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Editorial

On *Metschnikowia pulcherrima* and good systematics

In this issue, Matthias Sipiczki reports an important contribution in which he demonstrates that the many proposed new species in the *Metschnikowia pulcherrima* clade are in fact synonyms of *M. pulcherrima*. The paper deals with two things about which I care greatly, namely *Metschnikowia* and good systematics, for which reason I took the editorial liberty of adding the abstract of the paper to the entry. The proliferation of species proposals in that part of the big yeast tree of life may be explained in part by an unusual amount of barcode sequence polymorphism and heterogeneity in *M. pulcherrima*, which is the focus of Sipiczki's article and others that preceded. I suspect that the great interest for this yeast, owed to its many potential applications in winemaking, brewing, post-harvest biological control, and even clinical pursuits, also contributed to the desire to assign new specific epithets to what amount to population variants. A careful approach to species delineation should take into account all relevant aspects of yeast biology, and in particular, variation, which is inherent to biology. Isolates should be studied in context, and a typological application of simplistic rules that ignore variation should be avoided. The temptation exists to fit data to foregone conclusions, especially when it comes to yeasts with a lucrative potential. But good science must embrace good ethics.

M.A. Lachance, Editor

Recent publications.

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

To design a rapid, simple, and low-cost procedure for yeast selection with differential adsorption activities toward phenolics and ochratoxin A, 284 yeast strains were screened. This was done by evaluating the type of growth during grape must fermentation, acetic acid production on Chalk agar, H₂S production on BiGGY agar, and spore-formation on acetate agar. After that initial step, 84 strains were pre-selected and further studied by Petri plate tests and to determine their wine-making ability in trials and

evaluating their differential adsorption activities toward phenolics and ochratoxin A. Three yeast strains were selected based on the above evaluations. After confirming that they belonged to *Saccharomyces cerevisiae* species and were diploids, a spore clonal selection was performed. The strain Sc1741A_1D was selected and used in winemaking at six Calabrian wineries and found to be suitable as wine starter to improve quality and safety of red wines.

- 2 Caridi A, Nicolò A, Modafferi A, De Bruno A. 2022. Effect of pomegranate supplementation on the wine yeast response to acidic and osmotic stresses. *Eur Food Res Technol*, pp. 5, online 25 April <https://doi.org/10.1007/s00217-022-04024-9>.

The aim of the present work was to verify in winemaking the anti-stress efficacy due to the integration of the grape must with two protectants: pomegranate albedo and pomegranate arils; these substances had displayed in vitro anti-stress effects. The effect of pomegranate supplementation on stress tolerance of five strains of *Saccharomyces cerevisiae*, one wild type and four descendants, against fermentation in grape must with high sugar content (30°brix) and high acidity (pH 3.00) was studied. So, micro-winemaking trials were carried out using grape must, as it is or supplemented at 2% with pomegranate albedo or with pomegranate arils, inoculated in

duplicate with the yeast strains. At the end of winemaking, ethanol and acetic acid content, colour intensity, total phenolic content, and total antioxidant activity by DPPH and ABTS assays were analysed. The results shown the possibility to use pomegranate as protective agent in winemaking with high sugar content and high acidity giving wines in which the fermentable sugars will be fermented with acceptable acetic acid content, very high colour intensity values, very high total phenolic content, and very high antioxidant activity, expressed as DPPH and ABTS values.

Recent publications.

- 1 Cruz EL, Pajot HF, Martorell MM, Mac Cormack WP, de Figueroa LIC, Fernández PM. 2022. Isolated indigenous yeasts from Antarctica with the ability to remove toxic hexavalent chromium. *Chem Ecol* 1-18, April 2022 - <https://doi.org/10.1080/02757540.2022.2066084>.

Seven chromate-reducing yeasts isolated from Antarctic soils (with no history of Cr(VI) contamination) were evaluated as promising candidates for alleviating Cr(VI)-contamination. These yeast species have not been extensively studied regarding Cr(VI) tolerance and reduction abilities. Based on their tolerance (up to 2 mM) and ability to

remove Cr(VI) from culture media, two representative isolates of *Candida sake* #14 and *Debaryomyces hansenii* #35 were chosen. The resistant yeasts were cultivated in YM medium plus 1 mM Cr(VI) at 25°C, causing a removal of ~90 and ~70% hexavalent chromium before reaching 96 h of culture according to growth curves of *C. sake* #14 and *D. hansenii* #35,

respectively. Cr(VI) removal kinetic analysis indicated that both isolates removed Cr(VI) by reduction to Cr(III), and incorporated a small portion into the biomass. Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX) confirms the biosorption process, indicating the existence of a complex remediation mechanism. In conclusion,

Antarctica is an extreme environment with a potential to discover promising new candidates for the removal of Cr(VI). These results are interesting from a biotechnological point of view since psychrotolerant yeasts could be involved in the design of a profitable technology for the elimination of the toxic form of Cr.

Books chapters.

- 2 Viñarta SC, Maza DD, Fernández PM, Aybar MJ. de Figueroa LIC. 2022. Chapter 4. Integrated production of biodiesel and industrial wastewater treatment by culturing oleaginous microorganisms. In: *Integrated Environmental Technologies for Wastewater Treatment and Sustainable Development*, Vineet Kumar and Manish Kumar (Eds.), Elsevier Inc – <https://doi.org/10.1016/B978-0-323-91180-1.00021-1>.

Oleaginous microorganisms (bacteria, fungi, yeasts, and algae) are those that accumulate more than 20% (w/w) of lipids of their dry weight biomass. Depending on the fatty acids profile of microbial oils, they can be used as feedstock for the synthesis of biodiesel. However, the main drawback of commercialization is the high cost of the culture media components to obtain it. An integrated biodiesel production, combining the cultivation of oleaginous microorganisms with low-cost substrate (instead of glucose), such as industrial wastewater, may be a promising approach for improving the commercial and environmental viability of the process. In this chapter, several integrated strategies for the biological treatment of the different types of industrial

wastewater by culture of diverse oleaginous microorganisms are analyzed. Simple approaches using a single oleaginous microorganism are described, as well as a variety of integrated strategies using a consortium of different microorganisms. In turn, the types of oleaginous microorganisms used and their performance for the production of lipids are presented. Some critical aspects are discussed mainly considering co-cultivation methods, combination with other treatments, and advantages and disadvantages of these technologies. The potential of microbial oils as raw material for the synthesis of biodiesel and the possibility of industrial application of these technologies are also evaluated.

- 3 Ahmed PM, Pajot HP, Fernández PM. 2022. Chapter 12. Production of laccases from agricultural wastes: strain isolation and selection, enzymatic profiling, and lab-scale production. In: *Mycoremediation Protocols*, Dhanushka Udayanga et al. (eds.), Springer Protocols Handbooks https://doi.org/10.1007/978-1-0716-2006-9_12.

Fungal laccases (copper-containing oxidases) have profound applications in bioremediation and various other industrial and biotechnological areas. This chapter outlines recent developments in laccase production technologies, with a focus on the selection of the most promising producing fungal species, general considerations on media components and

culture conditions, lab-scale production, and partial purification of enzymes. Furthermore, protocols related to solid-state fermentation (utilizing agriculture wastes), such as one-step affinity chromatography (employing a mesoporous material as support), and electrophoresis (used to check the enzyme purity), are described.

Previous publications.

- 4 Maza DD, Viñarta SC, García-Ríos E, Guillamón JM, Aybar MJ. 2021. *Rhodotorula glutinis* T13 as a potential source of microbial lipids for biodiesel generation. *J Biotechnol* 331:14-18 <https://doi.org/10.1016/j.jbiotec.2021.03.002>

Single cell oils (SCO) are a promising source of oils that could be exploited in different industrial areas. SCO for biodiesel production circumvents the controversy food vs. fuel, does not require large land areas for culture, and is independent of climate and

seasonal variations, among other advantages in comparison to vegetable oils. In this study, a red yeast isolated from a mountain water source, identified as *Rhodotorula glutinis* T13, showed high potential for lipid production (40% w/w) with suitable growth

parameters, yields, and fatty acids profile. Yeast lipids showed a high content of unsaturated fatty acids (56.44%; C18:1, C18:2), and the fuel properties (cetane number, iodine value, density, kinematic viscosity, etc.) of yeast oil analysed were in good

agreement with international biodiesel standards. The results show that *R. glutinis* T13 can be used in the future as a promising microorganism for the commercial production of biodiesel.

- 5 Leguina AC del V, Fernández PM, de Figueroa LIC, Nieto-Peñalver CG. 2021. Quorum Quenching in copper-tolerant *Papiliotrema laurentii* strains. *Rhizosphere* 17, 100298 <https://doi.org/10.1016/j.rhisph.2020.100298>

In the rhizosphere, the role of yeasts in microbial interactions and signaling is an open question. To study the influence of fungicides on yeast inactivation of *N*-acyl homoserine lactone quorum sensing signals, we evaluated the resistance of *Papiliotrema laurentii* strains from tomato rhizosphere to CuSO_4 and $\text{Cu}_2(\text{OH})_3\text{Cl}$ fungicides, and the copper effect on yeast quorum quenching. Copper resistance profiles and colony morphologies allowed the distinction of two groups of *P. laurentii* strains: mucoid, green and resistant, and brown-orange and more sensitive. Most

of the strains inactivated C6-HSL and C10-HSL QS signals. Inactivation and copper divided the strains in three with weak activity independently of the metal, and 11 with activities affected by copper. The lack of alkalization allows the hypothesis of an enzymatic inactivation of the signals. These results suggest that yeasts contribute to communications in the rhizosphere, and that copper fungicides can modify their interactions with other rhizosphere microorganisms.

- 6 Bertini EV, Torres MA, Léger T, Garcia C, Hong KW, Chong TM, de Figueroa LIC, Chan KG, Dessaux Y, Camadro JM, Nieto-Peñalver CG. 2021. Insight in the quorum sensing-driven lifestyle of the non-pathogenic *Agrobacterium tumefaciens* 6N2 and the interactions with the yeast *Meyerozyma guilliermondii*. *Genomics* 113(6):4352-4360 – <https://doi.org/10.1016/j.ygeno.2021.11.017>

Agrobacterium tumefaciens is considered a prominent phytopathogen, though most isolates are nonpathogenic. Agrobacteria can inhabit plant tissues interacting with other microorganisms. Yeasts are likewise part of these communities. We analyzed the quorum sensing (QS) systems of *A. tumefaciens* strain 6N2, and its relevance for the interaction with the yeast *Meyerozyma guilliermondii*, both sugarcane endophytes. We show that strain 6N2 is nonpathogenic, produces OHC8-HSL, OHC10-HSL, OC12-HSL and OHC12-HSL as QS signals, and possesses a complex QS architecture, with one

truncated, two complete systems, and three additional QS-signal receptors. A proteomic approach showed differences in QS-regulated proteins between pure (64 proteins) and dual (33 proteins) cultures. Seven proteins were consistently regulated by quorum sensing in pure and dual cultures. *M. guilliermondii* proteins influenced by QS activity were also evaluated. Several up- and down-regulated proteins differed depending on the bacterial QS. These results show the QS regulation in the bacteria-yeast interactions.

- 7 Lacosegliaz MJ, Torres MA, Leguina AC. del V, Bertani I, Venturi V, de Figueroa LIC, Fernández PM, Nieto-Peñalver CG. 2021. Copper sulfate inhibition of quorum sensing in *Pseudomonas capeferrum* is dependent on biotic interactions. *Rhizosphere* 20:100434 <https://doi.org/10.1016/j.rhisph.2021.100434>

Quorum Sensing (QS) signaling, which allows the coordination of the microbial physiology, can be influenced by environmental factors. However, the impact of copper-based fungicides on the QS of rhizosphere microorganisms is currently unknown. The simultaneous presence of other microorganisms may also alter the QS signaling. In this work we report that the fungicide CuSO_4 modifies the expression from the promoter of *ppuI*, *ppoR* and *rsaL*, components of *Pseudomonas capeferrum* WCS358 QS system. *ppuI*

and *ppoR* promoters showed a biphasic behavior reducing their activities as CuSO_4 concentrations increased up to 1 mmol l^{-1} , but with increased values at higher metal concentrations. *rsaL* promoter attained a minimal activity with 1 mmol l^{-1} , though higher concentrations did not modify its expression. The impact of CuSO_4 on biofilm formation by WCS358 was dependent on the bacterial QS activity. Dual biofilms with *Papiliotrema laurentii* YL2 yeast were also affected by the bacterial QS and the metal. CuSO_4

was detrimental for the bacterial colonization, but YL2 exerted a protective effect in dual colonies, an effect that depended on the QS activity. These results suggest the importance of the WCS358 QS systems in the

rhizosphere, where copper-based fungicides and concomitant microorganisms conform a complex system.

III Centro Neotropical para la Investigación de la Biomasa, Colección de Levaduras Quito-Católica (CLQCA), Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Communicated by E.J. Carvajal Barriga <javiercarvajalbarriga@gmail.com>.

Recently published book chapter.

- 1 Carvajal Barriga EJ, Portero-Barahona P, Alexandra Narváez-Trujillo A. 2022. Yeast in a megadiverse country for sustainable futures. Chapter 12 in Kurtboke I, editor. Importance of Microbiology Teaching and Microbial Resource Management for Sustainable Futures. Elsevier <https://doi.org/10.1016/B978-0-12-818272-7.00001-8>

Chapter outline

- Introduction
- The significance of microbial collections in a megadiverse country like Ecuador
- Threats and opportunities of microbial collections in developing countries
- Case studies of sustainable technologies for biotechnology applications
- *Candida carvajalis*, the very first microorganism described in Ecuador
- Three yeasts with high potential for water decontamination
- Two Ecuadorian yeast species with probiotic potential in monogastric animals
- Antarctic yeast with potential for the biofuels industry
- From the Galapagos Islands to the pharmacy
- Novel yeast strains for fermented beverages and foods
- Raising the dead
- The yeast resuscitation method and ancient yeast applications
- Restoration of yeast cell compartment structures
- Controlled cell's hydration
- Metabolic activation
- Culture collection management
- Microorganisms culture collections system at PUCE
- Full description of the database and its importance for science
- Final remarks
- References
- Further reading

IV 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>.

Articles recently published.

- 1 Bermejo PM, Badino A, Zamberlan L, Raghavendran V, Basso TO, Gombert AK. 2021. Ethanol yield calculations in biorefineries. FEMS Yeast Res 21(8):foab065 – doi: 10.1093/femsyr/foab065.
- 2 Eliodório KP, de Gois E Cunha GC, White BA, Patel DHM, Zhang F, Hettema EH, Basso TO, Gombert AK, Raghavendran V. 2022. Blocking mitophagy does not significantly improve fuel ethanol production in bioethanol yeast *Saccharomyces cerevisiae*. Appl Environ Microbiol 88(5):e0206821 doi: 10.1128/aem.02068-21.

Pre-print published.

- 3 Carla Inês Soares Rodrigues, Bianca Eli Della-Bianca, Andreas K. Gombert et al. 2021. μ MAX of *Saccharomyces cerevisiae*: So often used, so seldom put into perspective PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-182823/v1>]

Ph.D. Thesis recently defended.

- 4 Pamela Magalí Bermejo. Towards an iSUCCELL *Saccharomyces cerevisiae* platform for fuel ethanol production, University of Campinas, Brazil, promotor Andreas K. Gombert

Book translated.

The book “The rise of yeast: how the sugar fungus shaped civilization”, written by Nicholas P. Money and published by Oxford University Press

(<https://global.oup.com/academic/product/the-rise-of-yeast-9780198749707?lang=en&cc=se>)

was translated into (Brazilian) Portuguese by Andreas K. Gombert and published by Editora Unicamp with the

title “A ascensão da levedura: como um simples fungo moldou nossa civilização”

(<https://www.editoraunicamp.com.br/produto/613/ascensao-da-levedura-a-como-um-simples-fungo-moldou-nossa-civilizacao>).

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

<https://doi.org/10.1101/2022.03.30.486421>

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

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

- 2 Steenwyk JL, Phillips MA, Yang F, Date SS, Graham TR, Berman J, Hittinger CT, Rokas A. 2022. An orthologous gene coevolution network provides insight into eukaryotic cellular and genomic structure and function. *Sci Adv* 8: eabn0105. <https://doi.org/10.1126/sciadv.abn0105>

The evolutionary rates of functionally related genes often covary. We present a gene coevolution network inferred from examining nearly 3 million orthologous gene pairs from 332 budding yeast species spanning ~400 million years of evolution. Network modules provide insight into cellular and genomic structure and function. Examination of the phenotypic impact of network perturbation using deletion mutant data from the baker's yeast *Saccharomyces cerevisiae*, which were obtained from previously published studies, suggests that fitness in diverse environments is

affected by orthologous gene neighborhood and connectivity. Mapping the network onto the chromosomes of *S. cerevisiae* and *Candida albicans* revealed that coevolving orthologous genes are not physically clustered in either species; rather, they are often located on different chromosomes or far apart on the same chromosome. The coevolution network captures the hierarchy of cellular structure and function, provides a roadmap for genotype-to-phenotype discovery, and portrays the genome as a linked ensemble of genes.

- 3 Gambacorta F, Wagner ER, Jacobson TB, Tremaine T, Muehlbauere LK, McGee MA, Baerwald JJ, Wrobel RL, Wolters JF, Place M, Dietrich JJ, Xie D, Serate J, Gajbhiye S, Liu L, Vang-Smith M, Coon JJ, Zhang Y, Gasch AP, Amador-Noguez D, Hittinger CT, Sato TK, Pflieger BF. 2022. Comparative functional genomics identifies an iron-limited bottleneck in a *Saccharomyces cerevisiae* strain with a cytosolic-localized isobutanol pathway. *Synth Syst Biotechnol* 7:738-749 <https://doi.org/10.1016/j.synbio.2022.02.007>

Metabolic engineering strategies have been successfully implemented to improve the production of isobutanol, a next-generation biofuel, in *Saccharomyces cerevisiae*. Here, we explore how two of these strategies, pathway re-localization and redox cofactor-balancing, affect the performance and physiology of isobutanol producing strains. We equipped yeast with isobutanol cassettes which had either a mitochondrial or cytosolic localized isobutanol pathway and used either a redox-imbalanced (NADPH-dependent) or redox-balanced (NADH-dependent) ketol-acid reductoisomerase enzyme. We then conducted transcriptomic, proteomic and metabolomic analyses to elucidate molecular differences between the engineered strains. Pathway localization had a large effect on isobutanol production with the strain expressing the mitochondrial-localized enzymes producing 3.8-fold more isobutanol than strains expressing the cytosolic

enzymes. Cofactor-balancing did not improve isobutanol titers and instead the strain with the redox-imbalanced pathway produced 1.5-fold more isobutanol than the balanced version, albeit at low overall pathway flux. Functional genomic analyses suggested that the poor performances of the cytosolic pathway strains were in part due to a shortage in cytosolic Fe-S clusters, which are required cofactors for the dihydroxyacid dehydratase enzyme. We then demonstrated that this cofactor limitation may be partially recovered by disrupting iron homeostasis with a *fra2* mutation, thereby increasing cellular iron levels. The resulting isobutanol titer of the *fra2* null strain harboring a cytosolic-localized isobutanol pathway outperformed the strain with the mitochondrial-localized pathway by 1.3-fold, demonstrating that both localizations can support flux to isobutanol.

- 4 Mozzachiodi S, Bai FY, Baldrian P, Bell G, Boundy-Mills K, Buzzini P, Čadež N, Cubillos FA, Dashko S, Dimitrov R, Fisher KJ, Gibson B, Gouliamova D, Greig D, Heisting L, Hittinger CT, Jecmenica M, Koufopanou V, Landry CR, Mašínová T, Naumova ES, Opulente D, Peña JJ, Petrovič U, Tsai IJ, Turchetti B, Villarreal P, Yurkov A, Liti G, Boynton P. 2022. Yeasts from temperate forests. *Yeast* 39:4-24 – <https://doi.org/10.1002/yea.3699>

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

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

- 5 Spurley WJ, Fisher KJ, Langdon QK, Buh KV, Jarzyna M, Haase MAB, Sylvester K, Moriarty RV, Rodriguez D, Sheddan A, Wright S, Sorlie L, Hulfachor AB, Opulente DA, Hittinger CT. 2022. Substrate, temperature, and geographical patterns among nearly 2,000 natural yeast isolates. *Yeast* 39:55-68 - <https://doi.org/10.1002/yea.3679>

Yeasts have broad importance as industrially and clinically relevant microbes and as powerful models for fundamental research, but we are only beginning to understand the roles yeasts play in natural ecosystems. Yeast ecology is often more difficult to study compared to other, more abundant microbes, but

growing collections of natural yeast isolates are beginning to shed light on fundamental ecological questions. Here we used environmental sampling and isolation to assemble a dataset of 1,962 isolates collected from throughout the contiguous United States of America (USA) and Alaska, which were then

used to uncover geographic patterns, along with substrate and temperature associations among yeast taxa. We found some taxa, including the common yeasts *Torulaspora delbrueckii* and *Saccharomyces paradoxus*, to be repeatedly isolated from multiple sampled regions of the US, and we classify these as broadly distributed cosmopolitan yeasts. A number of yeast taxon - substrate associations were identified,

some of which were novel and some of which support previously reported associations. Further, we found a strong effect of isolation temperature on the phyla of yeasts recovered, as well as for many species. We speculate that substrate and isolation temperature associations reflect the ecological diversity of and niche partitioning by yeast taxa.

- 6 Harrison MC, LaBella AL, Hittinger CT, Rokas A. 2022. The evolution of the *GAL*actose utilization pathway in budding yeasts. *Trends Genet* 38:97-106 – <https://doi.org/10.1016/j.tig.2021.08.013>

The Leloir galactose utilization or *GAL* pathway of budding yeasts, including that of the baker's yeast *Saccharomyces cerevisiae* and the opportunistic human pathogen *Candida albicans*, breaks down the sugar galactose for energy and biomass production. The *GAL* pathway has long served as a model system for understanding how eukaryotic metabolic pathways, including their modes of regulation, evolve. More

recently, the physical linkage of the structural genes *GAL1*, *GAL7*, and *GAL10* in diverse budding yeast genomes has been used as a model for understanding the evolution of gene clustering. In this review, we summarize exciting recent work on three different aspects of this iconic pathway's evolution: gene cluster organization, *GAL* gene regulation, and the population genetics of the *GAL* pathway.

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

The following papers have been published.

- 1 Dygas D, Janicka P, Berłowska J, Kregiel D. 2022. Conventional and unconventional yeasts able to grow on rapeseed meal hydrolysates. *BioResources* 17(2):3082-3094
DOI: 10.15376/biores.17.2.3082-3094

Yeast strains and appropriate hydrolysis conditions were selected for efficient single cell protein (SCP) production from waste rapeseed meal. High potential for effective biomass production was observed for *Yarrowia lipolytica* LOCK0264 with 10 g of rapeseed meal at $4.9 \times 10^8 \pm 1.5 \times 10^8$ (2.91

logarithmic units). The highest yeast multiplication rate was obtained for *Metschnikowia pulcherrima* NCYC747 with 12.5 g of rapeseed meal and with 15 g of rapeseed meal at $1.6 \times 10^8 \pm 3.8 \times 10^7$ and $4.5 \times 10^8 \pm 4.0 \times 10^7$ (2.75 and 2.86 logarithmic units, respectively).

- 2 Kregiel D, Nowacka M, Rygała A, Vadkertiova R. 2022. Biological activity of pulcherrimin from the *Metschnikowia pulcherrima* clade. *Molecules* 27:1855 – DOI: 10.3390/molecules27061855

Pulcherrimin is a secondary metabolite of yeasts belonging to the *Metschnikowia pulcherrima* clade, and pulcherrimin formation is responsible for the antimicrobial action of its producers. Understanding the environmental function of this metabolite can provide insight into various microbial interactions and enables the efficient development of new effective bioproducts and methods. In this study, we evaluated the antimicrobial and antiadhesive action of yeast pulcherrimin, as well as its protective properties under

selected stressful conditions. Classical microbiological plate methods, microscopy, and physico-chemical testing were used. The results show that pure pulcherrimin does not have antimicrobial properties, but its unique hydrophilic nature may hinder the adhesion of hydrophilic bacterial cells to abiotic surfaces. Pulcherrimin also proved to be a good cell protectant against UV-C radiation at both high and low temperatures.

- 3 Dygas D, Nowak Sz, Olszewska J, Szymanska M, Mroczynska Florczak M, Berłowska J, Dziugan P, Kregiel D. 2021. Ability of yeast metabolic activity to reduce sugars and stabilize betalains in red beet juice. *Fermentation* 7:105. DOI: 10.3390/fermentation7030105

To lower the risk of obesity, diabetes, and other related diseases, the WHO recommends that consumers reduce their consumption of sugars. Here, we propose a microbiological method to reduce the sugar content in red beet juice, while incurring only slight losses in the betalain content and maintaining the correct proportion of the other beet juice components. Several yeast strains with different metabolic activities were investigated for their ability

to reduce the sugar content in red beet juice, which resulted in a decrease in the extract level corresponding to sugar content from 49.7% to 58.2%. This strategy was found to have the additional advantage of increasing the chemical and microbial stability of the red beet juice. Only slight losses of betalain pigments were noted, to final concentrations of 5.11 % w/v and 2.56 % w/v for the red and yellow fractions, respectively.

VII State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), NRC “Kurchatov Institute”, I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by E.S. Naumova <lenna_naumova@yahoo.com>.

The following are papers for 2021 and 2022 or in press.

- 1 Naumova ES, Lee Ch-Fu, Naumov GI. 2021. Molecular genetic polymorphism of the yeast *Kluyveromyces dobzhanskii*. *Microbiology (Moscow)* 90(4):500–506.
- 2 Mozzachiodi S, Bai FY, Baldrian P, Bell G, Boundy-Mills K, Buzzini P, Čadež N, Cubillos FA, Dashko S, Dimitrov R, Fisher KJ, Gibson B, Gouliamova D, Greig D, Heisteringer L, Hittinger CT, Jecmenica M, Koufopanou V, Landry CR, Mašínová T, Naumova ES, Opulente D, Peña JJ, Petrovič U, Tsai IJ, Turchetti B, Villarreal P, Yurkov A, Liti G, Boynton P. 2022. Yeasts from temperate forests. *Yeast* 39 (1-2): 4–24.

See abstract under Dr. Hittinger’s entry.

- 3 Lyutova LV, Naumova ES. 2022. Inter-strain hybridization of *Kluyveromyces lactis* yeast for creating efficient lactose-fermenting strains. *Appl Biochem Microbiol* 58(8) (in press).

A molecular genetic study of the *Kluyveromyces lactis* yeasts isolated from various dairy products in the countries of the former Soviet Union and other regions of the world has been carried out. Based on physiological tests, four strains were selected that carry different *LAC* loci and are characterized by good fermentation intensity: VKM Y-1339 (*LAC3*), VKM Y-1333 (*LAC3*), NRRL Y-1118 (*LAC1*), and NRRL Y-1140 (*LAC2*). Eleven hybrids of the selected strains with different rates of lactose fermentation were obtained. No correlation was found between the intensity of lactose fermentation and the amino acid

sequences of the lactose permease *LAC12* gene of the *LAC1*, *LAC2*, and *LAC3* loci. Apparently, a specific combination of genotypes of crossed strains has a more significant effect on the fermentation activity. The results obtained showed that inter-strain hybridization of *K. lactis* dairy yeast is an effective method for creating new strains with high fermentation capacity. Hybrids H2-3 (NRRL Y-1118 × VKM Y-1333) and H3-3 (NRRL Y-1140 × VKM Y-1333) with the highest ability to ferment lactose are of interest for further molecular genetic research and breeding programs.

- 4 Lyutova LV, Naumov GI, Shnyreva AV, Naumova ES. 2022. Intraspecific polymorphism of the yeast *Kluyveromyces lactis*: genetic populations. *Microbiology (Moscow)* 91 (4) (in press).

According to the modern classification of yeasts, the species *Kluyveromyces lactis* includes two taxonomic varieties: cultural dairy yeast *K. lactis* var. *lactis* and nonlactose-fermenting environmental strains of *K. lactis* var. *drosophilarum*. This subdivision of the species, which is based only on phenotypic and ecological criteria, is formal and does not reflect the existing heterogeneity of *K. lactis*. Using various molecular methods and genetic hybridization analysis, we studied the genetic relationship of 35 *K. lactis* strains

isolated from dairy products and environmental sources in different regions of the world. The lactose-fermenting yeasts *K. lactis*, including dairy strains, clinical and soil isolates, had identical molecular karyotypes, did not differ in the nucleotide sequences of a number of molecular markers, and formed fertile hybrids with 84–99% ascospore viability. On the other hand, nonlactose-fermenting yeasts split into three genetically isolated populations: “krassilnikovii,” “drosophilarum,” and “phaseolosporus,” which differed in molecular

karyotypes, had unique SNP substitutions in the *ACT1* gene, and formed semi-sterile hybrids: 6–34% ascospore viability. Despite profound chromosome length polymorphism, *K. lactis* var. *lactis*, “krassilnikovii,” “drosophilarum,” and “phaseolosporus” probably have the same haploid number of chromosomes equal to six. The largest range of chromosomal sizes was observed in “krassilnikovii” (1000–2900 kb), and the smallest in “drosophilarum” (1600–2300 kb). The biogeography of

the yeast *K. lactis* is noteworthy. Lactose-fermenting strains of *K. lactis* var. *lactis* have been isolated in various regions of the world. Genetic “drosophilarum” and “phaseolosporus” populations are typical of North America, while “krassilnikovii” is represented by European and Central Asian isolates. Comparative analysis of the *ACT1* sequences should be applied for reliable delineation of all four genetic populations of *K. lactis*.

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

Margaretta Christita successfully defended her PhD thesis. We wish to express our thanks to Marc-André Lachance who acted as an external thesis examiner. Dr. Christita has returned to her home country of Indonesia, where she works at the Applied Microbiology Research Center, National Research and Innovation Agency, Cibinong, Indonesia. Publications below (Thesis, two published papers, and a pre-print) are work associated with her PhD on *Taphrina* species.

1 Margaretta Christita. PhD Thesis, defended 04 Feb 2022. *Taphrina* as model phytopathogenic yeasts infecting the model plant *Arabidopsis* and woody plant *Betula pendula*. Helsinki University, Dissertationes Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae <http://urn.fi/URN:ISBN:978-951-51-7824-4>

2 Christita M, and Overmyer K. 2021. Genetic resistance and tumour morphology in birch infected with *Taphrina betulina*. *Forest Pathology* 51(4): e12709. <https://doi.org/10.1111/efp.12709>

Witches’ broom of birch (*Betula* spp.) caused by *Taphrina betulina* is an understudied disease that causes the formation of woody tumours, from which ectopic axillary buds and branches grow to form a broom-like structure. We have addressed two aspects of this disease using naturally infected mature trees in the field. Broom symptoms offer a convenient means of scoring susceptibility in the field. Variation in broom symptom presentation suggests possible variation in resistance against witches’ broom disease. We tracked the local distribution of susceptible individuals among 721 trees at 159 independent sites. The analysis supports the hypothesis that there was genetic resistance segregating in these birch populations. Anatomical changes in

broom symptom bearing branches of European silver birch (*Betula pendula*) were also addressed by comparing sections of tissues from three locations in the same branch, which were normal, swollen in infected tissue adjacent to a tumour, and inside a tumour. Examination of tumours revealed disorganized and swollen xylem, expanded secondary phloem and expanded periderm. Swollen tissues newly infected from spreading disease adjacent to tumours exhibited enhanced growth only in secondary phloem and the periderm, which also exhibited distortions. This finding suggests that tumour formation and possibly pathogen colonization may initiate in these tissues.

3 Christita M, Sipilä TP, and Overmyer K. 2022. Distinct *Taphrina* strains from the phyllosphere of birch exhibiting a range of witches’ broom disease symptoms. *Environ Microbiol* in press. <https://doi.org/10.1111/1462-2920.16037>.

The phyllosphere is an important microbial habitat and reservoir of organisms that modify plant health. *Taphrina betulina* is the causal agent of birch witches’ broom disease. *Taphrina* species are dimorphic, infecting hosts in the filamentous form and residing in the host phyllosphere as non-infectious yeast. As such, they are expected to be found as resident yeasts on their hosts, even on healthy tissues; however, there is little

experimental data supporting this supposition. With the aim of exploring the local infection ecology of *T. betulina*, we isolated yeasts from the phyllosphere of birch leaves, using three sample classes; infected leaves inside symptom-bearing branches, healthy leaves from symptom-free branches on symptom-bearing trees, and leaves from symptom-free branches on symptom-free trees. Isolations yielded 224 yeast strains, representing

11 taxa, including *T. betulina*, which was the most common isolate and was found in all sample classes, including symptom-free samples. Genotyping revealed genetic diversity among these *T. betulina* isolates, with seven distinct genotypes differentiated by the markers

used. Twenty-two representative *T. betulina* strains were selected for further study, revealing further phenotypic differences. These findings support that *T. betulina* is ubiquitous on birch and that individual trees host a diversity of *T. betulina* strains.

- 4 Christita M, Auzane A, Wang K, Sipilä TP, Auvinen P, Paulin L, Salojärvi J, and Overmyer K. 2022. A *Taphrina* strain infecting the model plant *Arabidopsis thaliana*. PRE-PRINT BioRxiv, 460675 doi: <https://doi.org/10.1101/2021.09.16.460675>

Yeasts are important plant-associated organisms that can modulate host immunity to either promote or prevent disease. Mechanisms of plant-yeast interactions, specifically of yeast perception by the plant innate immune system, remain unknown. Progress has been hindered by the scarcity of yeast species associated with the model plant *Arabidopsis thaliana* (*Arabidopsis*). We have previously isolated *Taphrina* strain M11 from wild *Arabidopsis* in the field. *Taphrina* are poorly studied dimorphic yeast-like fungi that are plant pathogens, often producing plant hormones and causing tumour-like and leaf deformation symptoms on their hosts. Here we characterize the interaction of M11 with *Arabidopsis*. Infection of *Arabidopsis* with the birch pathogen

T. betulina, used as a non-host control, shows early HR, enhanced ROS accumulation, and limitation of growth, demonstrating that *Arabidopsis* had immunity against non-adapted yeasts. M11 triggered limited cell death, an attenuated ROS response, and grew *in planta*, as well as subtle but clear leaf deformation symptoms, demonstrating it is pathogenic. Hormone responsive promoter-reporter analysis demonstrated activation of cytokinin signalling during infection. Mutant infection assays indicate jasmonate and ethylene were required for immunity against M11. Analysis of the *Taphrina* M11 genome was used to mine evidence for yeast specific PAMPs, which may underlie host immune responses against yeast-like fungi.

IX Section for Genetics and Evolutionary Biology, Department of Biosciences, University of Oslo, Oslo, Norway and Department of Health, Valencian International University, C/ Pintor Sorolla, 21, 46002 Valencia, Spain. Communicated by David Peris Navarro <david.perisnavarro@gmail.com>.

New papers.

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

See abstract under Dr. Hittinger's entry.

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

Recent publications.

- 1 Csoma H, Kállai Z, Antunovics Z, Czentye K, Sipiczki M. 2021. Vinification without *Saccharomyces*: Interacting osmotolerant and “spoilage” yeast communities in fermenting and ageing botrytised high-sugar wines (Tokaj Essence). *Microorganisms* 9:19 – doi: 10.3390/microorganisms9010019.
- 2 Nemcová A, Gonová D, Samek O, Sipiczki M, Breierová E, Márová I. 2021. The use of Raman spectroscopy to monitor metabolic changes in stressed *Metschnikowia* sp. yeasts. *Microorganisms* 9:277 – doi: 10.3390/microorganisms9020277.
- 3 Nemcová A, Sztokowski M, Samek O, Cagánová L, Sipiczki M, Márová I. 2021. Use of waste substrates for the lipid production by yeasts of the genus *Metschnikowia* – Screening study. *Microorganisms* 9:2295 – DOI: 10.3390/microorganisms9112295

- 4 Horvah E, Dalyai L, Szabo E, Barna T, Kalmar L, Posta J, Sipiczki M, Csoma H, Miklos I. 2021. The antagonistic *Metschnikowia andauensis* produces extracellular enzymes and pulcherrimin, whose production can be promoted by the culture factors. *Sci Rep* 11:10593
doi: 10.1038/s41598-021-89982-y.
- 5 Yurkov A, Alves A, Bai FY, Boundy-Mills K, Buzzini P, Čadež N, Cardinali G, Casaregola S, Chaturvedi V, Collin V, Fell JW, Girard V, Groenewald M, Hagen F, Hittinger CT, Kachalkin AV, Kostrzewa M, Kouvelis V, Libkind D, Liu X, Maier T, Meyer W, Peter G, Piątek M, Robert V, Rosa CA, Sampaio JP, Sipiczki M, Stadler M, Sugita T, Sugiyama J, Takagi H, Takashima M, Turchetti B, Wang QM, Boekhout T. Nomenclatural issues concerning cultured yeasts and other fungi: why it is important to avoid unneeded name changes. *IMA Fungus*. 12:18 – doi: 10.1186/s43008-021-00067-x. PMID: 34256869.
- 6 Acs-Szabo L, Papp LA, Sipiczki M, Miklos I. 2021. Genome comparisons of the fission yeasts reveal ancient collinear loci maintained by natural selection. *J Fungi (Basel)* 7:864 – doi: 10.3390/jof7100864.
- 7 Pesti, B, Nagy, Z, Papp, L.A, Sipiczki, M, Sveiczler, A. 2021. Cell length growth in the fission yeast cell cycle: Is it (bi)linear or (bi)exponential? *Processes* 9:1533 – <https://doi.org/10.3390/pr9091533>
- 8 Sipiczki M. 2022. Wine yeasts 1.0. *Microorganisms*, 10:26.
<https://doi.org/10.3390/microorganisms10010026>
- 9 Sipiczki M. 2022. When barcoding fails: Genome chimerization (admiring) and reticulation obscure phylogenetic and taxonomic relationships. *Mol Ecol Resources*, 00:1–24
doi: 10.1111/1755-0998.13586.
- 10 Sipiczki M. 2022. Taxonomic revision of the *pulcherrima* clade of *Metschnikowia* (*Fungi*): Merger of species. *Taxonomy* 2:107-123 - <https://doi.org/10.3390/taxonomy2010009>

The type strains of 10 small-spored species of the ascomycetous yeast genus *Metschnikowia* usually form a compact group on the phylogenetic trees inferred from barcode sequences. Based on the name of the species, which was described first (*Metschnikowia pulcherrima*), the group is frequently referred to as the pulcherrima clade. All strains produce the iron-chelate pigment pulcherrimin and have antagonistic effects on many microorganisms. Recent results of molecular phylogenetic, genetic, and genomic research raised

doubts about the taxonomic division of the clade. Those data—combined with results obtained in this study by comprehensive analysis of primary and secondary barcode sequences, physiological tests, and hybridisation experiments—demonstrate that the species cannot be distinguished from each other by the criteria of any of the phenotypic, phylogenetic, and biological species concepts. Therefore, I propose that the species of the pulcherrima clade be merged into one species under the oldest species name, *M. pulcherrima*.

XI Department of Microbiology, Sikkim University, Gangtok 737102, Sikkim, India. Communicated by Jyoti Prakash Tamang <jyoti_tamang@hotmail.com>.

Recent publication.

- 1 Tamang JP, Lama S. 2022. Probiotic properties of yeasts in traditional fermented foods and beverages. *J Appl Microbiol* – <https://doi.org/10.1111/jam.15467>.

The interest in potentiality and functionality of probiotic yeasts from fermented foods has increased drastically over the years. In many fermented foods and beverages, lactic acid bacteria and yeasts exist synergistically by stimulating their growth and survival. Probiotic strains of lactic acid bacteria are

widely studied than potential probiotic yeasts. *Saccharomyces cerevisiae* variety *boulardii* is the only commercialized probiotic yeast, which is extensively studied. This review article provides information on the presence of potential probiotic yeasts in some traditional fermented foods and beverages.

Recent publication.

- 1 Stojiljković M, Claes A, Deparis Q, Demeke MM, Subotić A, Foulquié-Moreno MR, Thevelein JM. 2022. Whole-genome transformation of yeast promotes rare host mutations with a single causative SNP enhancing acetic acid tolerance. *Mol Cel Biol* e00560-21 – <https://doi.org/10.1128/mcb.00560-21>



Whole-genome (WG) transformation (WGT) with DNA from the same or another species has been used to obtain strains with superior traits. Very few examples have been reported in eukaryotes—most apparently involving integration of large fragments of foreign DNA into the host genome. We show that WGT of a haploid acetic acid-sensitive *Saccharomyces cerevisiae* strain with DNA from a tolerant strain, but not from nontolerant strains, generated many tolerant transformants, some of which were stable upon subculturing under nonselective conditions. The most tolerant stable transformant contained no foreign DNA but only seven nonsynonymous single nucleotide polymorphisms (SNPs), of which none was present in the donor genome. The *SNF4* mutation c.[805G→T], generating *Snf4E269**, was the main causative SNP. Allele exchange of *SNF4E269** or *snf4Δ* in industrial strains with unrelated genetic backgrounds enhanced

acetic acid tolerance during fermentation under industrially relevant conditions. Our work reveals a surprisingly small number of mutations introduced by WGT, which do not bear any sequence relatedness to the genomic DNA (gDNA) of the donor organism, including the causative mutation. Spontaneous mutagenesis under protection of a transient donor gDNA fragment, maintained as extrachromosomal circular DNA (eccDNA), might provide an explanation. Support for this mechanism was obtained by transformation with genomic DNA of a yeast strain containing *NatMX* and selection on medium with nourseothricin. Seven transformants were obtained that gradually lost their nourseothricin resistance upon subculturing in nonselective medium. Our work shows that WGT is an efficient strategy for rapidly generating and identifying superior alleles capable of improving selectable traits of interest in industrial yeast strains.

Recent peer reviewed publications.

- 1 Reid SJ, Josey M, MacIntosh AJ, Maskell D, Speers RA. 2021. Predicting fermentation rates in ale, lager and whisky. *Fermentation*, Special Issue Brewing & Distilling 2.0, 7:13
<https://doi.org/10.3390/fermentation 7010013>
- 2 Speers RA, Mishra A. 2020. Wort boil time and trub effects on fermentability. *JASBC* 79:46-52. (In top 10 most popular papers of 2021 – ASBC Buzz Jan. 2022)
- 3 Huismann M, Gormley F, Dzait D, Stewart K, Speers RA, Maskell DL. 2021. Unfilterable beer haze – Part II: Identifying suspect cell wall proteins. *JASBC*. Online
<https://doi.org/10.1080/03610470.2021.1937461>
- 4 Huismann M, Gormley F, Dzait D, Speers RA, Maskell DL. 2021. Part I: Unfilterable beer haze – Part I: The investigation of an India pale ale haze. *JASBC*. Online
<https://doi.org/10.1080/03610470.2021.1937460>
- 5 Reid SJ, Speers RA, Lumsden WB, Maskell DL. 2020. Pre-fermentation of malt whisky wort using *Lactobacillus plantarum* and its influence on new-make spirit character. *Food Chem* 320:1-9.

Presentations.

- 6 Speers RA, Killfoil G. 2022. PYF in the brewery. Craft Malt Conference. North American Craft Maltsters Guild. Invited Presentation. Online. Portland, ME, Feb. 18-19.
- 7 Reid SJ, Speers RA, Lumsden WB, Maskell DL. 2022. Scotch malt whisky new make spirit quality: The application of mathematical models. Submitted to the Young Scientist Symposium Cambridge, ENG. Sept. 4-7.
- 8 Speers RA, Killfoil G, Li Y, Onio A, Watts P. 2021. Virtual presentation – Further development of premature yeast methodology. Presented at the MBAA Conference, Cleveland, OH, Thursday, October 28 - Saturday, October 30, 2021 : Nov. 3-5.
- 9 Reid SJ, Lumsden WB, Speers RA, Maskell DL. 2021. The application of high-gravity fermentations for Scotch malt whisky production. Poster 85. Presented at the Worldwide Distilled Spirits Virtual Conference. Edinburgh, SCT. Sept. 6-8.
- 10 Reid SJ, Speers RA, Lumsden WB, Maskell DL. 2021. Modelling the Effects of Yeast Format and Pitching Rate for Scotch Malt Whisky Fermentations. Presented at the Worldwide Distilled Spirits Conference. Edinburgh, SCT. Sept. 6-8.
- 11 Speers RA. 2021. Bru-Lab Episode 025 | Boil length and trub level effects on fermentability. <https://www.stitcher.com/show/the-bru-lab/episode/episode-025-boil-length-and-trub-levels-w-dr-alex-speers-86020003>. (@7:20 min, total length 66 min).
- 12 Killfoil G, Roy L, Kishnani P, Speers RA. 2021. Brewing with craft floor malt: A pilot study. Presentation to CFI-ICA, Virtual meeting. Feb. 2021.
- 13 Speers RA, Reid SJ, Maskell DL. 2020. Characterising fermentation behaviour. World Brewing Congress, Presented. St. Paul, MN. Sept. 2020.
- 14 Reid SJ, Speers RA, Lumsden WB, Maskell DL. 2020. Novel yeast strains in Scotch malt whisky fermentations and impact on new-make spirit character. World Brewing Congress, Presented. St. Paul, MN. Sept. 2020.
- 15 Speers RA. 2020. Fermentation & flocculation: practical and physical Aspects. Invited lecture to UC Davis Brewing Class, Online. May 14. 2020.
- 16 Reid SJ, Speers RA, Lumsden WB, Maskell DL. 2019. Modelling Scotch whisky fermentations. MBAA Conf. Calgary, AB. Oct. 31-Nov. 2.

Comments on *Saccharomyces uvarum*
Nguyen Huu-Vang, France <vangng08@yahoo.fr>

A new genome of *Saccharomyces uvarum* CBS 7001 is available. It was submitted 24-AUG-2021 by the Department of Genetics, University of Georgia, 120 E. Green St, Athens, GA 30602, USA, and published by:

Chen J, Garfinkel DJ, Bergman CM. 2022. long-read genome assembly of *Saccharomyces uvarum* strain CBS 7001. Microbiol Resour Announc. 11(1):e0097221 – doi: 10.1128/mra.00972-21.

“Here, we report a long-read genome assembly for *Saccharomyces uvarum* strain CBS 7001 based on PacBio whole-genome shotgun sequence data. Our assembly provides an improved reference genome for

an important yeast in the *Saccharomyces sensu stricto* clade.”

Important points mentioned by the authors:

“*Saccharomyces uvarum* is a globally distributed yeast species that can be commonly extracted from low-temperature fermentations and other natural substrates (1–6). Low-coverage Sanger sequence assemblies were initially reported for an *S. uvarum* reference strain called CBS 7001 (also called MCYC 623) (7, 8). Later, Scannell et al. (9) generated a chromosome-level scaffolded assembly for CBS 7001 that combined Sanger and Illumina short-read sequences. All previous CBS 7001 assemblies contain

a large number of gaps (Table 1), limiting the comprehensive analysis of transposable elements (TEs) in this species (9, 10). Here, we generated a long-read genome assembly for strain CBS 7001 to improve the reference genome and support analysis of TEs in *S. uvarum*.

Our CBS 7001 assembly is 11,968,998 bp long (contig N50, 836,618 bp) with an overall GC content of 40.18% (Table 1). Of the chromosomes, 15/16 are assembled in scaffolds with one contig each; ChrXII is assembled in one scaffold with one gap at the rDNA locus. Data availability. The assembly produced here was deposited at NCBI under accession numbers CM034478 to CM034493. The PacBio data used to generate the assembly are available under SRA accession number PRJNA753102.”

Remark for genomists:

Sequences from the new genome of *S. uvarum* strain CBS 7001 can be retrieved by BlastN at NCBI database: Whole-genome shotgun contigs (wgs); Limit by Organism: *Saccharomyces uvarum* (taxid 230603). (One example of BlastN result: *Saccharomyces uvarum* strain CBS 7001 000013F_arrow, whole genome shotgun sequence. Sequence ID: JAIPTR010000014.1 Length: 482075 Number of Matches: 1).

Comments:

Until now researchers used three genomes of the same *S. uvarum* strain but deposited in two different databases, SGD and Wisconsin University, under different names or strain numbering. At SGD

(<https://www.yeastgenome.org/blast-fungal>)

the *S. uvarum* genome was labelled *Saccharomyces bayanus* for two strains: (1) MCYC 623 (Acc. AACAA01000043) as mentioned in the article above (Chen J, Garfinkel DJ, Bergman CM.2022) and (2) 623-6C (Acc. AACG02000000). MCYC 623 had been deposited by Santa-Maria at the CBS collection as CBS 7001. Users may think that strain 623-6C is another strain different from MCYC623 but in fact 623-6C is a “personal number” of a spore clone of strain MCYC 623, whose correct number was MCYC623-6c based on Table 1 of the article below.

Louis EJ, Naumova ES, Lee A, Naumov G, Haber JE. 1994. The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. *Genetics* 136(3):789-802 – doi: 10.1093/genetics/136.3.789

At Wisconsin University

(<http://sss.genetics.wisc.edu/cgi-bin/s3.cgi>)

the genome of *S. uvarum* CBS 7001 was re-sequenced by Scannel et al. (2011) and deposited under the name *S.bayanus* var. *uvarum*. Working with *S. uvarum* sequences from public databases, researchers, who are aware of the complexity of a situation in which two

names (*S. bayanus* as well as *S. uvarum*) was applied for one species, sometimes themselves change further the labelling of sequences retrieved as in the following example:

McCarthy GC, Morgan SC, Martiniuk JT, Newman BL, McCann SE, Measday V, Durall DM. 2021.

An indigenous *Saccharomyces uvarum* population with high genetic diversity dominates uninoculated Chardonnay fermentations at a Canadian winery. *PLoS One*. 16(2):e0225615

doi: 10.1371/journal.pone.0225615.

Important points:

“However, the history of *S. uvarum* research is difficult to trace, because *S. uvarum* has had many names, and has even shared names with now distinct species. In the past, *S. uvarum* has been referred to as *Saccharomyces bayanus* var. *uvarum* [13, 14, 21, 22] or simply *Saccharomyces bayanus* [23, 24]. To complicate matters, many commercial *S. cerevisiae* strains have been marketed incorrectly as strains of *S. bayanus* [25]. However, *S. uvarum* is now known to be a pure species, distinct from *S. bayanus*, which itself is a hybrid of the pure species *S. uvarum* and *S. eubayanus* [16, 19, 26, 27]. *S. uvarum* is a cryotolerant yeast usually found in association with white wine fermentations in cool-climate wine regions [12, 13, 15, 28], but has also been associated with cider production [23, 24] and some traditional fermentations [29, 30]. During fermentation, *S. uvarum* produces lower levels of ethanol, acetic acid, and acetaldehyde, and higher levels of glycerol, succinic acid, malic acid, isoamyl alcohol, isobutanol, and ethyl acetate, as compared to *S. cerevisiae* [31–34]. Additionally, because of its ability to conduct fermentation at lower temperatures, *S. uvarum* may produce wines with more balanced aroma profiles [35]. However, few studies have been conducted to investigate the origins, genetic diversity, and enological potential of this yeast, and thus more research is needed on this topic.”

Comments:

The authors mentioned “To complicate matters, many commercial *S. cerevisiae* strains have been marketed incorrectly as strains of *S. bayanus*”. Indeed producers of commercial yeast needed another name to qualify the their products, *S. cerevisiae* was so common and known as *Levure de Bière* (Brewing Yeast) and at the same time as *Levure de Boulangerie* (Baker’s yeast) so naming Gal- strains as *S. bayanus* make the Champagne and other wine yeasts in a different category of organism. Indeed galactose-negative is very easy to characterized but this is a mutable character cannot be used to define a species as mentioned in:

Vaughan-Martini A, Martini A. 1993. A taxonomic

key for the genus *Saccharomyces*. System Appl Microbiol 16:113-119.

Results of that study demonstrated that those physiological characteristics traditionally considered important for separation of the highly fermenting strains of *Saccharomyces* cannot be useful for species

distinction. For example, absence of galactose assimilation and fermentation, once considered paramount for the identification of *Sacch. bayanus*, was not a constant feature of that or of the other species of the group.

“Italian Group on Vine and Wine Microbiology”

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

The following are papers from the partners of GMV for 2021-2022

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

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

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

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

Nowadays, the interest toward products containing probiotics is growing due to their potential health benefits to the host and the research is focusing on search of new probiotic microorganisms. The present

work was focused on the characterization of indigenous *Saccharomyces cerevisiae* strains, isolated from different food matrixes, with the goal to select strains with probiotic or health-beneficial potential. A

preliminary screening performed on fifty *S. cerevisiae* indigenous strains, in comparison to a commercial probiotic strain, allowed to individuate the most suitable ones for potential probiotic aptitude. Fourteen selected strains were tested for survival ability in the gastrointestinal tract and finally, the strains characterized for the most important probiotic features were analyzed for health-beneficial traits, such as the content of glucan, antioxidant and potential anti-inflammatory activities. Three strains, 4LBI-3, LL-1,

TA4-10, showing better attributes compared to the commercial probiotic *S. cerevisiae* var. *bouardii* strain, were characterized by interesting health beneficial traits, such as high content of glucan, high antioxidant and potential anti-inflammatory activities. Our results suggest that some of the tested *S. cerevisiae* strains have potential as probiotics and candidate for different applications, such as dietary supplements, and starter for the production of functional foods or as probiotic to be used therapeutically.

- 2 Alberico, A, Capece, A, Mauriello, G, Pietrafesa, R, Siesto, G. 2021. Influence of microencapsulation on fermentative behavior of *Hanseniaspora osmophila* in wine mixed starter fermentation. *Fermentation*, , 7(3):112 – doi.org/10.3390/fermentation7030112

In recent years, as a consequence of the re-evaluation of the role of non *Saccharomyces* yeasts, several studies have been conducted on the use of controlled mixed fermentations with *Saccharomyces* and different non *Saccharomyces* yeast species from the winemaking environment. To benefit from the metabolic particularities of some non *Saccharomyces* yeasts, the management of a non *Saccharomyces* strain in mixed fermentation is a crucial step, in particular the use of procedures addressed to increase the persistence of non *Saccharomyces* strains during the fermentative process. The use of microencapsulation for cell immobilization might represent a strategy for enhancing the competitiveness of non *Saccharomyces* yeasts during mixed fermentation. This study was

aimed to assess the fermentative performance of a mixed starter culture, composed by a wild *Hanseniaspora osmophila* strain (ND1) and a commercial *Saccharomyces cerevisiae* strain (EC1118). For this purpose, free and microencapsulated cells of ND1 strain were tested in co culture with EC1118 during mixed fermentations in order to evaluate the effect of the microencapsulation on fermentative behavior of mixed starter and final wine composition. The data have shown that *H. osmophila* cell formulation affects the persistence of both ND1 and EC1118 strains during fermentations and microencapsulation resulted in a suitable system to increase the fermentative efficiency of ND1 strain during mixed starter fermentation.

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- 1 Cardinale M, Trinchera R, Natrella G, Difonzo G, De Benedittis C, D'amato I, Mascellani M, Paradiso V.M, Rustioni L. 2021. Dynamics of the fermentation process and chemical profiling of pomegranate (*Punica granatum* L.) wines obtained by different cultivar×yeast combinations. *Foods* 10(8):1913 <https://doi.org/10.3390/foods10081913>

Pomegranate (*Punica granatum* L.) is one of the historical tree crops in the Mediterranean region and is nowadays commercialized for its beneficial properties in the form of fruits, juice, jams and, in some East countries, as fermented juice (pomegranate wine). However, pomegranate wines are not established as a common beverage in Western countries. In this work, we produced pomegranate wines using two cultivars and two yeasts (*Saccharomyces cerevisiae* strain Clos and *S. cerevisiae* ex-bayanus strain EC1118) with contrasting characteristics. A comprehensive chemical profile of the

wines was obtained. Notable differences were observed in the function of the cultivars and the yeasts. Different cultivar×yeast combinations provided wines with clearly different chemical profiles and specific features in the patterns of organic acids, phenolics, and volatile compounds. This highlights the opportunity to obtain tailored pomegranate wines with desired chemical profiles and, consequently, sensory properties, through management optimization of pomegranate winemaking. In this view, pomegranate wines have the potential to become an established beverage in Western countries.

- 1 De Vero L, Iosca G, Gullo M, Pulvirenti A. 2021. Functional and healthy features of conventional and non-conventional sourdoughs. *Appl Sci* 11(8):3694 – doi.org/10.3390/app11083694

Sourdough is a composite ecosystem largely characterized by yeasts and lactic acid bacteria which are the main players in the fermentation process. The specific strains involved are influenced by several factors including the chemical and enzyme composition of the flour and the sourdough production technology. For many decades the scientific community has explored the microbiological, biochemical, technological and nutritional potential of sourdoughs. Traditionally, sourdoughs have been used to improve the organoleptic properties, texture, digestibility, palatability, and safety of bread and other kinds of baked products. Recently, novel sourdough-based biotechnological applications have

been proposed to meet the demand of consumers for healthier and more natural food and offer new inputs for the food industry. Many researchers have focused on the beneficial effects of specific enzymatic activities or compounds, such as exopolysaccharides, with both technological and functional roles. Additionally, many studies have explored the ability of sourdough lactic acid bacteria to produce antifungal compounds for use as bio-preservatives. This review provides an overview of the fundamental features of sourdoughs and their exploitation to develop high value-added products with beneficial microorganisms and/or their metabolites, which can positively impact human health.

- 2 De Vero L, Iosca G, La China S, Licciardello F, Gullo M, Pulvirenti A. 2021. Yeasts and lactic acid bacteria for panettone production: an assessment of candidate strains. *Microorganisms*, 9(5):1093 doi.org/10.3390/microorganisms9051093

The recovery of yeasts and lactic acid bacteria (LAB) involved in sourdough fermentation is the first step in the selection of starters with suitable technological aptitude and capable of producing desired aromas and/or aromatic precursors. In this work, two sourdoughs samples (MA and MB) and the derived doughs (samples A and B) were collected from a bakery during artisanal Panettone manufacture. Yeasts and bacteria were isolated at different fermentation steps on selective agar media. A total of 77 isolates were obtained and characterized. Representative strains of yeasts and LAB were identified by sequencing the D1/D2 domain of the 26S rRNA and the 16S rRNA

genes, respectively. Moreover, the volatile organic compounds (VOCs) produced in the collected samples were detected and correlated to the species found in the same samples. The results highlighted the occurrence of *Kazachstania humilis* in both samples A and B, while *Saccharomyces cerevisiae* strains were detected only in samples B. Among LAB, *Fructilactobacillus sanfranciscensis* was the main species detected in both sourdoughs. Furthermore, strains belonging to the species *Lactiplantibacillus plantarum*, *Furfurilactobacillus rossiae*, *Lactobacillus parabuchneri*, *Leuconostoc citreum*, and *Leuconostoc mesenteroides* were assessed in the dough samples.

- 1 Barone E, Ponticello G, Giaramida P, Squadrito M, Fasciana F, Gandolfo V, Ardizzone F, Monteleone M, Corona O, Francesca N, Oliva D. 2021. Use of *Kluyveromyces marxianus* to increase free monoterpenes and aliphatic esters in white wines. *Fermentation* 7:79 doi.org/10.3390/fermentation7020079

An increasing interest in novel wine productions is focused on non-*Saccharomyces* yeasts due to their potential in improving sensory profiles. Although *Kluyveromyces marxianus* has been originally isolated from grapes and its enzymatic activities are used in oenology, rarely it has been used as co-starter. The

K. marxianus Km L2009 strain has been characterized here and selected as a co-starter both at laboratory- and winery-scale fermentation. The Km L2009 strain showed growth of up to 40 (mg/L) of sulfites and 6% (v/v) of ethanol. Gas chromatographic analysis demonstrates that wines produced by mixed

fermentation contain remarkably higher quantities of free monoterpenes and aliphatic esters than wines produced only by commercial strains of *Saccharomyces cerevisiae*. Differences in the volatile organic compound composition produced sensorially

distinct wines. In light of these results, it is possible to state that even within the *K. marxianus* species it is possible to select strains capable of improving the aromatic quality of wines.

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

Wine yeasts can be natural hosts for dsRNA, ssRNA viruses and retrotransposon elements. In this study, high-throughput RNA sequencing combined with bioinformatic analyses unveiled the virome associated to 16 *Saccharomyces cerevisiae* and 8 non-*Saccharomyces* strains of oenological interest. Results showed the presence of six viruses and two satellite dsRNAs from four different families, two of which—Partitiviridae and Mitoviridae—were not reported before in yeasts, as well as two ORFan contigs of viral origin. According to phylogenetic analysis, four new putative mycoviruses distributed in Totivirus, Crispovirus, and Mitovirus genera were identified. The majority of commercial *S. cerevisiae* strains were confirmed to be the host for helper L-A

type totiviruses and satellite M dsRNAs associated with the killer phenotype, both in single and mixed infections with L-BC totiviruses, and two viral sequences belonging to a new crispovirus putative species discovered here for the first time. Moreover, single infection by a narnavirus 20S-related sequence was also found in one *S. cerevisiae* strain. Considering the non-*Saccharomyces* yeasts, *Starmerella bacillaris* hosted four RNAs of viral origin—two clustering in Totivirus and Mitovirus genera, and two ORFans with putative satellite behavior. This study confirmed the infection of wine yeasts by viruses associated with useful technological characteristics and demonstrated the presence of complex mixed infections with unpredictable biological effects.

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

High throughput sequencing allowed the discovery of many new viruses and viral organizations increasing our comprehension of virus origin and evolution. Most RNA viruses are currently characterized through similarity searches of annotated virus databases. This approach limits the possibility to detect completely new virus-encoded proteins with no detectable similarities to existing ones, i.e., ORFan proteins. A strong indication of the ORFan viral origin in a metatranscriptome is the lack of DNA corresponding to an assembled RNA sequence in the biological sample. Furthermore, sequence homology among ORFans and evidence of co-occurrence of these ORFans in specific host individuals, provides further indication of a viral origin. We use this theoretical framework to report the finding of three conserved clades of protein-coding

RNA segments without a corresponding DNA in fungi. Protein sequence and structural alignment suggest these proteins are distantly related to viral RNA dependent RNA polymerases (RdRP). In these new putative viral RdRP clades no GDD catalytic triad is present, but the most common putative catalytic triad is NDD, and a clade with GDQ, a triad previously unreported at that site. SDD, HDD ADD are also represented. For most members of these three clades, we were able to associate a second genomic segment, coding for a protein of unknown function. We provisionally named this new group of viruses ormycovirus. Interestingly, all the members of one of these sub-clades (gammaormycovirus) accumulate more minus sense RNA than plus sense RNA during infection.

- 1 Rossi S, Parrotta L, Gottardi D, Glicerina VT, Del Duca S, Dalla Rosa M, Patrignani F, Schlüter O, Lanciotti R. 2022. Unravelling the potential of cricket-based hydrolysed sourdough on the quality of an innovative bakery product. *J Insects Food Feed*, in press – doi:10.3920/JIFF2021.0184

The purpose of this research was to evaluate the potential of a sourdough containing cricket powder hydrolysate by the RO25 *Yarrowia lipolytica* strain to produce an innovative bakery product. RO25 hydrolysed cricket bread (RO25H-CB) was compared with control bread obtained from a traditional sourdough using wheat flour and with an additional bread control obtained from no-hydrolysed cricket powder sourdough. The results obtained showed that RO25H-CB had a highest amount of proteins and free fatty acids than wheat control bread, attributed to the well-known proteolytic and lipolytic activities of *Y. lipolytica*. Moreover, RO25H-CB sample was characterised by high content of health-promoting and

aroma precursors lipids as well as a lowest biogenic amine index among samples analysed, suggesting for this sample a high overall quality respect to no hydrolysed cricket powder bread. Finally, the data relating the sensory analysis highlighted good application opportunities for RO25 cricket hydrolysate as ingredients for baking. In fact, RO25H-CB had received positive evaluations for almost all the parameters considered. These results demonstrated that hydrolysates from *Y. lipolytica*, compared to the no hydrolysed cricket, were able to impart specific sensory and qualitative characteristics to the final product, with positive feedback from the involved panellists.

- 2 Gottardi D, Siroli L, Vannini L, Patrignani F, Lanciotti R. 2021. Recovery and valorization of agri-food wastes and by-products using the non-conventional yeast *Yarrowia lipolytica*. *Trends Food Sci Technol* 115:74–86 – doi.org/10.1016/j.tifs.2021.06.025

Yarrowia lipolytica is one of the most studied “non-conventional” yeasts. Due to its intrinsic biological characteristics and variability, the potential applications of wild type isolates in the agri-food sector are broad (production of biomasses, enzymes, and metabolites). By-products generated by the food industry are rising environmental and economic concerns worldwide. These side streams have different compositions and stability. However, they can still be applied as substrates for microbial growth. The fitness of wild type strains to adverse and different environments can be exploited not only for food production but also for recovery and valorization of agri-food wastes and by-products. This review brings together a selection of the most relevant and recent data about the physiology, nutritional requirements,

and metabolites produced by wild type isolates of *Y. lipolytica*. Moreover, the principal agri-food side streams, their specific productions and valorization using wild type strains have been critically discussed. Critical aspects of side streams and by-products can be solved (e.g. reduction of COD and pollutants before discharging), novel ingredients can be generated (e.g. lipases, single cell oils and citric acid) and, eventually, yeast biomasses can be produced for further applications (food adjuncts or supplements). Selecting and characterizing wild type isolates able to consume or convert or valorize the different waste/by-product components into added value products is extremely important in the view of a sustainable process and sustainable economy.

- 3 Sabaghian S, Braschi G, Vannini L, Patrignani F, Samsulrizal NH, Lanciotti R. 2021. Isolation and identification of wild yeast from Malaysian grapevine and evaluation of their potential antimicrobial activity against grapevine fungal pathogens. *Microorganisms* 9:2582 <https://doi.org/10.3390/microorganisms9122582>

Pathogenic fungi belonging to the genera *Botrytis*, *Phaeomoniella*, *Fusarium*, *Alternaria* and *Aspergillus* are responsible for vines diseases that affect the growth, grapevine yield and organoleptic quality. Among innovative strategies for in field plant

disease control, one of the most promising is represented by biocontrol agents, including wild epiphytic yeast strains of grapevine berries. Twenty wild yeasts, isolated and molecularly identified from three different Malaysian regions (Perlis, Perak and

Pahang), were evaluated in a preliminary screening test on agar to select isolates with inhibition against *Botrytis cinerea*. On the basis of the results, nine yeasts belonging to genera *Hanseniaspora*, *Starmerella*, *Metschnikowia*, *Candida* were selected and then tested against five grape berry pathogens: *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Fusarium oxysporum*, *Alternaria alternata* and *Phaeoconiella chlamydospora*. *Starmerella bacillaris* FE08.05 and *Metschnikowia pulcherrima* GP8 and *Hanseniaspora uvarum* GM19 showed the highest

effect on inhibiting mycelial growth, which ranged between 15.1 and 4.3 mm for the inhibition ring. The quantitative analysis of the volatile organic compound profiles highlighted the presence of isoamyl and phenylethyl alcohols and an overall higher presence of low chain fatty acids and volatile ethyl esters. The results of this study suggest that antagonist yeasts, potentially effective for the biological control of pathogenic moulds, can be found among the epiphytic microbiota associated with grape berries.

- 4 Rossi S, Parrotta L, Del Duca S, Dalla Rosa M, Patrignani F, Schluter O, Lanciotti R. 2021. Effect of *Yarrowia lipolytica* RO25 cricket-based hydrolysates on sourdough quality parameters. *LWT Food Sci Technol* 148:111760 – <https://doi.org/10.1016/j.lwt.2021.111760>

The principal aim of this research was to use *Yarrowia lipolytica* RO25 to obtain a cricket powder-based hydrolysate to produce sourdough for bread production. RO25 hydrolysed cricket sourdough (RO25H-CS) was compared with a control traditional sourdough and a control containing the no-hydrolysed cricket powder. Microbiological analyses evidenced a good growth of *Yarrowia lipolytica* in RO25 cricket hydrolysate and the RO25H-CS was characterized, in comparison with the controls, by a marked and peculiar total protein profile, attributed to the well-known proteolytic activities of *Yarrowia lipolytica*. RO25H-CS was also endowed with a specific profile

in free fatty acids, including arachidonic and linolenic having a functional role. Moreover, RO25H-CS was characterized, compared with the control samples, by the highest releases of C18:2, C18:1 and C16:1, which are considered aroma precursors. In fact, the presence of highest proteolytic activity and the highest amount of free fatty acids detected in RO25H-CS sample underline a specific volatile molecules profile. The results obtained showed the great potential of *Yarrowia lipolytica* RO25 to produce sourdough characterized by specific sensory and functional fingerprints.

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- 1 Binati RL, Larini I, Salvetti E, Torriani S. 2022. Glutathione production by non-*Saccharomyces* yeasts and its impact on winemaking: A review. *Food Res Internat* 156:111333 doi: 10.1016/j.foodres.2022.111333.

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

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

- 2 Binati RL, Salvetti E, Bzducha-Wróbel A, Bašinskienė L, Čižeikienė D, Bolzonella D, Felis GE. 2021. Non-conventional yeasts for food and additives production in a circular economy perspective. *FEMS Yeast Res* 21(7):foab052 – doi: 10.1093/femsyr/foab052

Yeast species have been spontaneously participating in food production for millennia, but the scope of applications was greatly expanded since their key role in beer and wine fermentations was clearly acknowledged. The workhorse for industry and scientific research has always been *Saccharomyces cerevisiae*. It occupies the largest share of the dynamic yeast market, that could further increase thanks to the better exploitation of other yeast species. Food-related ‘non-conventional’ yeasts (NCY) represent a treasure trove for bioprospecting, with their huge untapped potential related to a great diversity of metabolic capabilities linked to niche adaptations. They are at the crossroad of bioprocesses and biorefineries,

characterized by low biosafety risk and produce food and additives, being also able to contribute to production of building blocks and energy recovered from the generated waste and by-products. Considering that the usual pattern for bioprocess development focuses on single strains or species, in this review we suggest that bioprospecting at the genus level could be very promising. *Candida*, *Starmerella*, *Kluyveromyces* and *Lachancea* were briefly reviewed as case studies, showing that a taxonomy- and genome-based rationale could open multiple possibilities to unlock the biotechnological potential of NCY bioresources.

- 3 Checchia I, Binati RL, Troiano E, Ugliano M, Felis GE, Torriani S. 2021. Unravelling the impact of grape washing, SO₂, and multi-starter inoculation in lab-scale vinification trials of withered black grapes. *Fermentation* 7(1):43 – doi: 10.3390/fermentation7010043

Wine quality is strongly affected by chemical composition and microbial population of grape must, which, in turn, are influenced by several post-harvest treatments, including grape withering. Different strategies have been suggested to manage the fermenting must microbiota, as it plays a central role in the outcomes of both spontaneous and guided fermentations. This study aimed at evaluating the impact of grape washing, SO₂ addition, and selected starter culture inoculation on population dynamics, fermentation kinetics, and main oenological parameters in lab-scale trials, focusing on withered grapes usually used for Amarone production. Although grape washing treatment was effective in removing heavy metals and undesirable micro-

organisms from grape berry surface, inoculation of multi-starter cultures impacted more fermentation rates. Further, both grape washing and starter inoculation procedures had a remarkable impact on wine chemical characteristics, while 30 mg/L SO₂ addition did not significantly affect the fermentation process. In summary, the best strategy in terms of limiting off-flavors and potentially reducing the need for SO₂ addition in wine from withered grapes was the use of yeast starters, particularly mixed cultures composed by selected strains of *Metschnikowia* spp. and *Saccharomyces cerevisiae*. Application of a washing step before winemaking showed a potential to improve organoleptic characteristics of wine.

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- 1 Perpetuini G, Rossetti AP, Tittarelli F, Battistelli N, Arfelli G, Suzzi G, Tofalo R. 2021. Promoting *Candida zemplinina* adhesion on oak chips: A strategy to enhance esters and glycerol content of Montepulciano d’Abruzzo organic wines. *Food Research International*, 150:1107721 doi.org/10.1016/j.foodres.2021.110772

In this study cell surface hydrophobicity and the ability to adhere on abiotic surfaces (polystyrene plates, stainless steel and oak chips) of 10 *Candida zemplinina* strains were assessed. Moreover, the impact of *C. zemplinina* cells adhered on oak surface on fermentation kinetics and volatile profile of Montepulciano d’Abruzzo organic wines was

evaluated. All strains showed a hydrophobic nature with a certain affinity for the apolar solvents tested (hexadecane and decane). In agreement with this data strains were able to adhere on abiotic surfaces in a strain dependent way. On polystyrene plates all strains mainly grew as planktonic cells. On stainless steel surfaces sessile cells ranged from 2.6 Log CFU/mL

(SB2) to 4.1 Log CFU/mL (SB8), while on oak chips were about 2 log higher Log CFU/mL (SB10). *Candida zemplinina* sessile state resulted in an increase of glycerol (from 6.98 g/L to 11.92 g/L) and esters amount (from 55.47 g/L to 91.5 mg/L), and a reduction of ethanol content (from 14.13% to 9.12% v/v). As for esters, methyl vanillate, ethyl isobutyrate, and ethyl isovalerate were present only when *C.*

zemplinina was adhered on oak chips. This study revealed that changes of concentrations in esters and glycerol content reflected the fermentation bioactivity of yeast cells attached on oak chips. Surface-adhered behaviours should be considered in the improvement of strategies for the development of high quality organic wines and eventually obtain novel wine styles.

- 2 Tofalo R, Suzzi G, Perpetuini G. 2021. Discovering the influence of microorganisms on wine color. *Frontiers Microbiol* 12:790935 – doi: 10.3389/fmicb.2021.790935

Flavor, composition and quality of wine are influenced by microorganisms present on the grapevine surface which are transferred to the must during vinification. The microbiota is highly variable with a prevalence of non-*Saccharomyces* yeasts, whereas *Saccharomyces cerevisiae* is present at low number. For wine production an essential step is the fermentation carried out by different starter cultures of *S. cerevisiae* alone or in mixed fermentation with non-*Saccharomyces* species that produce wines with significant differences in chemical composition. During vinification wine color can be influenced by yeasts interacting with anthocyanin. Yeasts can influence wine phenolic composition in different manners: direct interactions – cell wall adsorption or enzyme activities – and/or indirectly – production of primary and secondary metabolites and fermentation products. Some of these characteristics are heritable trait in yeast and/or can be strain dependent. For this reason, the stability, aroma, and color of wines depend on strain/strains used during must fermentation. *Saccharomyces cerevisiae* or non-*Saccharomyces* can produce metabolites reacting with

anthocyanins and favor the formation of vitisin A and B type pyranoanthocyanins, contributing to color stability. In addition, yeasts affect the intensity and tonality of wine color by the action of β -glycosidase on anthocyanins or anthocyanidase enzymes or by the pigments adsorption on the yeast cell wall. These activities are strain dependent and are characterized by a great inter-species variability. Therefore, they should be considered a target for yeast strain selection and considered during the development of tailored mixed fermentations to improve wine production. In addition, some lactic acid bacteria seem to influence the color of red wines affecting anthocyanins' profile. In fact, the increase of the pH or the ability to degrade pyruvic acid and acetaldehyde, as well as anthocyanin adsorption by bacterial cells are responsible for color loss during malolactic fermentation. Lactic acid bacteria show different adsorption capacity probably because of the variable composition of the cell walls. The aim of this review is to offer a critical overview of the roles played by wine microorganisms in the definition of intensity and tonality of wines' color.

- 3 Perpetuini G, Battistelli N, Tittarelli F, Suzzi G, Tofalo R. 2021. Influence of FLO1 and FLO5 genes on aroma profile of sparkling wines. *LWT - Food Sci Technol* 146:111407 doi.org/10.1016/j.lwt.2021.1114

This study investigated the influence of *S. cerevisiae* F6789A strain and its derivative mutants – harbouring *FLO1* gene deletion (F6789A- Δ FLO1) and *FLO5* gene deletion (F6789A- Δ FLO5) – on secondary fermentation, autolysis outcome and aroma compounds production. Data revealed differences in terms of metabolic behaviour cells. An early autolysis was observed for F6789A- Δ FLO5. Differences were observed especially for esters in terms of number and quantity of esters released. In particular, the parental strains produced 39 different esters while F6789A- Δ FLO1 and F6789A- Δ FLO5 27 and 35, respectively. F6789A- Δ FLO5 was the main ester producer with a total amount of leading to the production of sparkling

wines with different characteristics. F6789A showed the best fermentation kinetic reaching a pressure of 5 bar inside the bottle, while F6789A- Δ FLO1 and F6789A- Δ FLO5 reached 4 bar and 3.8 bar, respectively. Cell viability was in agreement with fermentation kinetics. In fact, F6789A showed the highest number of about 89 mg/L. Sensory analysis showed that all the strains produced balanced sparkling wines with negative and positive attributes arranged in good proportions, showing good aroma descriptors. Obtained data suggested that *FLO1* or *FLO5* genes had a pleiotropic effect affecting not only flocculation ability but also other metabolic traits.

- 4 Perpetuini G, Rossetti AP, Battistelli N, Arfelli G, Tofalo R. 2021. Adhesion properties, biofilm forming potential, and susceptibility to disinfectants of contaminant wine yeasts. *Microorganisms* 9(3):654 – <https://doi.org/10.3390/microorganisms9030654>

In this study, yeasts isolated from filter membranes used for the quality control of bottled wines were identified and tested for their resistance to some cleaning agents and potassium metabisulphite, adhesion to polystyrene and stainless-steel surfaces, and formation of a thin round biofilm, referred to as a MAT. A total of 40 strains were identified by rRNA internal transcribed spacer (ITS) restriction analysis and sequence analysis of D1/D2 domain of 26S rRNA gene. Strains belong to *Pichia manshurica* (12), *Pichia kudriavzevii* (9), *Pichia membranifaciens* (1), *Candida sojae* (6), *Candida parapsilosis* (3), *Candida sonorensis* (1), *Lodderomyces elongisporus* (2), *Sporopachydermia lactativora* (3), and *Clavispora lusitaniae* (3) species.

Regarding the adhesion properties, differences were observed among species. Yeasts preferred planktonic state when tested on polystyrene plates. On stainless-steel supports, adhered cells reached values of about 6 log CFU/mL. MAT structures were formed only by yeasts belonging to the *Pichia* genus. Yeast species showed different resistance to sanitizers, with peracetic acid being the most effective and active at low concentrations, with minimum inhibitory concentration (MIC) values ranging from 0.08% (v/v) to 1% (v/v). *C. parapsilosis* was the most sensible species. Data could be exploited to develop sustainable strategies to reduce wine contamination and establish tailored sanitizing procedures.

- 5 Battistelli N, Perpetuini G, Piva A, Pepe A, Sidari R, Wache Y, Tofalo R. 2021. Cultivable microbial ecology and aromatic profile of “mothers” for Vino cotto wine production. *Food Research International*, 143: 110311 – <https://doi.org/10.1016/j.foodres.2021.110311>

The aim of the present study was to assess the cultivable microbiota of “mothers” of Vino cotto collected from production of different years 1890, 1895, 1920, 1975, 2008. A total of 73 yeasts and 81 bacteria were isolated. *Starmerella lactis-condensi*, *Starmerella bacillaris*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Hanseniaspora guillermondi* and *Metschnikowia pulcherrima* were identified. Bacteria isolates belonged to lactic acid bacteria (*Lactiplantibacillus plantarum* and *Pediococcus pentosaceus*) and acetic acid bacteria (*Gluconobacter oxydans*). Remarkable biodiversity was observed for *Starm. bacillaris*, as well as *L. plantarum* and *G.*

oxydans. Organic acids and volatile compounds were also determined. Malic and succinic acids were the main ones with values ranging from 8.49 g/L to 11.76 g/L and from 4.15 g/L to 7.73 g/L respectively, while citric acid was present at low concentrations (<0.2 g/L) in all samples. Esters and higher alcohols were the main volatile compounds detected followed by alkanes. This study permits to better understand the microbial communities associated to this product and could be considered a starting point for the definition of tailored starter cultures to improve the quality of Vino cotto preserving its typical traits.

GMV VIII Department of Agraria, Mediterranean University of Reggio Calabria, Loc. Feo di Vito, 89122 Reggio Calabria (RC), Italy. Communicated by Dr. Rossana Sidari <rossana.sidari@unirc.it>.

- 1 Sidari R, Martorana A, De Bruno A. 2019. Effect of brine composition on yeast biota associated with naturally fermented *Nocellara messinese* table olives. *LWT - Food Science and Technology* 109:163–170 – doi.org/10.1016/j.lwt.2019.04.010

The study aims to assess the yeast biota associated with table olives of the cultivar *Nocellara messinese* naturally fermented using different brines. Microbial counts, physico-chemical parameters, and yeast consortia was evaluated at days 0, 3, 7, 15, 30, 60, 90, 180, and 240. Yeasts were identified by PCR-RFLP of the 5.8S ITS rRNA region and sequencing of D1/D2 domain of the 26S rRNA gene. At the fermentation end, the yeast population was assessed by PCR-DGGE analysis. Throughout the eight months of fermentation, the brine modification determined different evolution of

the yeast species. The salt concentration shift from 5% to 8% delayed the growth of some species at the end of the fermentation. The experimental brine acidification determined a growth of *Pichia mexicana* and *Saccharomyces cerevisiae* that were not found in the other brine formulations. *Pichia kudriavzevii* was the most isolated species followed by *Wickerhamomyces anomalus*, *Zygoascus hellenicus*, *Candida boidinii*, *Saccharomyces cerevisiae*, *Candida aaseri*, *Candida tropicalis*, *Candida diddensiae*, *Zygoascus meyeriae*, and *Pichia mexicana*. *P. kudriav-zevii* and *W. anomalus*

species were present in all the processed olives. Some of the strains exhibited suitable technological properties so

they could be good candidates as starter in table olive fermentations.

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- 1 Zara G, Nardi T. 2021. Yeast metabolism and its exploitation in emerging winemaking trends: from sulfite tolerance to sulfite reduction. *Fermentation*, 7:57
<https://doi.org/10.3390/fermentation7020057>

Sulfite is widely used as a preservative in foods and beverages for its antimicrobial and antioxidant activities, particularly in winemaking where SO₂ is frequently added. Thus, sulfite resistance mechanisms have been extensively studied in the fermenting yeast *Saccharomyces cerevisiae*. Nevertheless, in recent years, a negative perception has developed towards sulfites in wine, because of human health and environmental concerns. Increasing consumer demand for wines with low SO₂ content is pushing the winemaking sector to develop new practices in order to reduce sulfite content in wine, including the use of

physical and chemical alternatives to SO₂, and the exploitation of microbial resources to the same purpose. For this reason, the formation of sulfur-containing compounds by wine yeast has become a crucial point of research during the last decades. In this context, the aim of this review is to examine the main mechanisms weaponized by *Saccharomyces cerevisiae* for coping with sulfite, with a particular emphasis on the production of sulfite and glutathione, sulfite detoxification through membrane efflux (together with the genetic determinants thereof), and production of SO₂-binding compounds.

GMV X Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100, Sassari, Italy. Communicated by Professor Ilaria Mannazzu <imannazzu@uniss.it>.

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

Schizosaccharomyces japonicus and *Saccharomyces cerevisiae* were inoculated in Sangiovese grape must and the impact of simultaneous (co-inoculation) and sequential inoculation protocols on growth and fermentation kinetics, and on the analytical and sensory profiles of the experimental wines, was evaluated at the end of the alcoholic fermentation and after aging in bottle. While *Sch. japonicus* proved to affect *S. cerevisiae* growth and fermentative performances solely in sequential inoculation, the combined activity of the two yeasts always resulted in significantly higher total polysaccharides concentrations ($p < 0.05$), no matter the inoculation protocol utilized. Moreover, *Sch. japonicus* modulated

the concentration of some volatile compounds when in mixed culture. In particular, ethyl acetate, that reached high levels at the end of alcoholic fermentation, showed a significant decrease after twenty-four months of bottle aging ($p < 0.05$), when the occurrence of hydrolysis/esterification reactions led to a significant increase of other acetate esters ($p < 0.05$). As a result, sensory analyses showed no significant differences for the ethyl acetate perception in mixed fermentation and control aged wines. Moreover, sequentially inoculated wines showed significantly higher sweetness compared to control wines, possibly due to the higher glycerol and polysaccharide content.

- 2 Tkáčová J, Zara G, Ianiri G, Castoria R, Čertík M, Mannazzu I. 2021. Impairment of carotenoid biosynthesis through *CARI* gene mutation results in CoQ₁₀, sterols, and phytoene accumulation in *Rhodotorula mucilaginosa*. *Applied Microbiology and Biotechnology* –
<https://doi.org/10.1007/s00253-021-11673-5>

Red yeasts, mainly included in the genera *Rhodotorula*, *Rhodospiridiobolus*, and *Sporobolomyces*, are renowned biocatalysts for the production of a wide

range of secondary metabolites of commercial interest, among which lipids, carotenoids, and other isoprenoids. The production of all these compounds is tightly

interrelated as they share acetyl-CoA and the mevalonate pathway as common intermediates. Here, T-DNA insertional mutagenesis was applied to the wild type strain C2.5t1 of *Rhodotorula mucilaginosa* for the isolation of albino mutants with impaired carotenoids biosynthesis. The rationale behind this approach was that a blockage in carotenoid biosynthetic pathway could divert carbon flux toward the production of lipids and/or other molecules deriving from terpenoid precursors. One characterized albino mutant, namely, strain W4, carries a T-DNA insertion in the *CAR1* gene coding for phytoene desaturase. When cultured in

glycerol-containing medium, W4 strain showed significant decreases in cell density and fatty acids content in respect to the wild type strain. Conversely, it reached significantly higher productions of phytoene, CoQ₁₀, and sterols. These were supported by an increased expression of *CAR 2* gene that codes for phytoene synthase/lycopene cyclase. Thus, in accordance with the starting hypothesis, the impairment of carotenoids biosynthesis can be explored to pursue the biotechnological exploitation of red yeasts for enhanced production of secondary metabolites with several commercial applications.

GMV XI National Research Council of Italy, Institute of Sciences of Food Production (ISPA) c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy. Communicated by Dr Vittorio Capozzi <vittorio.capozzi@ispa.cnr.it> <https://orcid.org/0000-0002-0717-0753>

- 1 Sellitto VM, Zara S, Fracchetti F, Capozzi V, Nardi T. 2021. Microbial biocontrol as an alternative to synthetic fungicides: boundaries between pre-and postharvest applications on vegetables and fruits. *Fermentation*, 7(2), 60 – <https://doi.org/10.3390/fermentation7020060>

From the field to the consumption, there are several steps in the fruits and vegetables production in which undesired microbial contaminations can develop. In managing these diseases, harvest is a crucial phase for tailoring the intervention approaches. While in preharvest, pest management consists of tailored agricultural practices, in postharvest, the contaminations are treated using specific (bio)technological approaches (physical, chemical, biological). However, in some cases, postharvest disease found its origin in the colonisation of undesired microorganisms in preharvest. In these cases, postharvest practices can ‘amplify’ the

contamination, favouring microbial spread and provoking injures of the product, which can sustain microbial growth. In this context, microbial biocontrol is a biological strategy receiving increasing interest as sustainable innovation. Among the microbial-based biotools, yeasts can find applications both to control plant diseases and to reduce contaminations on the product, and therefore, can be considered biocontrol solutions in preharvest or in postharvest, overtaking the boundaries between pre- and postharvest applications and protecting quality against microbial decay.

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

- 1 Guerrini S, Barbato D, Guerrini L, Mari E, Buscioni G, Mangani S, Romboli Y, Galli V, Parenti A, Granchi L. 2021. Selection of indigenous *Saccharomyces cerevisiae* strains and exploitation of a pilot-plant to produce fresh yeast starter cultures in a winery. *Fermentation* 7:99 <https://doi.org/10.3390/fermentation7030099>

The inoculation of grape juice with *Saccharomyces cerevisiae* strains selected from indigenous yeast populations can be a suitable tool to control alcoholic fermentation, contributing to producing wines with typical flavour and aroma and, hence, the demand for native starter cultures is increasing. However, since low amounts of indigenous yeast biomasses are usually required for local winemaking, the industrial production of these yeasts can be expensive. Therefore, in this study, after selecting an indigenous *S. cerevisiae* strain based on relevant oenological and technological

features, a pilot-plant for easy and rapid production of fresh yeast biomass directly in a winery located in Tuscany, was exploited. The selected yeast strain was used as a starter to carry out 25 and 100 hL fermentations and its oenological performance was compared with that of the commercial starter normally used in the winery. Chemical and sensory analysis of the resulting wines showed that they differentiated according to the used yeast strain, with the wines produced by the indigenous *S. cerevisiae* strain being characterized by a distinctive aromatic and sensory

profile. In conclusion, the pilot-plant effectively resulted in producing fresh yeast starter cultures in the winery to

be successfully used to carry out alcoholic fermentations.

2. Galli V, Romboli Y, Barbato D, Mari E, Venturi M, Guerrini S, Granchi L. 2021. Indigenous *Aureobasidium pullulans* strains as biocontrol agents of *Botrytis cinerea* on grape berries. Sustainability 13:9389 – <https://doi.org/10.3390/su13169389>

Aureobasidium pullulans is a yeast like fungus found on the surface of the grape berries that has been proven to act as a biocontrol agent for the management of grey mould disease caused by *Botrytis cinerea*. In this work, an indigenous strain of *A. pullulans* isolated from grape berries and selected according to the in vitro activity against *B. cinerea*, was used in vineyards of the winery where it originated, in comparison with a commercial product containing two *A. pullulans* strains with the aim of assessing its effectiveness as a biocontrol agent. The experimental design included daily meteorological data registration and the early defoliation of grapevines as treatments. The monitoring

of *A. pullulans* strains on grapes berries by plate counts and molecular methods as well as of *B. cinerea* symptoms on grape bunches was performed in the different trials from the end of flowering to the harvest time. Results highlighted that although no significant differences ($p < 0.05$) in the occurrence of *B. cinerea* were detected according to different treatments, the mean incidence of symptomatic berries ranged from 7 to 16%, with the lowest values recorded in bunches treated with the indigenous *A. pullulans* strain. The efficacy of the biocontrol agent was affected more by meteorological conditions than the defoliation practice.

GMV XIII Department of Food, Environmental and Nutritional Sciences (DEFENS), University of Milan, Food Microbiology and Bioprocesses Section, via G. Celoria 2, Milan, 20133, Italy. Communicated by Professor Ileana Vigentini <ileana.vigentini@unimi.it> and Professor Roberto Foschino <roberto.foschino@unimi.it>.

1 Di Canito A, Mateo-Vargas MA, Mazzieri M, Cantoral J, Foschino R, Cordero-Bueso G, Vigentini I. 2021. The role of yeasts as biocontrol agents for pathogenic fungi on postharvest grapes: a review. Foods 10:1650 – doi: 10.3390/foods10071650

In view of the growing concern about the impact of synthetic fungicides on human health and the environment, several government bodies have decided to ban them. As a result, a great number of studies have been carried out in recent decades with the aim of finding a biological alternative to inhibit the growth of fungal pathogens. In order to avoid the large losses of fruit and vegetables that these pathogens cause every

year, the biological alternative's efficacy should be the same as that of a chemical pesticide. In this review, the main studies discussed concern *Saccharomyces* and non-*Saccharomyces* yeasts as potential antagonists against phytopathogenic fungi of the genera *Penicillium* and *Aspergillus* and the species *Botrytis cinerea* on table grapes, wine grapes, and raisins.

2 Di Canito A, Foschino R, Mazzieri M, Vigentini I. 2021. Molecular tools to exploit the biotechnological potential of *Brettanomyces bruxellensis*: a review. Appl Sci 11:7302 <https://doi.org/10.3390/app11167302>

The *Brettanomyces bruxellensis* species plays various roles in both the industrial and food sectors. At the biotechnological level, *B. bruxellensis* is considered to be a promising species for biofuel production. Its presence in alcoholic beverages can be detrimental or beneficial to the final product; *B. bruxellensis* can contribute to spoilage of wine and beer but can also produce good aromas. However, little is known about its genetic characteristics and, despite the complete sequencing of several

B. bruxellensis genomes and knowledge of its metabolic pathways, the toolkits for its efficient and easy genetic modification are still underdeveloped. Moreover, the different ploidy states and the high level of genotype diversity within this species makes the development of effective genetic manipulation tools challenging. This review summarizes the available tools for the genetic manipulation of *B. bruxellensis* and how they may be employed to improve the quality of wine and beer.

- 3 Dei Cas M, Vigentini I, Vitalini S, Laganaro A, Iriti M, Paroni R, Foschino R. 2021. Tryptophan derivatives by *Saccharomyces cerevisiae* EC1118: evaluation, optimization, and production in a soybean-based medium. *Int J Mol Sci* 22:472 – <https://doi.org/10.3390/ijms22010472>

Given the pharmacological properties and the potential role of kynurenic acid (KYNA) in human physiology and the pleiotropic activity of the neurohormone melatonin (MEL) involved in physiological and immunological functions and as regulator of antioxidant enzymes, this study aimed at evaluating the capability of *Saccharomyces cerevisiae* EC1118 to release tryptophan derivatives (dTRPs) from the kynurenine (KYN) and melatonin pathways. The setting up of the spectroscopic and chromatographic conditions for the quantification of the dTRPs in LC-MS/MS system, the optimization of dTRPs' production in fermentative and whole-cell biotransformation approaches and the production of

dTRPs in a soybean-based cultural medium naturally enriched in tryptophan, as a case of study, were included in the experimental plan. Variable amounts of dTRPs, with a prevalence of metabolites of the KYN pathway, were detected. The LC-MS/MS analysis showed that the compound synthesized at highest concentration is KYNA that reached 9.146 ± 0.585 mg/L in fermentation trials in a chemically defined medium at 400 mg/L TRP. Further experiments in a soybean-based medium confirm KYNA as the main dTRPs, whereas the other dTRPs reached very lower concentrations. While detectable quantities of melatonin were never observed, two MEL isomers were successfully measured in laboratory media.

- 4 Fracassetti D, Di Canito A, Bodon R, Messina N, Vigentini I, Foschino R, Tirelli A. 2021. Light-struck taste in white wine: reaction mechanisms, preventive strategies and future perspectives to preserve wine quality. *Trends Food Sci Technol* 112:547-558
<https://doi.org/10.1016/j.tifs.2021.04.013>

Background: The light-dependent reactions involving riboflavin (RF) and methionine (Met) as substrates are responsible for the light-struck taste (LST). This fault is associated to cabbage-like odours due to the formation of methanethiol and dimethyl disulfide impacting negatively on the sensory properties of white wine. The reaction can occur for a relatively short period of time in white wine bottled in clear glass under both natural and artificial lights. **Scope and approach:** This review aimed to point out the aspects related to the mechanisms of light-dependent reactions and the oenological strategies applicable to counteract the appearance of this

detrimental fault. **Key findings and conclusions:** LST can be prevented through the proper choice of the fermenting yeast, the addition of certain adjuvants being able to remove RF, and additives with a protective effect, such as hydrolysable tannins. As the use of these oenological tools plays an important role in limiting the detrimental change, they represent the strategies applicable in productive approaches. In this context, the more recent findings are summarized also to update the knowledge about the complex reaction mechanisms allowing to overcome the formation of this fault and supporting the wine industry.

GMV XIV Department of Agriculture Food and Environment (DAFE), University of Pisa, via G. Borghetto 80, 56124 Pisa, Italy. Communicated by Professor Monica Agnolucci <monica.agnolucci@unipi.it>.

- 1 Palla M, Conte G, Grassi A, Esin S, Serra A, Mele M, Giovannetti M, Agnolucci M. 2021. Novel yeasts producing high levels of conjugated linoleic acid and organic acids in fermented doughs. *Foods* 10(9):2087 – <https://doi.org/10.3390/foods10092087>

Traditional fermented foods are obtained by a complex consortium of autochthonous microorganisms producing a wide variety of bioactive compounds, thus representing a reservoir of strains with new functional properties. Here, doughs obtained using five different wholegrain flours were singly fermented with selected yeast strains, which were evaluated for their functional traits. Lactate, volatile fatty acids and conjugated

linoleic acid isomers produced by fermented doughs were detected by HPLC, while dough anti-inflammatory capacity was measured on human peripheral blood mononuclear cells by flow cytometry. Yeast potential probiotic activity was assessed by evaluating their resistance to simulated gastric and intestinal fluids. For the first time we report evidence of yeast strains producing high levels of the

conjugated linoleic acid (CLA) isomer CLA 10-12tc and propionic acid, which are known for their specific health benefits. Moreover, such yeast strains showed an anti-inflammatory capacity, as revealed by a significantly decreased production of the strongly pro-inflammatory cytokine IL-1 β . All our *Saccharomyces cerevisiae* strains were remarkably resistant to

simulated gastric and intestinal fluids, as compared to the commercial probiotic strain. The two strains *S. cerevisiae* IMA D18Y and L10Y showed the best survival percentage. Our novel yeast strains may be exploited as valuable functional starters for the industrial production of cereal-based innovative and health-promoting fermented foods.

Obituary

RNDr. Ivan Hapala, PhD (1954-2022)

With a great sadness we must inform the yeast research community of the death of Dr. Ivan Hapala, a prominent scientist of the Slovak Academy of Sciences in Bratislava. He passed away on January 2nd, 2022, after a long and severe illness, at the age of 67. He left behind his wife and the families of his two daughters. As a leading yeast researcher and Director of the Institute of Animal Biochemistry and Genetics, he served for several years as Chair of the Czech and Slovak Yeast Commission and Slovak representative for the International Commission on Yeasts. His death is a great loss for Slovak science and particularly for yeast research focused on biogenesis and function of yeast membranes. His most recognized scientific contribution was in the area of the transport of sterols and the production of lipid by yeasts. He will be remembered not only as an outstanding scientist, a favorite teacher, and a responsible leader, but also as a generous, gentle, and good-natured man who organized a number of Annual Yeast Conferences at the Smolenice Castle and the 30th International Specialized Symposium on Yeasts in High Tatras in 2013.



Peter Biely

Future Meeting

ISSY36 – Sea to Sky
36th International Specialized Symposium on Yeasts
Yeasts in the Genomics Era
July 12-16, 2022
The University of British Columbia, Vancouver, BC, Canada



ISSY36 will take place at the University of British Columbia, in Vancouver, British Columbia, which is on the west coast of Canada. The focus of the meeting is ‘Yeast in the Genomics Era’. The sessions at this meeting will include yeast ecology and biogeography, evolutionary genomics, yeast culture collections, yeast physiology and metabolism, beverage and food fermentation, genome engineering in non-traditional yeasts, industrial ethanol production and synthetic biology.

Visit <https://issy36.com>. Contact Vivien Measday for inquiries: <vmeasday@mail.ubc.ca>

Fermenting Futures: A recap on “Yeast technology meets art”

Marina Jecmenica and Simone Bachleitner

The art exhibition “Fermenting Futures” at Künstlerhaus Vienna was shown March 2–22, 2022, and it’s time to recap and draw conclusions on the impressions left with the audience. After all, the goal of the event was to emphasize on the importance of yeast in modern biotechnology and highlight on how tightly entangled the history of humankind and this unicellular organism used to be, but most importantly how it continues to be. By the means of BioArt, the British artists Anna Dumitriu and Alex May showed the aesthetics behind biotechnology and revealed if and how BioArt can play a role for knowledge transfer to the general public. In the end, the question on how the visitors perceived the intersection between art and biology remains and what impression was left behind by the exhibition.



The majority of people visiting the exhibition was familiar with the term “yeast”, mainly with regard to its usage in baking, brewing and wine making. That the baker’s yeast belongs to the group of unicellular fungi, however, and within this only represents one member next to many more interesting species, was equally new and fascinating for most of them. Especially the topic of biodegradable plastic production raised many questions, and people seemed very intrigued by potential solutions using synthetic CO₂ fixing yeast. Contrary to other exhibitions, “Fermenting Futures” reached out for the direct dialogue with visitors. Discussion panels, interviews with the artists, scientific talks and workshops were offered and enabled visitors to directly dive into the yeast

universe – and people loved it. Workshops were fully booked, and additional dates were demanded. Next to the art pieces, bubbling bioreactors, shaking yeast cultures and steady microscopes turned half of the art exhibition into a laboratory, covering every topic from both areas of expertise. In the end, visitors left personal notes on huge panels that described different yeast species designated as the “Wall of Yeast” with words like “Climate savior!”, “Make *Pichia pastoris* great again!” or “Great Exhibition!”.

All in all, communicating science by pointing at art sculptures turned out to be remarkably easy and excited the people as much as it created space for novel perspectives.



Photo credit: Left Studio

Communicated by Diethard Mattanovich

Brief News Item

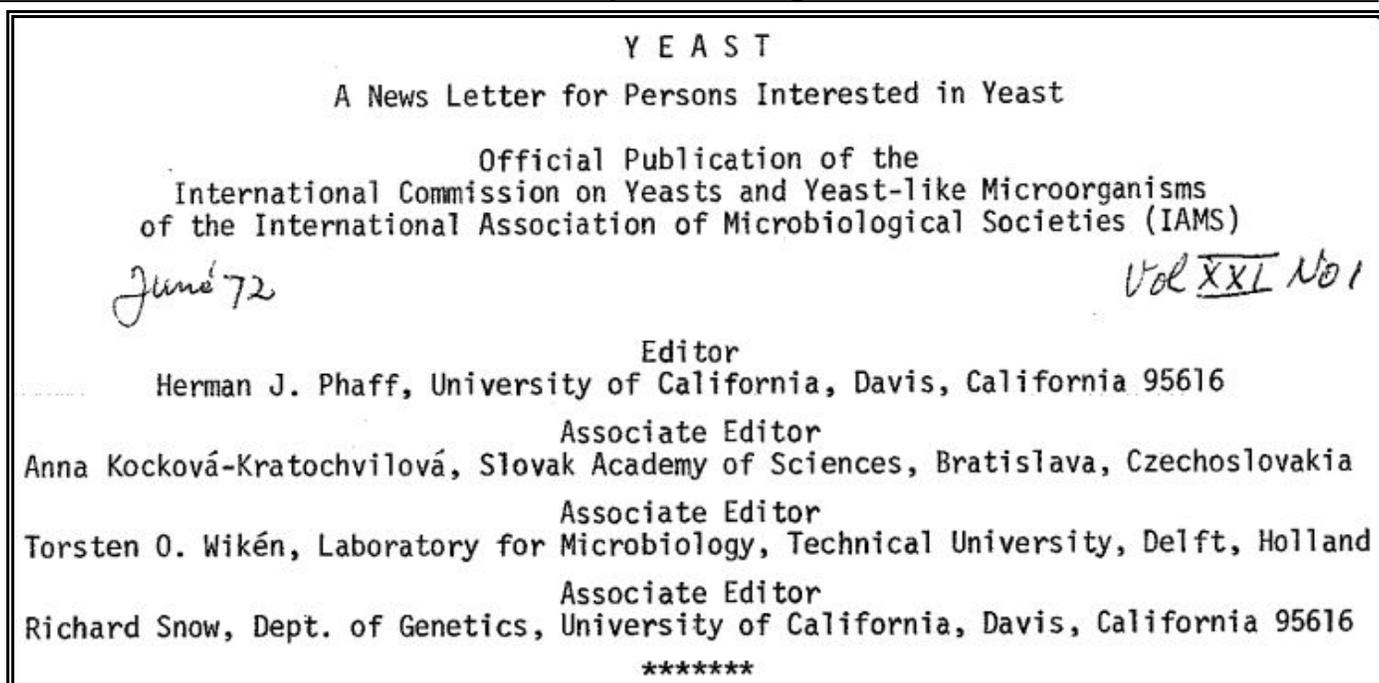
Closure of laboratory and genetic resources, Jeremy Thorner

As announced at the SGD web site, after 47 years at the University of California, Berkeley, the laboratory of Professor Jeremy Thorner closed permanently, as of 30 June 2021. Henceforth, 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. I have since continued to receive a deluge of requests for research materials that, regrettably, I can no longer honor.

Jeremy Thorner
Distinguished Professor Emeritus
BBS Division, MCB Department
University of California, Berkeley, USA

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Fifty Years Ago



Mr. **D. Yarrow** of CBS communicated receipt of type strains of 39 new yeast species: *Brettanomyces abstinens*, 10 *Candida* species, *Cryptococcus himalayensis*, *Filobasidium floriforme*, *Hansenula dryadoides*, *Kluyveromyces thermotolerans*, 8 *Pichia* species, three *Saccharomyces* species, *Sympodiomyces parvus*, 11 *Torulopsis* species, and *Trichosporon brassicae*.

S. Poncet of Université de Lyon, France listed publications on yeasts from vegetable tanning extracts, and use of GC content for *Kluyveromyces* and *Pichia* taxonomy.

I. Campbell of the Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh, Scotland described their studies of metabolism, cell cycle and histones in *Schizosaccharomyces pombe* and *Kluyveromyces fragilis*, and identification of yeasts from sherry flor fermentations and clean and polluted water by serological and morphological analyses.

S.A. Meyer assumed a position at the American Type Culture Collection, after applying DNA base composition to systematics under the direction of **H.J. Phaff** at the University of California Davis and under **A. Stenderup** at the University of Aarhus, Denmark She presented a paper on taxonomy using 100 species of *Candida* and 50 species of *Torulopsis* at the Second Specialized Symposium on Yeasts and Yeast-like Microorganisms in Kyoto,

Japan, March 1972. She used a combination of molecular techniques: DNA base composition, DNA-DNA hybridization, genome size, and amount of repetitive DNA. Perfect and imperfect species pairs had nearly identical GC content. She invited readers to deposit type cultures; medically, industrially and ecologically important strains; and assay organisms into ATCC.

J.J. Miller of McMaster University, Hamilton, Canada submitted a publication on non-reductional nuclear divisions of two-spored yeasts. **M. Banerjee** completed his PhD thesis titled, "The role of carbohydrate in the germination of yeast ascospores", studying *Saccharomyces chevalieri*.

P. Galzy of Ecole Nationale Supérieure Agronomique de Montpellier, France published articles on conditions that induce sporulation and metabolism of acetic acid during sporulation of *S. cerevisiae*.

R.V. Chudyk of the University of Nottingham, England summarized their PhD thesis, which was on effects of high and low temperatures and microwaves on yeast ascospores and vegetative cells.

H.J. Phaff of the University of California Davis reported that Dr. **L. Mendonça** was studying psychrophobic enteric yeasts. Dr. **S. Douglass** was developing improved DNA methods including extraction, separation of mitochondrial and nuclear DNA, and determination of repetitive sequences. Molecular studies were revealing that yeasts that differ in one or two physiological traits may have identical base sequences. The GC content of 29 strains, 18 species of *Kluyveromyces* fell into 5 groups. At the 4th International Fermentation Symposium in Kyoto, Japan in March 1972, **Phaff** presented a study of yeast associated with trees in Japan and the West Coast of North America. [Note: In following years, this survey resulted in discovery of *Phaffia* and many other new genera and species of yeast.]

A. Kahanpää of the University of Helsinki, Finland published a dissertation on examination of adult bronchopulmonary fungal cultures. 4,539 yeast strains belonging to 41 species were identified. The most common species was *Candida albicans*, followed by *Torulopsis glabrata*.

A. Widra of the University of Illinois Medical Center shared a rapid method for identification of *Candida albicans*, using Zein-Lactose-Tween 80 agar with a 1% Tween overlay. At 2-3 hours after inoculation and covering with a coverslip, filament "tubes" can be detected under the microscope.

L.R. Travassos of Universidade Federal do Rio de Janeiro shared the summaries of publications on metabolism of ethionine in *Candida slooffii*, the cell walls of *Torulopsis pintolopesii*, and vitamin requirements of psychrophobic yeasts.

H. Suomalainen of the Research Laboratories of State Alcohol Monopoly, Helsinki, Finland communicated the summaries of work on alpha-glucosidase, long-chain fatty acids, fusel oils, plasma membrane and glycolipids in *S. cerevisiae*.

N.P. Elinov of the Chemical-Pharmaceutical Institute of Leningrad, USSR summarized work on structural cell wall polysaccharides of red and yellow yeasts, *C. albicans*, and *C. tropicalis*.

J.R. Villanueva of Departamento de Microbiología, Universidad de Salamanca, Spain isolated and characterized yeast lytic enzymes including invertase, and also studied spore germination.

M. Crandall, Indiana University, presented a lecture at the 4th International Fermentation Symposium in Kyoto, Japan, on the effects of trace metals on mating factors in *Hansenula wingei*.

N.R. Eaton of Brooklyn College, New York summarized their studies MAL genes, using a yeast mutant that constitutively expressed sucrose fermentation. Strains with different MAL4 alleles were crossed, asci were dissected, and sucrose fermentation was determined, which revealed presence of a recessive gene responsible for constitutive expression of sucrose fermentation. Other alleles were identified that prevented sucrose fermentation, revealing that at least 6 loci are required for maltose and sucrose fermentation.

E. Moustacchi of Institut du Radium, Orsay, France listed publications on a mitotic recombination and a UV-supersensitive mutant of *Schizosaccharomyces pombe*, mitochondrial DNA synthesis in *S. cerevisiae*, repair of mitochondrial UV damage. They proposed roles of nuclear and mitochondrial Rad genes in nuclear and/or mitochondrial repair.

H. Gutz of the University of Texas at Dallas reported recent work on intragenic recombination, gene conversion and mating types of *Schizosaccharomyces pombe*.

A. Nasim and **N.E. Gentner** of the Atomic Energy of Canada Limited, Chalk River, Ontario, Canada summarized their work on radiation sensitivity and dark repair in *S. pombe*.

A. Goffeau of L'Université de Louvain, Belgium reported their work on glucose repression using "petite-negative" mutants of *S. pombe*.

R.A. Woods of the University of Sheffield, England worked on genetic control of purine metabolism, sterol metabolism and physiology, and resistance to polyene antibiotics in *S. cerevisiae*.

M. Luzzati of Centre National de la Recherche Scientifique, Gif-sur-Yvette, France studied mitotic recombination, using *ade3* mutants of *S. cerevisiae*.

J. Johnston of the University of Strathclyde, Royal College, Glasgow, Scotland published on spontaneous, heat-induced and X-ray induced mitotic recombination in *S. cerevisiae*.

F. Lacroute of Université Louis Pasteur, Strasbourg, France summarized their lab's recent work on regulation of the pyrimidine pathway and chromosome replication.

H. Mori of Noda Institute for Scientific Research, Chiba-ken, Japan studied the induction of auxotrophic mutants in *Saccharomyces rouxii* by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine.

G.A. Howard of Allied Breweries (Production) Limited, Burton-on-Trent, England presented a paper at the Am. Soc. of Brewing Chemists Convention on breeding of industrial yeasts by hybridization by mass mating.

G.G. Stewart of Labatt Breweries, London, Ontario, Canada presented a paper at the American Society of Brewing Chemists meeting on induction of flocculation of *S. cerevisiae* by wort-associated compounds, especially polypeptides.

Dr. **E. Minárik** of the Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia published a paper on the ecology of yeasts in the Hlohovek-Trnava wine region in western Slovakia. Early wine fermentation yeasts included *Kloeckera apiculata* and *M. pulcherrima*, followed by *Saccharomyces cerevisiae*. Sweet table wines had *S. cerevisiae* and *S. oviformis* (*bayanus*), and dry wines had *Candida vini* and *C. zeylanoides*.

O. Paredes-Lopez of Laboratorios Nacionales de Fomento Industrial, Mexico produced single cell protein using alfalfa residual juice, comparing several yeast species including *S. cerevisiae*, *S. carlsbergensis*, *C. utilis*, *C. tropicalis*, and *Rhodoturla glutinis*. The amino acid profile was comparable to soybean oil-meals.

The speech given by **T. Wikén**, Chairman of the Commission on Yeasts, at the Fourth International Fermentation Symposium in Kyoto, Japan, March 1972 was printed in the Newsletter, as were the detailed plans for upcoming Symposia, including registration and housing costs, and preregistration information:

- August 1972 in Tokyo, Specialized Symposium on the importance of yeasts in medical science
- October 1972, Third International Symposium on Yeast Protoplasts, Salamanca, Spain
- June 1973 in Aulanko, Finland, Specialized Symposium on metabolism and regulation of cellular processes
- July 1974 in Vienna, Austria, General Symposium

T. Takahashi of the Suita Laboratory, Asahi Breweries, Japan summarized the presentations at the Yeast Genetics Conference, March 1972, Kyoto University.

L.S. Baskin and **I.R. Lapidus** described the first meeting of the New York Metropolitan Yeast Club in Hoboken, New Jersey in February 1972.

The **Institute for Fermentation**, Osaka, Japan published the prices for IFO cultures (\$5 or \$13.25 each) and catalog (\$3 each).

The **Yeast Genetic Stock Center** presented an inventory of strains of *S. cerevisiae* and their genetic loci, including wild type strains, nutritional tester strains, temperature-sensitive lethal strains.

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