to

assist the SO₂ tolerance; namely, the metabolisms related to sugar alcohol (polyols) and oxidative stress, and structural compounds.

- 2 Fracassetti D, Massaglia S, Viberti A, Motta G, Foschino R, Vigentini I. 2020. Wine industry's attitude towards oenological yeasts: Italy as a case study. *Beverages*, 6: 33; doi:10.3390/beverages6020033.

Yeast inoculation is a widespread practice in winemaking in order to control the must fermentation. However, the use of indigenous wine yeasts can enrich wine quality and differentiate wine styles. Yeast cream preparation (CRY), recently accepted by the International Organization of Vine and Wine, could allow an easier usage of autochthonous yeasts. This work aimed at investigating the actual Italian wine industry's attitude towards the available formulations of commercial wine yeasts with attention to CRY. Moreover, this study evaluated the perception of wineries toward indigenous yeasts in both winemaking and marketing viewpoints. Data show different levels of knowledge and use about the available yeast

formulations. In general, there is not a predominantly positive or negative participants' opinion regarding the use of indigenous yeasts. Wineries using CRY (4% of the sample) mainly adopt them as a part of the production in order to compare the wines with the ones traditionally obtained with commercial yeasts. CRY is perceived by some interviewees as a potential tool to increase communication and product differentiation. This survey could have anticipated future trends in the use of yeast formulations, determined by the market demands for diversified, unique, and environmentally sustainable products, that can allow an accessible application of precision enology.

- 3 Fracassetti D, Bottelli P, Corona O, Foschino R, Vigentini I. 2019. Innovative alcoholic drinks obtained by co-fermenting grape must and fruit juice. *Metabolites*, 9 (5): 86 doi:10.3390/metabo9050086.

In this study, Cabernet Sauvignon and Chardonnay musts, and fruit juices from cherry, kiwi, peach, and strawberry were co-fermented with *Saccharomyces cerevisiae* EC1118 and *Torulaspora delbrueckii* UMY196 at two different proportions (80:20 (v/v) and 60:40 (v/v)). The most pleasant fruitbased drink was obtained with Cabernet Sauvignon must and kiwi juice in a proportion of 60:40 and fermented with *T. delbrueckii*. This beverage was produced in higher volume to simulate a scaleup, and the aromatic profile, sensory description, and consumer acceptability were determined. The most powerful odorants of the kiwi-based drink were ethyl octanoate, phenylethanal, ethyl hexanoate, vinyl-guaiacol, benzaldehyde, and

nonanal, for which the odor activity values were 21.1, 3.3, 2.6, 2.2, 1.9, and 1.6, respectively. These findings were in accordance with the sensory analysis, since the emerged descriptors were fruity (ethyl octanoate), honey and floral (phenylethanal), apple and peach (ethyl hexanoate), and citrus (nonanal). The consumers judged the kiwi-based drink acceptable (67%) and 39% of them would buy it. The reliable fermentation of a grape must/fruit juice was demonstrated. The kiwi-based drink represents an innovative and pleasant beverage with a positive impact on sustainability as its production can limit the loss of fresh fruits, as well as contribute to the enological field. © 2019 by the authors.

- 4 Fracassetti D, Vigentini I, Marai S, Ferrari E, Tirelli A, Foschino R, Guidetti R. 2019. Suitability of ultraviolet-C irradiation for white grape must. *Australian Journal of Grape and Wine Research*, 25 (2): 224-232; doi:10.1111/ajgw.12388.

Background and Aims: Ultraviolet (UV)-C irradiation is used for the reduction of microbial spoilage in food. Its effectiveness for the treatment of white grape must has been investigated considering different microbial species and modification of must composition. Methods and Results: Static and dynamic laboratory-scale systems emitting at 254 nm were used for the treatment of *Vitis vinifera* cv. Chardonnay and cv. Crimson grape juices of variable

turbidity. The must samples were singularly inoculated with a pure culture of wine spoilage strains belonging to three species, *Dekkera bruxellensis*, *Acetobacter aceti* and *Lactobacillus brevis*, and treated with UV-C ranging from 300 to 1800 J/L. Cell counts and microbial reduction (derived from α values, according to the Weibull model), colour change, polyphenol oxidase (PPO) inactivation and formation of α -dicarbonyl compounds were evaluated. After

treatment at the maximum UV-C dosage under static conditions, reductions of yeast counts of 4.91-5.99 log 10 colony-forming units (CFU)/mL and bacterial counts of 4.12–5.05 log 10 CFU/mL were achieved, depending on the strain being tested. Polyphenol oxidase activity decreased by up to $7.8 \pm 1.4\%$ with variation attributed to must turbidity, which indicated that clarification of must before UV-C treatment can lead to PPO inactivation. No significant variation in the colour or the concentration of α -dicarbonyl compounds was detected. Results were confirmed for grape juice with the continuous flow apparatus.

Conclusions: The UV-C systems achieved microbial reduction without formation of oxidative compounds, suggesting that the experimental conditions employed did not cause any noticeable oxidative phenomena. Significance of the Study: The UV-C treatment of grape juice can be considered a potential alternative to sulfur dioxide addition for processing of white must. Improved efficacy of treatment can be achieved by increasing the flow rate and by clarification of must, making the system potentially applicable under industrial conditions.

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

- 1 Mangani S, Buscioni G, Guerrini S, Granchi L. 2020. Influence of sequential inoculum of *Starmerella bacillaris* and *Saccharomyces cerevisiae* on flavonoid composition of monovarietal Sangiovese wines Yeast. 2020; 37:549–557; doi: 10.1002/yea.3474.

The selection of *Starmerella bacillaris* strains to be used with *Saccharomyces cerevisiae* as mixed cultures has been recently suggested in order to produce wines containing lower ethanol and higher glycerol concentrations and to promote fructose degradation due to their fructophilic character. However, studies about effects of such mixed starter cultures on phenolic compounds, which are responsible for the color and health-enhancing properties in red wines, are currently lacking. Therefore, in this work the influence of sequential fermentation (SIF) with *Starm. bacillaris* and *S. cerevisiae* on phenolic content of monovarietal Sangiovese wine was evaluated by fermentations at laboratory scale. Axenic fermentations (AXF) with *S. cerevisiae* were performed as control. *S. cerevisiae* attained higher cell densities in AXF compared to SIF. The experimental wines obtained by SIF showed

significant lower ethanol and higher glycerol concentrations, whereas no significant difference was detected in color intensity. The total phenol index reached significantly lower values in SIF. Furthermore, the wines produced by SIF contained higher concentrations of vitisin A that has a greater color stability than the anthocyanins monomer. Finally, a lower content of both free anthocyanins and flavan-3-ols, key compounds for wine quality possessing also health-enhancing properties, was found in wines obtained by SIF. On the contrary, no significant difference was detected on flavonol concentration between SIF and AXF. This study highlighted that the use of sequential inoculum of *Starm. bacillaris* and *S. cerevisiae* can contribute to increasing the color stability of red wines, even if at the expense of compounds with health properties.

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

- 1 Gerardi C, Tristezza M, Giordano L, Rampino P, Perrotta C, Baruzzi F, Capozzi V, Mita G, Grieco F. 2019. Exploitation of *Prunus mahaleb* fruit by fermentation with selected strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. Food Microbiology, 84: 103262 doi:10.1016/j.fm.2019.103262).

The organoleptic attributes of *Prunus mahaleb*, a fruit representing a new source of bioactive

compounds, are so pronounced that it can be considered non-edible. This study was designed to evaluate the

acceptance of *P. mahaleb* fruits after fermentation with different *Saccharomyces cerevisiae* and *Lactobacillus plantarum* protechnological strains. Four different bacterial and one yeast strains, as single or mixed starter formulation, were used to inoculate an aqueous suspension of *P. mahaleb* fruits. The fermented fruits and fermentation broths were subjected to physico-chemical characterization and the organoleptic properties of both samples were also assessed by a hedonic panel. The obtained results indicated that all the employed strains were able to grow and to ferment the matrix. However, the mixed

starter FG69+Li180-7 (*L. plantarum*/*S. cerevisiae*) had the best impact on sensory characteristics of *P. mahaleb* fruit and fermented medium. The adopted protocol allowed us to attain edible fruits and a new fermented non-dairy drink with valuable probiotic health-promoting properties. In our knowledge, this is the first study concerning the exploitation of *P. mahaleb* fruits. This investigation confirmed the potential of yeasts and lactic acid bacteria co-inoculation in the design of starter tailored for this kind of food applications.

- 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: 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.

- 3 Ramires F.A, Durante M, Maiorano G, Migoni D, Rampino P, Fanizzi F.P, Perrotta C, Mita G, Grieco F, Bleve G. 2020. Industrial scale bio-detoxification of raw olive mill wastewaters by the use of selected microbial yeast and bacterial strains to obtain a new source for fertigation. *Journal of Environmental Management*, 110: 110574; doi: 10.1016/j.jenvman.2020.110574.

The Olive Mill Wastewaters (OMWs) are one of the most important agro-industrial wastes of the Mediterranean Countries and the disposal by draining them onto land has been proved to be damaging for soils, plants and groundwater due to their polluting

power. The present report describes a new method for bio-detoxification of undiluted fresh OMW based on the driven selection of aerobic yeasts and bacteria. The identified yeast *Candida boidinii* A5y and the bacterium *Paenibacillus albidus* R32b strains allowed

the treatment of freshly produced raw OMW characterized by very high COD value and phenolic content, when applied as sequential inoculum. The treated OMW showed the absence of antimicrobial effects and a strongly reduction of phytotoxic activity on the germination of several plant seeds. The process was successfully validated on an industrial scale without any pre-treatment, dilution and/or

supplementation of the raw waste. Bio-detoxified OMW produced by this sustainable and low-cost process would be suitable for new non-chemical fertigation or soilless applications. The described procedure represents a virtuous example of circular economy efficaciously applied for a depleting agri-food resource.

GMV-XIII Fondazione Edmund Mach Fdn Edmund Mach, Ctr Trasferimento Tecnologico, Via Mach 1, I-38010 San Michele all Adige, Trento, Italy. Communicated by Dr. Raffaele Guzzon <rafafele.guzzon@fmach.it> www.fmach.it.

- 1 Guzzon R, Roman T, Larcher R. 2020. Impact of different temperature profiles on simultaneous yeast and bacteria fermentation. *Annals of Microbiology*. 70: 44; doi: 10.1186/s13213-020-01565-w.

Purpose The role of fermentation temperature was studied for its impact on the evolution of malolactic fermentation performed by simultaneous inoculum of yeast and bacteria in grape must. Results were discussed considering the different fermentative kinetics and the composition of obtained wines. **Methods:** Two strains of bacteria belonging to the *O. oeni* and *L. plantarum* species were inoculated 24 h after the beginning of the alcoholic fermentation in 2 grape musts having different acidic and sugar profiles. Fermentations were conducted at 3 different temperature profiles (16/22 degrees C in 3 days, 18/24 degrees C in 3 days, 22/32 degrees C in 5 days). Evolution of microbiota was followed by flow cytometry and plate count. Chemical analysis of grape musts and wines were performed by instrumental approaches (FT-IR, enzymatic quantification of malic acid, GC-MS). **Results:** *L. plantarum* resulted more efficient in malic acid consumption in the entire set of tests. These results are unexpected because, generally, *Lactobacillus* has been reported to be more sensitive to

an oenological environment than *O. oeni*. In our experiments, *O. oeni* resulted inhibited by the highest fermentation temperature profile, causing incomplete malic acid degradation. Similarly, *S. cerevisiae* showed a higher sensitivity to environmental limiting factors in respect to what is generally known. Differences in the chemical composition of wines were observed in relation to the bacteria strain and the temperature profile. However, the statistical treatment of data identified temperature as the main variable able to influence the features of wines. **Conclusions:** Simultaneous inoculum of yeast and bacteria in grape must is an alternative approach in the management of malolactic fermentation which showed some interesting features. However, it is necessary to consider that the dynamics of the microbial population are different to that observed in traditional winemaking and the environmental variables act against the microorganisms in a peculiar, and in certain cases unexpected, way.

- 2 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. *Journal of Applied Microbiology*, 128, 209-224; 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. Chemical and

sensory characterization of wine was performed. 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 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). Carbonic maceration impacts on the evolution of the microbiota and the wine features. The absence of addition of starters and sulphur dioxide would seem to be correlated with the high microbial biodiversity. Significance and Impact of the Study Carbonic maceration is a traditional winemaking practice, today there are difficulties in its managing because the anaerobiosis stimulates spoilage micro-

organisms. 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.

GMV-XIV DISTAL, Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Viale Fanin 44, Bologna, Italy. Communicated by Prof Dr. Rosalba Lanciotti <rosalba.lanciotti@unibo.it>.

- 1 Parpinello G.P, Ricci A, Folegatti B, Patrignani F, Lanciotti R, Versari A. 2020. Unraveling the potential of cryotolerant *Saccharomyces eubayanus* in Chardonnay white wine production. LWT-Food Science and Technology, 134: 110183; doi: 10.1016/j.lwt.2020.110183.

This work investigated the suitability of the cryotolerant yeast *Saccharomyces eubayanus* to ferment Chardonnay must at different temperatures (10°C, 12°C, 16°C, and 26°C) over two vintages (2013 and 2014). The effect of added nitrogen was also evaluated. The strain's fermentation parameters (maximum growth rate, lag phase, and asymptotic maximum) and cell growth were compared to the values for two reference *Saccharomyces cerevisiae* strains. *Saccharomyces eubayanus* showed its best fermentation performance at low temperatures (10°C and 12°C), with optimal kinetic parameters and high sugar consumption. Moreover, wines from the cryotolerant yeast showed a volatile acidity reduction of approximately 50%, and a 9% increase in total

polyphenols, compared to the reference yeasts. At 16°C the cryotolerant and control yeasts performed quite similarly, whereas at 26°C the former displayed stuck fermentation. For both yeasts, at 10°C the nitrogen content did not affect maximum growth rate, whereas it did at 16°C. Sensory analyses were run on the 2014 trials, showing differences in color, sweetness, and overall liking among wines obtained at 10°C, whereas wines obtained at 16°C differed only in color. Results demonstrate for the first time the *Saccharomyces eubayanus* suitability for low-temperature fermentation in white wine production, potentially enriching yeast biodiversity in winemaking.

- 2 Patrignani, F, Parrotta, L, Del Duca, S, Vannini L, Camprini L, Dalla Rosa M, Schlüter, O, Lanciotti, R. 2020. Potential of *Yarrowia lipolytica* and *Debaryomyces hansenii* strains to produce high quality food ingredients based on cricket powder. LWT-Food Science and Technology, 11-27; doi.org/10.1016/j.lwt.2019.108866.

This research was aimed at producing, by the use of *Yarrowia lipolytica* and *Debaryomyces hansenii* strains, cricket powder hydrolysates endowed with high food safety, functionality, sensory and technological properties to be further used as food ingredients. The hydrolysates were characterised, in comparison to a control, by a reduced chitin content and higher contents of antimicrobial substances (acetic acid, short chain fatty acids, chitosan, and GABA) and health-promoting molecules (arachidonic and linolenic acids, GABA, AABA, and BABA). Additionally, in the cricket powder hydrolysates, a major release of

free amino acids was detected, highlighting the high matrix digestibility. The hydrolysates obtained by the use of GRAS yeasts were characterised by the presence of many volatile molecules and specific amino acids typical of ripened and fermented foods that can impart good sensory properties. In this study, the ability of each tested strain, based on their physiological and metabolic features in the system taken into consideration, was shown to give rise to cricket-based hydrolysates characterised by specific physicochemical, sensory and functional fingerprints.

- 1 Alfonzo A, Francesca N, Mercurio V, Prestianni R, Settanni L, Spanò G, Naselli V, Moschetti G. 2020. Use of grape racemes from Grillo cultivar to increase the acidity level of sparkling base wines produced with different *Saccharomyces cerevisiae* strains. *Yeast*, 37(9-10), 475-486; doi.org/10.1002/yea.3505.

The most important oenological characteristics of high quality sparkling wines are aromatic aspect, taste persistence, perlage, high levels of acidity and low pH. Due to hot climate and reduced rainfall that characterize Sicily region, white grape varieties such as Grillo cultivar cultivated in this area are characterized by very low concentrations of malic and tartaric acids. Grillo cultivar is characterized by an intense production of raceme grapes with low pH and high content of tartaric and malic acids. These fruits possess the chemical properties useful to increase the amounts of acids in the final wines. With this in mind, the present research was carried out to test the ability of four *Saccharomyces cerevisiae* strains (CS182, GR1, MSE13 and MSE41) to ferment a raceme must

with a pH of 2.9 at two concentrations (14° and 16° Babo degree) of total sugars. The inoculation of the strains was performed after a preadaptation at pH 2.5. The chemical parameters and kinetics of the fermentations were monitored. The experimental sparkling base wines were characterized by a very high total acidity with 16–17 g/L of tartaric acid and 9–10 g/L of malic acids. On the other hand, ethanol was detected at low values in the range 9–10% (v/v). The base wine obtained with GR1 differed in their high acidity values, whereas trials inoculated with CS182 showed more intense odors and exotic fruit. Experimental wines produced in this study represent an innovative strategy for “blending wines” to produce sparkling wines in dry Mediterranean climate.

- 2 Alfonzo A, Francesca N, Matraxia M, Craparo V, Naselli V, Mercurio V, Moschetti G. 2020. Diversity of *Saccharomyces cerevisiae* strains associated to racemes of Grillo grape variety. *FEMS Microbiology Letters*, 367(12); doi.org/10.1093/femsle/fnaa079.

The most important oenological characteristics of high-quality sparkling wines are high content of acidity and low pH. Racemes are late-maturing grapes of Grillo variety characterized by low pH and high content of tartaric and malic acids and, due to their intrinsic characteristics, might represent an interesting technological solution to increase acid quality of base sparkling wine. To this purpose, the use of yeasts able to ferment grape must at very low pH is mandatory for the success of the process. In this work, 261 *Saccharomyces cerevisiae* isolated from spontaneous vinifications of Grillo grape racemes were subject to intraspecific characterization by interdelta.. analysis

which evidenced a total population consisting of 82 strains which were screened for their basis of technological traits including SO₂ and alcohol tolerance, flocculence, growth at low temperatures and qualitative features such as H₂S production. A total of 11 strains with interesting technological performance in vitro were inoculated into musts obtained from racemes of Grillo grape variety and microfermentation were monitored. For the first time an ecological investigation of yeast associated to raceme grapes has been carried out and provided an innovative strategy to improve the acidity of a Sicilian sparkling base wine from Grillo grape variety.

- 3 Alfonzo A, Gaglio R, Barbera M, Francesca N, Moschetti G, Settanni L. 2020. Evaluation of the fermentation dynamics of commercial baker's yeast in presence of pistachio powder to produce lysine-enriched breads. *Fermentation*, 6(1): 2; doi.org/10.3390/fermentation6010002.

The present work was carried out to evaluate the microbiological, physicochemical, and sensory characteristics of fortified pistachio breads. Pistachio powder (5% w/w) was added to flour or semolina and fermented by a commercial baker's yeast (*Saccharomyces cerevisiae*). Pistachio powder did not influence the biological leavening of the doughs. The

kinetics of pH and total titratable acidity (TTA) during dough fermentation showed that the leavening process occurred similarly for all trials. The concentration of yeasts increased during fermentation and reached levels of 10⁸ CFU/g after 2 h. Pistachio powder decreased the height and softness of the final breads and increased cell density of the central slices. The

amount of lysine after baking increased in pistachio breads and this effect was stronger for semolina rather than flour trials. Sensory evaluation indicated that fortified breads processed from semolina were those more appreciated by the judges. This work clearly

indicated that the addition of pistachio powder in bread production represents a promising strategy to increase the availability of lysine in cereal-based fermented products.

GMV-XVI CREA - Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Conegliano (TV), Italy. Communicated by Dr Tiziana Nardi
<tiziana.nardi@crea.gov.it> <http://www.crea.gov.it/>.

- 1 Nardi T, Panero L, Petrozziello M, Guaita M, Tsolakis,C, Cassino,C, Vagnoli P, Bosso A.. 2019. Managing wine quality using *Torulaspora delbrueckii* and *Oenococcus oeni* starters in mixed fermentations of a red Barbera wine. Eur. Food Res. Technol, 245: 293–307; doi: 10.1007/s00217-018-3161-x.

The use of selected starter cultures for grape must fermentation is nowadays practiced in most wine regions of the world, and as the range of available microorganisms increases this gives winemakers the possibility to increase biodiversity in guided fermentations. Nevertheless, little information is available regarding the oenological use of *Saccharomyces* and non-*Saccharomyces* yeasts in combination with lactic acid bacteria. The present study evaluates the effect of the inoculation of a non-*Saccharomyces* yeast, *Torulaspora delbrueckii*, in an alcoholic fermentation (in combination with a *Saccharomyces* starter), together with different modalities of malolactic fermentation management (co-inoculation, sequential inoculation and no

inoculation of an *Oenococcus oeni* starter), on the quality of a red Barbera wine. The fermentation dynamics were verified and the final wine properties were evaluated in terms of physico-chemical composition determined by 1H-NMR, free volatile compounds measured by GC-MS and organoleptic characteristics assessed via sensory analysis. Overall, the results show that the joint employment of non-*Saccharomyces* yeasts did not delay alcoholic nor malolactic fermentation progress, did not interfere with the positive effect of the co-inoculation of malolactic bacteria (beneficial for both fermentation length and for the chemical, aromatic and sensory properties of wine) and enabled the production of wines with a more intense colour.

- 2 Benucci I, Cerreti M, Liburdi K, Nardi T, Vagnoli P, Ortiz-Julien A, Esti M, 2018. Pre-fermentative cold maceration in presence of non-*Saccharomyces* strains: Evolution of chromatic characteristics of Sangiovese red wine elaborated by sequential inoculation. Food Res. Int, 107: 257–266; doi: 10.1016/j.foodres.2018.02.029.

Two different *Metschnikowia* strains (*M. pulcherrima* MP 346 or *M. fructicola* MF 98-3) were applied for the first time, during pre-fermentative cold maceration (PCM) in order to enhance the properties and stability of Sangiovese wine color. During the 2014 and 2015 vintages a total of eight wines were produced with 24 h of cold maceration (PCM 24 h) or 72 h (PCM 72 h), respectively. PCM was carried out in presence of MP 346 or MF 98-3 or pectic enzyme (Cuvée Rouge). The sequential inoculation of *S. cerevisiae* strain was carried out at the end of PCM. After 12 months in the bottle, the MP 346 and MF 98-3 wines contained much higher levels of total

flavonoids than the Control sample for both vintages and regardless PCM duration. Moreover, in both vintages only MF 98-3 showed a higher color intensity than the Control sample after 12 months in the bottle. However, neither PCM duration nor the microbial/enzymatic treatment increased the level of anthocyanins at draining off. Both wines produced by the pre-fermentative inoculum with *Metschnikowia* strains (MP 346 and MF 98-3) retained their red hue, regardless the duration of pre-fermentative and fermentative macerations, while the Control wines were characterized by faster rates of color loss.

- 3 Benucci I, Luziatelli F, Cerreti M, Liburdi K, Nardi T, Vagnoli P, Ruzzi M, Esti M, 2018. Pre-fermentative cold maceration in the presence of non-*Saccharomyces* strains: effect on fermentation behaviour and volatile composition of a red wine. *Aust. J. Grape Wine Res*, 24: 267–274; doi: 10.1111/ajgw.12326.

This study evaluated the impact of pre-fermentative cold maceration (PCM), in the presence of two non-*Saccharomyces* yeasts, *Metschnikowia pulcherrima* MP 346 and *Metschnikowia fructicola* MF 98-3, and of a commercial pectic enzyme, on fermentation kinetics and on the volatile composition of a Sangiovese red wine. Sangiovese grape must was inoculated with MP 346 or MF 98-3 or treated with a pectic enzyme preparation during PCM, at 5°C for 24 or 72 h. A Control wine was produced by a pure culture of *Saccharomyces cerevisiae*. Both non-*Saccharomyces* strains affected the initial yeast population dynamics and the persistence of *S. cerevisiae* at the end of malolactic fermentation. Irrespective of the duration of PCM, the inoculum of *Metschnikowia* strains did not influence the rate of

sugar consumption or the kinetics of malolactic fermentation. The volatile composition of the final wines was evaluated with solid-phase extraction, followed by GC/MS. The concentration of some terpenes and C13-norisoprenoids, nerol, geraniol, 8-hydroxy-linalool (cis) and 3-oxo- α -ionol, and of some esters, isoamyl lactate and ethyl isoamyl succinate, was higher in wines inoculated with *Metschnikowia* strains than in the Control and wine treated with pectic enzyme. *Metschnikowia* yeast strains MP 346 and MF 98-3 affect wine volatile composition. This study shows for the first time that an inoculum of *Metschnikowia* strains MP 346 and MF 98-3 during PCM is effective in modulating the volatile composition of a Sangiovese red wine.

GMV-XVII Department of Agriculture, Food, Natural resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy. Communicated by Professor Dr. Giuseppe Spano <giuseppe.spano@unifg.it> www.unifg.it.

- 1 Berbegal C, Khomenko I, Russo P, Spano G, Fragasso M, Biasioli F, Capozzi V. 2020. PTR-ToF-MS for the online monitoring of alcoholic fermentation in wine: Assessment of VOCs variability associated with different combinations of *Saccharomyces*/non-*Saccharomyces* as a case-study. *Fermentation*, 6:55; doi: 10.3390/fermentation6020055.

Alcoholic fermentation (AF) is a bioprocess of interest in the production of important beverages. Among alcoholic beverages, wine represent a model production worldwide diffused. *Saccharomyces cerevisiae* has a key role in driving AF. However, non-*Saccharomyces* yeasts received increasing interest also to differentiate sensory quality. The combination of *S. cerevisiae* and non-*Saccharomyces* strains can differently modulate the content of Volatile Organic Compounds (VOCs). We tested the potential of proton-transfer reaction-mass spectrometry coupled to a time-of-flight mass analyzer (PTR-ToF-MS) to

monitor the progress of this bioprocess, evaluating the impact of the different yeast combinations on wine quality. In particular, as model strains, we tested non-*Saccharomyces* belonging to the species *Metschnikowia pulcherrima* and *Torulasporea delbrueckii*. We inoculated the different combinations both in commercial grape juice and fresh grape must. It was one of the first studies evaluating the impact on VOCs contribution of the simultaneous inoculation of two non-*Saccharomyces* species together with a *S. cerevisiae* strain in terms.

- 2 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.

The heterogeneous category of non-*Saccharomyces* yeasts represents interesting reservoirs of innovation in the field of starter cultures for the wine industry. Even if non-*Saccharomyces* species/strains have been extensively studied and

reviewed, poor attention has been deserved to the commercial microbial formulation containing these yeasts. We formulated a review paper proposing an overview of scientific findings, literature patents, and biotechnological solutions that rely on the application

of non-*Saccharomyces* species/strains. In particular, we revised the present literature about 26 different scale micro-vinification tests and then the commercial yeasts starter cultures formulated including, at least, one non-*Saccharomyces* strain. We reported a brief history of the commercialization of non-*Saccharomyces* in the oenological field. In parallel, we followed the evolution of the normative

environment. We pointed out asymmetries among scientific evidence and the amount of patents using taxonomic units as targets for our analysis. In addition, we considered the possible drivers of innovation and harmonization in the wine industry. Finally, we discussed future perspectives in this field of wine biotechnologies.

GMV-XIX Laboratory of Microbial Biotechnology, Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, via Balzarini 1, 64100 Teramo, Italy. Communicated by Professor Rosanna Tofalo <rtofalo@unite.it> www.unite.it.

- 1 Tofalo R, Fusco V, Böhnlein C, Kabisch J, Logrieco A.F, Habermann D, Cho G.S, Benomar N, Abriouel H, Schmidt-Heydt M, Neve H, Bockelmann W, Franz C.M.A.P. 2020. The life and times of yeasts in traditional food fermentations. *Crit. Rev. Food Science Nutr*, 60(18): 3103-3132; doi: 10.1080/10408398.2019.1677553.

Yeasts are eukaryotic microorganisms which have a long history in the biotechnology of food production, as they have been used since centuries in bread-making or in the production of alcoholic beverages such as wines or beers. Relative to this importance, a lot of research has been devoted to the study of yeasts involved in making these important products. The role of yeasts in other fermentations in association with other microorganisms – mainly lactic acid bacteria – has been relatively less studied, and often it is not clear if yeasts occurring in such fermentations are

contaminants with no role in the fermentation, spoilage microorganisms or whether they actually serve a technological or functional purpose. Some knowledge is available for yeasts used as starter cultures in fermented raw sausages or in the production of acid curd cheeses. This review aimed to summarize the current knowledge on the taxonomy, the presence and potential functional or technological roles of yeasts in traditional fermented plant, dairy, fish and meat fermentations.

- 2 Perpetuini G, Tittarelli F, Battistelli N, Suzzi G, Tofalo R. 2020. γ -Aminobutyric acid production by *Kluyveromyces marxianus* strains. *J. Appl. Microbiol*, 129(6): 1609-1619; doi:10.1111/jam.14736.

Aims. *Kluyveromyces marxianus* dairy strains were tested for γ aminobutyric acid (GABA) production. The genes involved in GABA catabolism (*UGA1* and *UGA2*) and anabolism (*GAD1*) were found in *K. marxianus* genome. Their relative expression was evaluated with primer designed ad hoc. **Methods and Results.** Strains were grouped on the basis of *GAD1* gene sequence. Representative strains for each group were tested for GABA production by high performance liquid chromatography. All strains produced it at low levels. qRT PCR showed the absence of a relation between GABA production and *GAD1* gene expression. *UGA1* and *UGA2* genes were not upregulated and low amounts of succinic acid were

detected. **Conclusions.** All strains released a low amount of GABA suggesting that probably it was stored within cells. The different behaviour of strains in terms of GABA and succinic acid production as well as gene expression highlighted the genetic and phenotypic biodiversity of this species. **Significance and Impact of the Study.** GABA production and genes involved in its catabolism and anabolism were described in a population of dairy *K. marxianus* for the first time. The variability observed in terms of genetic and phenotypic biodiversity is important especially to exploit this non conventional yeast as microbial platform.

- 3 Perpetuini G, Tittarelli F, Suzzi G, Tofalo R. 2019. Cell wall surface properties of *Kluyveromyces marxianus* strains from dairy-products. *Front. Microbiol*, 10: 79; doi: 10.3389/fmicb.2019.00079.

Thirty-three *Kluyveromyces marxianus* strains were tested for the ability to form biofilm and mat structures in YPD and whey and for cell surface

hydrophobicity. To identify genes potentially involved in adhesion properties, a RT-qPCR analysis was performed. Eight strains were able to adhere on

polystyrene plates in both media and formed a mature mat structure. These strains showed a different level of hydrophobicity ranging from 55 to 66% in YPD and from 69 to 81% in whey. Four *K. marxianus* orthologs genes (*FLO11*, *STE12*, *TPK3*, and *WSC4*), known from studies in other yeast to be involved in biofilm formation, have been studied. *FLO11* and *STE12* showed the highest fold changes in all conditions tested and especially in whey: 15.05 and 11.21,

respectively. *TPK3* was upregulated only in a strain, and *WSC4* in 3 strains. In YPD, fold changes were lower than in whey with *STE12* and *FLO11* genes showing the highest fold changes. In mat structures *FLO11* and *STE12* fold changes ranged from 3.6–1.3 to 2–1.17, respectively. Further studies are necessary to better understand the role of these genes in *K. marxianus* adhesion ability.

GMV-XX 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> www.agr.unipi.it

- 1 Palla M, Blandino M, Grassi A, Giordano D, Sgherri C, Quartacci F.M, Reyneri A, Agnolucci M, Giovannetti M. 2020. Characterization and selection of functional yeast strains during sourdough fermentation of different cereal wholegrain flours. *Scientific Reports*, 10:12856;doi: 10.1038/s41598-020-69774-6.

The increasing demand for healthy baked goods boosted studies on sourdough microbiota with beneficial metabolic traits, to be used as potential functional starters. Here, 139 yeasts isolated from cereal-based fermented foods were in vitro characterized for their phytase and antioxidant activities. The molecular characterization at strain level of the best 39 performing isolates showed that they did not derive from cross contamination by baker's yeast. Afterwards, the 39 isolates were in vivo analyzed for their leavening ability, phytase activity and polyphenols content using five different wholegrain flours, obtained from conventional and pigmented common wheat, emmer and hull-less barley. Combining these findings, through

multivariate permutation analysis, we identified the 2 best performing strains, which resulted diverse for each flour. Doughs singly inoculated with the selected strains were further analyzed for their antioxidant capacity, phenolic acids, xanthophylls and anthocyanins content. All the selected yeasts significantly increased the total antioxidant activity, the soluble, free and conjugated, forms of phenolic acids and anthocyanins of fermented doughs. This study revealed the importance of a specific selection of yeast strains for wholegrain flours obtained from different cereals or cultivars, in order to enhance the pro-technological, nutritional and nutraceutical traits of fermented doughs.

- 2 Palla M, Agnolucci M, Calzone A, Giovannetti M, Di Cagno R, Gobbetti M, Rizzello C.G, Pontonio E. 2019. Exploitation of autochthonous Tuscan sourdough yeasts as potential starters. *International Journal of Food Microbiology*, 302: 59-68; doi: 10.1016/j.ijfoodmicro.2018.08.004

The increasing demand for healthy baked goods boosted studies on sourdough microbiota with beneficial metabolic traits, to be used as potential functional starters. Here, yeast populations of traditional sourdoughs collected from four Tuscan bakeries were investigated. Among 200 isolated strains, 78 were randomly selected and molecularly characterized. *Saccharomyces cerevisiae* was dominant, representing the only species detected in three out of the four sourdoughs. The fourth one harbored also *Kazachstania humilis*. Inter-delta regions analysis revealed a high intraspecific polymorphism discriminating 16 biotypes of *S. cerevisiae* isolates, which clustered based on their

origin. Representative isolates from each biotype group were individually used to ferment soft and durum wheat flour, aiming at evaluating their pro-technological, nutritional and functional features. During fermentation under standardized conditions, all strains were able to grow of ca. 2 log cycles, but only *S. cerevisiae* L10Y, D18Y and D20Y had a significantly shorter latency phase in both flours. Overall, values of phytase activity were significantly higher in durum compared to the corresponding soft fermented dough. *K. humilis* G23Y and *S. cerevisiae* D20Y, D24Y showed a threefold higher phytase activity than spontaneously fermented control, and the highest concentration of total phenols. Almost all the

strains led to increases of antioxidant activity, without significant differences among them. Investigations on the resistance of the strains to simulated gastric and intestinal conditions, that is considered a pre-requisite for the selection of probiotics, revealed the ability to survive in vitro by many of the strains considered. This study proposed the best performing yeast strains selected among autochthonous sourdough yeasts based

on their protechnological, nutritional and functional traits to be used as starters for making sourdough baked goods or functional cereal-based beverages. Although some yeast strains combined several technological and nutritional traits, the association of more selected strains seemed to be a requisite to get optimal sourdough characteristics.

- 3 Guzzon R, Labagnara T, Toffanin A, Roman Villegas T. 2018. Oenological characterisation of indigenous strains of *S. cerevisiae* isolated in a biodynamic winery in the Cortona DOC area. *Annals of Microbiol*, 68: 963; doi: 10.1007/s13213-018-1405-4.

The work relates about the genotypic and technological characterisation of the *S. cerevisiae* population isolated in a biodynamic winery in the Cortona DOC area. The oenological performance of 11 *S. cerevisiae* strains was evaluated with physiological tests. Strain typing was performed through analysis of interdelta sequences and 26S rDNA sequencing. The analysis revealed a remarkable variability in terms of *S. cerevisiae* strains, despite the homogeneity of wine features, underlining the high levels of biodiversity characterising biodynamic agriculture. Some strains were found in wines of different vintages, suggesting the presence of an established microbiota in the winery. Oenological

tests demonstrated that while some yeasts provided reliable oenological performance, other strains were not able to accomplish prompt and effective alcoholic fermentation, or were characterised by spoilage characteristics, such as excessive production of volatile phenols or acetic acid. Indigenous strains of *S. cerevisiae* could be a useful instrument for reliable winemaking without altering the native microbiota of each oenological environment. However, characterisation of their oenological suitability, and the application of practices able to drive the evolution of microbiota, must be employed to reduce the risk of wine spoilage.

GMV-XXI Laboratory of Food Microbiology, Department of Biotechnology, University of Verona (UNIVR), Strada le Grazie 15, Ca' Vignal 2, 37134, Verona, Italy. Communicated by Professor Dr. Sandra Torriani <sandra.torriani@univr.it> www.dbt.univr.it.

- 1 Gatto V, Binati R.L, Lemos Junior W.J.F, Basile A, Treu L, Almeida O.G.G, Innocente G, Campanaro S, Torriani S. 2020. New insights into the variability of lactic acid production in *Lachancea thermotolerans* at the phenotypic and genomic level. *Microbiological Research*, 238:126525; doi: 10.1016/j.micres.2020.126525.

Non-conventional yeasts are increasingly applied in fermented beverage industry to obtain distinctive products with improved quality. Among these yeasts, *Lachancea thermotolerans* has multiple features of industrial relevance, especially the production of L(+)-lactic acid (LA), that is useful for the biological acidification of wine and beer. Since few information is available on this peculiar activity, the current study aimed to explore the physiological and genetic variability among *L. thermotolerans* strains. From a collection of strains, mostly isolated from wine, a huge phenotypic diversity was acknowledged and allowed the selection of a high (SOL13) and a low (COLC27) LA producer for whole-genome sequencing Comparative genome analysis of these two selected

strains and the type strain CBS 6340T showed a high similarity in terms of gene content and functional annotation. Notwithstanding, target gene-based analysis revealed variations between high and low producers in the key gene sequences related to LA accumulation. More in-depth investigation of the core promoters and expression analysis of the genes *ldh*, encoding lactate dehydrogenase, indicated that the transcriptional regulation may be the principal cause behind phenotypic differences. These findings highlighted the usefulness of whole-genome sequencing coupled with expression analysis to provide crucial genetic insights for a deeper investigation of the intraspecific variability in LA production pathway.

Obituary

Prof. Arnold L. Demain (1927-2020)

Arnold Lester Demain, professor emeritus of biology, passed away on Apr. 3 at the age of 92 from complications due to Covid-19. He was just shy of celebrating his 93rd birthday.

Demain advanced the field of fermentation biology, and made major contributions to the study of antibiotics like penicillin, cephalosporin, and beta-lactam. Over the course of his 60-year career, he made a name for himself as one of the world's leading industrial microbiologists, and mentored hundreds of budding scientists around the world.

“Arny was a prolific industrial biologist, as well as a colleague and friend,” says Alan Grossman, head of the Department of Biology and the Praecis Professor of Biology. “His work on antibiotic fermentations spurred a new wave. He was kind and supportive to all, and a dedicated mentor to many students and postdocs.”



Photo MIT

Demain was born on April 26, 1927 in Brooklyn, New York, and grew up during the Great Depression. He graduated from high school at the age of 16, and attended Michigan State College (now Michigan State University). At 17, he put his education on pause to join the U.S. Navy and fight in World War II. After the war ended, he returned to Michigan State and resumed his studies, earning his bachelor's degree in 1949 and his master's in microbiology in 1950, with a focus on food fermentation — specifically, how pickles spoil. During this time, Demain met his wife Joanna (Kaye) Demain, and they were married on August 2, 1952.

Demain began his PhD in food science at the University of California at Berkeley, but relocated to Davis when that campus opened. Under the guidance of his research advisor and prominent yeast scholar, Herman Jan Phaff, Demain studied the degradation of pectic acid by an extracellular enzyme in the yeast *Klyveromyces fragilis*, publishing four papers, including one in *Nature*. Demain and Phaff were also among the first researchers to perform affinity chromatography, which later became a standard biochemical procedure.

After earning his PhD in 1954, Demain was recruited by Merck Sharp & Dohme Research Laboratories, first to research penicillin biosynthesis and later to study cephalosporin C. In 1965, he established Merck's Fermentation Microbiology Department.

After 16 years at Merck, Demain joined MIT's former Department of Nutrition and Food Science, and in 1988 he joined the Department of Biology. When he first arrived, no one at MIT was conducting research on antibiotics. He was eager to continue investigating penicillins and cephalosporins, and his hard work culminated in the breakthrough discovery of a key enzyme in cephalosporin biosynthesis: deacetoxycephalosporin C synthase.

Rich Losick PhD '69 was finishing his graduate work at MIT when Demain arrived on campus. Demain later interacted with Losick's wife Janice Pero and former postdoc John Perkins because all three shared an interest in vitamin B2 research. “I was drawn to Arny due to his warm and engaging personality and my interest in microbiology,” Losick recalls. “He pioneered industrial production of vitamins, antibiotics, and fine chemicals, and was revered for his many contributions to industrial microbiology. He was a big-hearted human being, an excellent and productive scientist, and a dedicated teacher. He will be greatly missed.”

While at MIT, Demain also helped catalyze the biotech industry by serving as the founding consultant for the biotech company, Cetus Corporation. By the mid-1990s, he'd spearheaded a series of NASA-sponsored experiments to probe the effects of simulated microgravity on secondary metabolism. Toward the end of his 32 years at MIT, he began examining *Clostridium tetani* and *Clostridium difficile* bacteria in hopes of devising tetanus and antibiotic-associated diarrhea vaccines. He ultimately authored more than 500 publications and 21 U.S. patents.

"Arny had a keen mind and a gentle disposition that put you at ease," says Gerald Fink, professor in the Department of Biology and founding member and former director of the Whitehead Institute. When Fink started at MIT, Demain was the first to greet him. "He dropped into my office in Building 56 and he said, 'You are going to like it here,'" Fink recalls.

"Arny was a wonderful colleague," adds Robert Sauer, the Salvador E. Luria Professor of Biology. "He was always upbeat and happy to talk about science or anything else on your mind."

Professor of Biology Anthony Sinskey shared an office with Demain, and remembers him as a pioneer who applied genetics and biochemistry to improve antibiotic production processes. He says Demain was instrumental in forming important interdisciplinary programs at MIT — including using anaerobic microorganisms to convert cellulose to fuels, as well as strategies for cell free synthesis of antibiotics and other projects.

"I learned a tremendous amount from our interactions," Sinskey says. "He taught industrial microbiology and fermentation technology to hundreds of students both at MIT and from industry."

While Demain was at MIT, an informal group of students formed called Arny's Army and Friends. Since his "early" retirement from MIT at age 75, Demain's students have held the Arny's Army and Friends Symposia in his honor every three years.

Later, Demain would recall that he "was very lucky ... to have had a fantastic group of bright and hardworking visiting scientists, postdoctoral associates, graduate students, undergraduate students and high school students. ... Success at MIT would not have been possible without them."

Abraham L. "Linc" Sonenshein first crossed paths with Demain as a graduate student at MIT. From the beginning, Sonenshein could tell Demain was "a very important scientist to interact with," because of the way he applied his knowledge of microbiology to prevent and treat infections. "I was amazed that he continued to contribute to science publication and training for decades after officially retiring as a full-time faculty member," Sonenshein says. "The number of scientists he educated and trained is phenomenal."

In 2000, Demain moved to Madison, New Jersey, and joined the Research Institute for Scientists Emeriti (RISE) at Drew University. He continued to conduct research and mentor students until May 2019, when he fully retired at the age of 92. That same year, Drew University established an endowed scholarship in his name.

Over the course of his career, Demain earned numerous awards, including one from the king of Spain and another from the emperor of Japan. He was also a member of the National Academy of Sciences, president of the Society for Industrial Microbiology, and on the Board of Governors for the American Academy of Microbiology.

He is survived by his wife of 68 years, Joanna (Jody) Demain; his daughter, Pamela Demain; his son, Jeffrey Demain; his daughter-in-law, Lauren Brener; his grandchildren; and his great-grandchildren. A memorial service for family, friends, colleagues, and former students will be held when it is safe to do so. Donations in his memory can be made to the Alzheimer's Foundation of America.

Raleigh McElvery, Department of Biology

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Future Meetings

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

The 47th Annual Conference on Yeasts (ACY), originally organized for May 2020, is now planned for 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/>

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

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

With kind regards,

Diethard Mattanovich and the “ICY15 meets ICYGMB30” organizing team

Brief News Item

Thorner Laboratory Closure

After 47 years at the University of California, Berkeley, the laboratory of Professor Jeremy Thorner will be closing permanently, as of 30 June 2021. After that date, there will be no way to distribute any strains, plasmids, enzymes, or antibodies generated during the course of the studies on *Saccharomyces cerevisiae* conducted by the Thorner laboratory over those many years.

Jeremy W. Thorner
jthorner@berkeley.edu

Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

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Editor

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

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Associate Editor

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

Associate Editor

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

Mr. D. Yarrow of CBS communicated receipt of type strains of new species *Aessosporon salmonicolor*, *Brettanomyces naardenensis*, *Bullera dendrophila*, *Candida australis*, *C. banhamii*, *C. bombi*, *C. chilensis*, *C. chiropterorum*, *C. edax*, *C. guilliermondii* var. *japonica*, *C. incommunis*, *C. ishiwadae*, *C. boidinii*, *C. oleophila*, *C. parapsilosis* var. *hokkai*, *C. punicea*, *C. requinyii*, *C. steatolytica*, *C. suecica*, *C. tepae*, *C. terebra*, *C. valdiviana*, *Debaryomyces halotolerans*, *D. nepalensis*, *Endomycopsis lipolytica*, *E. muscicola*, *Hanesnula philodendra*, *H. sydowiorum*, *Pichia castillae*, *P. krusei*, *Rhodotorula araucariae*, *Rhodotorula glutinis* var. *rufusa*, *Saccharomyces beticus*, *S. cordubensis*, *S. gaditensis*, *Selenotila intestinalis*, *Sel. peltata*, *Sporobolomyces antarcticus*, *Sterigmatomyces elviae*, *St. polyborus*, *Syringospora albicans*, *Syr. clausenii*, *Syr. stellatiodea*, *Torulopsis bombicola*, *T. humilis*, *T. karawaiewi*, *T. kestonii*, *T. mannifaciens*, *T. psychrophila*, *T. xestobii*, *Trichosporon aquatile*, *Tr. cutaneum* var. *antarcticum*, *Tr. eriense*, *Tr. fennicum*, and *Tr. melibiosaceum*. [Note: most of these names have been changed, due in large part to use of DNA technologies for systematics, see next item.]

Dr. H. J. Phaff, department of Food Science and Technology, University of California Davis submitted abstracts of two articles published by UC Davis Professor M. W. Miller and coworkers. Both of these studies delineated species based primarily on morphology of sexual structures: the number and shapes of spores.

“*Endomyces tetrasperma*, a new species” by J. M. Macy and M. W. Miller

“Synonymy of *Metschnikowia pulcherrima* and *Torulopsis burgeffiana*” by M. W. Miller, Ellen R. Johnson and J. I. Pitt

Phaff also shared the abstract of the Ph.D. dissertation of Dr. Sally Meyer, titled “DNA base composition and homology in *Candida* species and related yeasts”. Dr. Meyer used GC content and DNA-DNA hybridization to compare 55 strains of *Candida*, representing 30 species and four varieties. Previous close relation of many species and varieties based on morphology and physiological properties were not supported. Clusters of related species were revealed. Relationship of *C. bogoriensis* (now *Pseudohyphozyma bogoriensis*) to heterobasidiomycetous yeasts was suggested due to GC content of 57.3%, much higher than all other yeasts examined. Two key sentences in the abstract: “In general, the

physiological similarities of the organisms in the group are not indicative of a high degree of genetic relatedness.... Separation of species on the basis of a single assimilative or fermentative property is unsound. It is suggested that GC values, and preferably DNA homology, should be included in future taxonomic studies before new organisms are described and named or new relationships are proposed.” [Note that this work solidified the use of DNA technologies as tools for systematics. Current efforts to include genome sequences to circumscribe yeast species parallel this work.]

Dr. Samuel P. Meyers of Louisiana State University contributed abstracts of two recent publications in partnership with D. G. Ahearn of Georgia State University on hydrocarbonoclastic yeasts, titled “The role of yeasts in the decomposition of oils in marine environments” and “Mycological degradation of petroleum products in marine environments”.

Maruhiko Mori of the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan submitted a paper entitled, “A yeast isolated from tomato catchup”. The yeast *Saccharomyces acidifaciens* caused gas formation and produced large amounts of acetic acid and ethyl alcohol. They also worked on genetic improvement of *S. rouxii* for shoyu and miso fermentation.

Dr. Chr. Schönborn of Karl-Marx-Universität, Leipzig, D.D.R. [East Germany] isolated 762 yeasts from excreta of wild birds, pigeons, and birds from a zoo, in an effort to study association of *Cryptococcus neoformans* with birds. No strains of *C. neoformans* were found. The most common yeasts were *Rhodotorula*, *Torulopsis*, *Candida* and *Geotrichum*. Several species potentially pathogenic to humans included *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii* and *Torulopsis glabrata*.

Prof. Dr. S. Windisch of the Lehrstuhl für Mikrobiologie der Technischen Universität Berlin, West Berlin reported publication of the thesis of U. Steckowski titled “Regulationsmechanismen der alpha-Glucosidase-Synthese bei *Saccharomyces*-Hefen,” as well as publications on yeasts in ground almond and sweets, *Saccharomyces carlsbergensis* and *S. rouxii* in marzipan, osmotolerance of yeasts, and the effect of osmotic value on growth and ethanol formation by yeast.

Yasuji Oshima of Osaka University, Japan moved from the Central Research Institute, Suntory Ltd. To Osaka University, Osaka, Japan, where they study homothallism genes and mating type switching in *Saccharomyces*.

John Johnston of the University of Strathclyde was spending a nine month sabbatical leave studying genetic analysis of polyene antibiotic resistance in yeast at the Centro de Investigación y de Estudios Avanzados del Instituto Politecnico Nacional, Departamento Genética y Biología Celular, Mexico. Mr. John Coulson recently completed his Ph.D. thesis, studying antibiotic resistance in yeasts.

T. Takahashi of Suita Laboratory, Brewing Science Research Institute, Asahi Breweries Ltd., Suita Japan summarized the second meeting of the Yeast Genetics Conference-Japan, held August, 1970 at Osaka University.

A. Maxwell of the Stanford Research Institute presented an abstract at the Cell Biology meeting in San Diego in November 1970 titled, “Ultrastructural changes in *Saccharomyces cerevisiae* treated with iodoacetic acid.

H. C. Birnboim of Atomic Energy of Canada described a study of nutritional requirement of “wild type” *Schizosaccharomyces pombe* for cysteine at low cell concentrations.

K. W. van de Poll of State University of Utrecht, The Netherlands recently completed a thesis titled, “Regulation of allantoinase synthesis in *Saccharomyces carlsbergensis*”, and five related papers.

J. O. Lampen of Rutgers University, New Jersey published five papers related to yeast, pertaining to biosynthesis of yeast mannan, staining of mannan in yeast cell walls with fluorescein-conjugated concanavalin A, grooves in the plasmalemma, and yeast and *Neurospora* invertases.

Prof. Heikki Suomalainen of the Research Laboratories of the State Alcohol Monopoly, Helsinki, Finland published studies of contents of cytochromes in yeast, yeast and its effect on the flavor of alcoholic beverages, alpha-hydroxy ketones in wines, and diacetyl and formic acid decomposition/

M. Hilmi Pamir of A. Ü. Ziraat Fakültesi Fermantasyon Teknolojisi Kürsüsü, Ankara, Turkey published a paper on the effect of yeast strains on the formation of SO₂-binding compounds and rates in wines.

T. M. Enari of the State Institute for Technical Research, Helsinki, Finland published studies of the effect of wort amino acids on fermentation, and influence of nitrogenous compounds on beer fermentation.

Brief news items:

Professor Augusto Capriotti was killed in an automobile accident in April 1970.

In January 1970, the National Collection of Yeast Cultures (Norwich, UK) published their new catalogue.

F. Schlenk and **G. Svihla** of Argonne National Laboratory, Argonne, Illinois published work on they yeast cell wall.

Fred Sherman of the University of Rochester, New York published studies of relationship of gene structure and protein structure, and control of organelle development.

Leslie Hedrick, Illinois Institute of Technology published work on the ecology of yeasts in polluted water.

Mr. Raja Seshadri, a predoctoral student at the University of Rhode Island, isolated and examined the biochemical aspects of 300 yeast strains from seaweeds on the Rhode Island coast, and requested any publications related to utilization of phenolic compounds by yeasts.

N. J. W. Kreger-van Rij of the Bacteriologisch-Serologisch Laboratorium der Rijksuniversiteit, Groningen, Holland published an electron microscope study of *Pityrosporum ovale*, and septal pores in *Trichosporon cutaneum*.

Professor Pierre Galzy published work on modification of the cell wall of smooth colony mutant strains of *Saccharomyces cerevisiae*, protoplast preparation, and cadmium, cobalt and arsenic-resistant mutants.

Professor A. Castellani, Escola Nacional de Saude Publica e de Medicina Tropical, Lisobn, Portugal published work on infantile and juvenile leucodermata and palliative cosmetic treatment.

Dr. William A. Clark, director of the American Type Culture Collection in Rockville, Maryland, requested that workers who describe new yeast species deposit type cultures in ATCC as well as other collections, and also announced that **Dr. Sally Meyer** would join the staff of ATCC in 1972, responsible for the yeast collection.

Prof. J. Santa Maria of Institute Nacional de Investigaciones Agronomie, Madrid, Spain published work on abnormal segregation of mating type in *Saccharomyces*, new species *Saccharomyces gaditensis* and *S. cordubensis*, and taxonomy of *Kluyveromyces*.

The new edition of "The Yeasts – A Taxonomic Treatise", J. Lodder, editor, was published in Fall 1970. The book chapters were listed. The species discussions were divided into four chapters:

Ascomycetous yeasts by Wickerham, Kreger-van Rij, van der Walt, Phaff, Slooff, Miller, van Uden and do Carmo-Sousa

Ustilaginales by Fell, Phaff and Newell

Sporobolomycetaceae by Phaff

Asporogenous yeasts not belonging to Sporobolomycetaceae by van der Walt, van Uden, Buckley, Phaff, Fell, do Carmo-Sousa, Sloof, Ahearn, Kreger-van Rij, and Vidal-Leiria

Donald G. Ahearn of Georgia State University announced that the proceedings of the symposium “Recent Trends in Yeast Research” held in Plattsburgh, New York were published in *Spectrum, Monograph Series in the Arts and Sciences* by Georgia State University.

Dr. S. M. Martin announced that the Section on Culture Collections, IAMS, voted to dissolve and be reconstituted as the World Federation for Culture Collections at its meeting in Mexico City, August 1970. Martin, the first WFCC Chairman, expressed gratitude for the financial support of the Japanese Federation of Culture Collections.

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