

Yeast

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Editorial

Celebrating Peter Biely's 80th birthday

On the occasion of his 80th birthday, RNDr. Peter Biely, DrSc. was honoured with a special issue of the journal *MOLECULES* (2021; 26:4857). The special issue was entitled: "Peter Biely, A Pioneering Researcher in the Enzymology of Plant Biomass Degradation". In an editorial to the special issue, Maria Hrmova describes how Peter began his career with the publication of seminal papers on xylanases. Peter's two top papers in this field have been cited over 4000 times, which is quite an achievement. His interest in xylanases led to the discovery of several new enzymes as well as the development of chromogenic substrates for the study of polysaccharide-hydrolysing enzymes. The reach of Peter's science is global, with collaborations with European, American, Canadian, South African, and Australian laboratories, as well as visiting professorships in Chile, Brazil, Japan, New Zealand, South Africa, and Sweden. He is the recipient of several awards in Slovakia and overseas.

Peter served for many years as Associate Editor of the *Yeast Newsletter*, assiduously providing detailed accounts of the meetings of the Czech and Slovak Yeast Commission, held annually at Smolenice Castle, which incidentally is the birthplace of the International Yeast Commission. Peter is also a talented musician. I can personally attest to Peter's piano virtuosity from our musical collaboration at the Smolenice Castle in 1990, when duets between my harmonica, tuned on the natural scale, and the castle's piano, off concert pitch by nearly a half tone, forced him to play in impossible keys, which he did with superb results.

On behalf of our readers, I wish Peter the very best for years to come.

M.A. Lachance, Editor

Thesis by Martina Daute.

- 1 Daute M. 2021. Exploiting yeast diversity in whisky fermentations for biocatalysis of desirable flavour compounds. PhD Thesis, Abertay University, Dundee, Scotland

Yeast is an important component in producing flavourful fermented products. The Scotch Whisky industry currently only uses four different yeast strains all belonging to *Saccharomyces cerevisiae*. In recent years, the brewing and wine industries have proven that a variety of yeasts can impart flavourful notes, and this has prompted research into alternative yeasts for whisky production. In this study, a variety of non-*Saccharomyces* yeasts were investigated for their potential of becoming new Scotch Whisky yeasts. While none of the yeast strains tested had as good fermentation performance as the distilling strains, two yeasts (*Dekkera bruxellensis*, *Schizosacchaomyces pombe*) showed a high performance. Flavour wise, the yeast strains tested showed a variety of different flavours resulting in different ratios of detected congeners opening a field of wider flavour research. The flavour differences could not be linked to differences between species, *Saccharomyces* spp. tested showed a large variety in produced congener. A correlation between degree of fermentation and flavour could be found with completed fermentations being more *sweet*, *fruity*, and *floral* and less complete fermentations having increased *feinty*, *cereal* and *sulphur* notes. *Dek. bruxellensis* CBS 11269 was shown to be especially *fruity*. The congener production across four different yeast strains and how

they were influenced by different fermentation conditions (time, temperature, gravity, pitching rate) was investigated. The results showed that the yeast strain had a large impact, but the fermentation conditions also significantly influenced the congener ratios. Of the assessed factors, temperature and gravity were discovered to have the largest impact.

To examine the flavour differences between yeast strains, Napping was implemented as a new quick sensory technique for Scotch Whisky and produced similar product separation as Quantitative Descriptive Analysis and Gas chromatography – Mass spectrometry. Furthermore, the flavour and congener changes from wash to low wines and new make spirit was investigated. The result was that assessing the flavour and congener profile on wash and low wine level is sufficient for screening purposes and drastically reduces the workload. Additionally, the ABV before distillation does not impact the flavour and congeners detected in the spirit if the same distilling regime is used. Pre-treatment (boiling, autoclaving, filtration) of wort before fermentation was also assessed to investigate different ways of reducing the impact of the microflora in wort but all three techniques had too large an impact on the fermentation performance and congener development and would not be recommended.

Thesis by Kirsty Black.

- 2 Black K. 2021. From field-to-glass: Optimising field beans (*Vicia faba* L.) and peas (*Pisum sativum* L.) for brewing and distilling. PhD Thesis, Abertay University, Dundee, Scotland (In collaboration with The James Hutton Institute and Arbikie Distillery).

The starch of pulse (*Fabaceae*) grains, such as faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.), offers an environmentally sustainable raw material for the beverage alcohol industry due to their ability to satisfy their entire nitrogen fertiliser requirement through the natural process of biological nitrogen fixation (BNF). As a result of this legume crops present a significantly lower greenhouse gas (GHG) footprint than crops such as spring barley (*Hordeum vulgare* L.) or winter wheat (*Triticum aestivum* L.), which require large amounts of mineral nitrogen (N) fertiliser. Mitigation of agriculture derived GHG

emissions through utilisation of legumes can therefore have a positive environmental impact. To this end, the potential of utilising legumes, when grown as a mixed crop (intercrop, IC), to support traditional cereal crops was evaluated. The suitability of utilising the harvested pulse crop as a source of fermentable material in the production of beer and distilled spirits was then assessed along with the associated protein rich by-products.

The IC studies successfully demonstrated the potential of growing malting quality barley in the presence of legumes without the application of N

fertiliser. Although variability in grain yield and quality was observed across trial years opportunities for breeding specifically for IC conditions, for example, increased cereal tillering and N availability complementarity, combined with a refresh of malting barleys desired characteristics (e.g. preferred N concentration and enzyme activity levels) could allow ICs to develop as a viable low input agriculture alternative.

The use of pulses as an adjunct in brewing and as the sole raw material in distilling was investigated. The gelatinisation properties of pulse starch were assessed with respect to swelling power, pasting properties and diastatic power, identifying a high temperature pre-cook and exogenous starch degrading enzymes to be necessary. The mashing process was further optimised through the investigation of different temperature regimes and additional exogenous enzymes. The processing conditions were found to be species specific with faba bean wort run-off rate significantly benefiting from the addition of protease, producing a wort with a run-off rate, primary amino nitrogen (PAN) content, and fermentability comparable to a 100% malted barley wort. Conversely, pea wort required no additions beyond the starch degrading enzymes.

Unlike brewing, the separation of insoluble materials during the mashing process in distilled spirit production is optional as, ultimately, the process of distilling will separate such material from the final product. The impact of removal point on fermentation kinetics and distillate quality was assessed. Removal of the insoluble material during the mashing step resulted in a faster fermentation and a spirit with lower congeners levels. However, fermentable materials were also lost during the separation process resulting

in a decrease in ethanol yield. An increased availability of pulse starch has arisen during the duration of this research project due to an increased demand for plant protein as a replacement for meat. The high starch concentration of such materials would suggest a high potential ethanol yield within a distillery. However, fermentation trials found fermentation to progress slowly and would fail to reach completion within a commercial fermentation schedule. Although additions of commercially available yeast nutrients had no significant impact on fermentation rate a portion of whole peas (25% by weight) provided sufficient nutrition to support the complete conversion of the fermentable sugars to ethanol thus presenting a new market for this pulse protein by-product.

Commercially available pulse-based beers and spirits have been released during, and as a direct result of, this research. Consumer analysis of beer brewed using 30% (by weight) dehulled bean (kernel) flour as an adjunct to malted barley, was conducted using a series of quantitative sensory tests. The results suggest that the use of faba beans in brewing does not impact negatively on the taste or acceptability of the resultant beer. Assessment of the nutritional (minerals, B vitamins) and antinutritional (vicine, convicine) qualities of beer produced with faba beans as an adjunct concluded that pulses could be used to produce a nutritionally enriched beer with limited concerns around antinutritional properties.

Finally, an assessment of the impact of pulses on by-product value (feed and commercial) demonstrated the potential benefits and further valorisation of these products in their existing markets.

See also:

<https://www.youtube.com/watch?v=ZfJUtoz2AIM&t=3s>

Conference presentations.

- 3 Daute M, Walker J, Harrison B, Maskell P, Grigor J, Jack F, Walker G. 2021. Influence of non-*Saccharomyces* yeast on Scotch whisky fermentation and flavour development. Worldwide Distilled Spirits Conference, Edinburgh, September 2021.

Scotch whisky is a flagship of Scotland's economy with an export value of over £4.7 billion. The ingredients behind this successful product are simple: water, barley, yeast and maturation in oak casks. During fermentation the yeast not only convert wort sugars into ethanol, they also produce flavour-active secondary metabolites. These metabolites are subsequently concentrated and separated in distillation processes. Due to different metabolic pathways expressed by different yeast strains, the concentrations of metabolites contributed by fermentation are highly

yeast strain dependent. Research from other alcoholic beverage fermentations, such as brewing and winemaking, has demonstrated a clear influence of yeast diversity on the final flavour of beer and wine, including contributions from non-*Saccharomyces* yeasts like *Torulasporea delbrueckii* for beer or *Metschnikowia pulcherrima* for wine. The Scotch whisky industry, however, generally sources its yeast from a limited number of yeast suppliers and all of these commercially available strains are *Saccharomyces cerevisiae*. We have investigated the influence

of non-*Saccharomyces* strains on Scotch whisky fermentation, with a focus on the development of diverse flavours. Substrate utilisation tests were used to evaluate the potential of over 80 different yeast strains to ferment the main sugars in wort compared to commercial distilling strains. Well-performing strains were selected for small-scale fermentations. Kinetics of fermentation were monitored by assessing carbon dioxide accumulation. Yeast growth and sugar utilisation (by HPLC) were also monitored to assess fermentation performance. Following distillation, the

resulting low wines were chemically analysed using GC-MS and congener characteristics assessed by a trained sensory panel. In a further selection step, the best non-*Saccharomyces* strains were selected to distil on a larger scale to produce new make spirit. This presentation describes differences in spirit composition and flavour produced by selected non-*Saccharomyces* yeasts and discusses the potential of using diverse yeast species to elaborate whisky sensory characteristics.

- 4 Daute M, Baxter I, Walker G, Jack F. 2021. Comparison of *Napping* and standard sensory profiling to explore the sensory impact of various yeast strains on spirit flavour. Worldwide Distilled Spirits Conference, Edinburgh, September 2021.

A set of experimental Scotch-type spirits were produced in the laboratory using a range of *Saccharomyces* and non-*Saccharomyces* yeast. The aim of this study was to determine the impact of yeast strains on flavour. The standard approach for this would be to use a flavour profiling method, like Quantitative Descriptive Analysis (QDA). Samples are presented to trained panellists who rate the intensity of selected attributes. Differences between samples can be determined from these panel scores. The assessment of a large set of samples is difficult and may require several sessions, because their high strength makes repeated nosing of large sample sets leads to sensory fatigue.. In this study, we explored the use of *Napping* as an alternative rapid approach to determine flavour differences across our set of samples. With *Napping*, panellists are asked to nose the samples and arrange them on a large piece of paper or tablecloth (*French: Nappe*) based on their flavour characteristics. Samples that are alike should be

placed close to each other, while those that are different further apart. Panellists can chose their own criteria for arranging the samples, no training is required, and re-nosing or rearrangement of the samples is possible. Finally, panellists are asked to write short descriptions next to each sample/ group. All samples can be evaluated in one session. In our study, data were analysed by recording the X-Y coordinates of each sample on the *nappe*. Multivariate statistical techniques were then applied to create a consensus map showing relative differences and grouping of samples according to their flavour. In this presentation, data produced using both sensory approaches were compared and discussed. An approach like *Napping* may be advantageous as a screening tool for distilled spirits because the high strength makes repeated nosing of large sample sets difficult, allowing a more detailed QDA on a smaller subset.

- 5 Daute M, Jack F, Walker G. 2021. Non-*Saccharomyces* yeasts for whisky fermentations. International Workshop on brewing Yeasts, Bariloche, Argentina, November 2021.

Using diverse yeast is typical for brewing and wine making. A wide variety of yeasts are commercially offered with most breweries having their own proprietary strains. However, brewers and wine makers also experiment with alternative or non-conventional yeasts to produce products with enhanced and special flavours. In comparison, the Scotch Whisky industry is a traditional industry. Distilleries cannot re-pitch their yeasts and buy them from commercial yeast suppliers which offer only a limited selection of yeasts. Distillers have high expectations of their yeasts because no enzymes can be added and the fermentation process is not sterile,

resulting in the need of yeasts with fast fermentation and high attenuation. While brewers showed that yeasts can impart a variety of flavours, distillers are now becoming more interested in non-conventional yeasts for flavour enhancement. In this study, 90 yeast strains belonging mostly to non-*Saccharomyces* yeasts were screened for their potential in Scotch Whisky fermentation. After an initial fermentation screening they were tested for their fermentation performance with the Ankom Gas Monitoring System to monitor CO₂ production kinetics. Eight yeast strains were selected for upscaled fermentation with subsequent distillation for spirit analysis. The new make spirits

were assessed with a trained sensory panel and gas chromatography- mass spectrometry. The results showed that the commercial yeast strains had the best fermentation performance but unconventional yeasts could enhance alternative flavour characteristics with

the ratio of esters being especially yeast strain dependent. Further research will be needed to improve the fermentation performance of selected yeast strains or investigate the potential of co-fermentation for Scotch Whisky fermentation.

Pending Publication.

- 6 Daute M, Jack F, Harrison B, Walker G. 2021. Experimental whisky fermentations: influence of wort pre-treatments. *Foods*.

In addition to ethanol yield, the production of flavour congeners during fermentation is a major consideration for Scotch Whisky producers. Experimental whisky fermentations can provide useful information to industry and this is the focus of this paper. This study investigated the impact of wort pre-treatments (boiled, autoclaved, filtered) on fermentation performance and flavour development in Scotch Whisky distillates as alternatives to freezing wort for storage. Our study showed that no significant sensorial differences were detected in low wines (first distillates), while the chemical compositions showed clear changes in increased levels of esters and higher alcohols in boiled and autoclaved wort. In contrast,

fermented and distilled filtered wort comprised overall lower levels of congeners. had an overall decrease in congeners. Regarding alcohol yield, all three pre-treatments resulted in decreased yields. In practice, pre-treatment of wort prior to fermentation requires additional process operations while freezing requires large storage units. The pre-treatments adopted in this study significantly influence the composition of malt wort used for experimental whisky fermentations and this results in a poorer fermentation performance compared with un-treated wort. We recommend the use of fresh or frozen wort as the best options for small-scale fermentation trials.

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

Recent publication.

- 1 Ksiezopolska E, Schikora-Tamarit MÀ, Beyer R, Nunez-Rodriguez JC, Schüller C, Gabaldón T. 2021. Narrow mutational signatures drive acquisition of multidrug resistance in the fungal pathogen *Candida glabrata*. *Curr Biol* 21:S0960-9822(21):01352-X. doi: 10.1016/j.cub.2021.09.084. Epub ahead of print. PMID: 34699784.

Fungal infections are a growing medical concern, in part due to increased resistance to one or multiple antifungal drugs. However, the evolutionary processes underpinning the acquisition of antifungal drug resistance are poorly understood. Here, we used experimental microevolution to study the adaptation of the yeast pathogen *Candida glabrata* to fluconazole and anidulafungin, two widely used antifungal drugs with different modes of action. Our results show widespread ability of rapid adaptation to one or both

drugs. Resistance, including multidrug resistance, is often acquired at moderate fitness costs and mediated by mutations in a limited set of genes that are recurrently and specifically mutated in strains adapted to each of the drugs. Importantly, we uncover a dual role of ERG3 mutations in resistance to anidulafungin and cross-resistance to fluconazole in a subset of anidulafungin-adapted strains. Our results shed light on the mutational paths leading to resistance and cross-resistance to antifungal drugs.

Recent publications.

- 1 Jamiu AT, Albertyn J, Sebolai OM and Pohl CH. 2021. Update on *Candida krusei*, a potential multidrug-resistant pathogen. *Med Mycol* 59:14–30.

Although *Candida albicans* remains the main cause of candidiasis, in recent years a significant number of infections has been attributed to non-*albicans Candida* (NAC) species, including *Candida krusei*. This epidemiological change can be partly explained by the increased resistance of NAC species to antifungal drugs. *C. krusei* is a diploid, dimorphic ascomycetous yeast that inhabits the mucosal membrane of healthy individuals. However, this yeast can cause life-threatening infections in immunocompromised patients, with hematologic malignancy patients and those using prolonged azole prophylaxis being at higher risk. Fungal infections are usually treated with five major classes of antifungal agents which include azoles, echinocandins, polyenes, allylamines, and nucleoside analogues. Fluconazole, an azole, is the most commonly used antifungal drug due to its low host toxicity, high water solubility, and

high bioavailability. However, *C. krusei* possesses intrinsic resistance to this drug while also rapidly developing acquired resistance to other antifungal drugs. The mechanisms of antifungal resistance of this yeast involve the alteration and overexpression of drug target, reduction in intracellular drug concentration and development of a bypass pathway. Antifungal resistance menace coupled with the paucity of the antifungal arsenal as well as challenges involved in antifungal drug development, partly due to the eukaryotic nature of both fungi and humans, have left researchers to exploit alternative therapies. Here we briefly review our current knowledge of the biology, pathophysiology and epidemiology of a potential multidrug-resistant fungal pathogen, *C. krusei*, while also discussing the mechanisms of drug resistance of *Candida* species and alternative therapeutic approaches.

- 2 Fourie R, Cason ED, Albertyn J, Pohl CH. 2021. Transcriptional response of *Candida albicans* to *Pseudomonas aeruginosa* in a polymicrobial biofilm. *G3* 11(4): jkab042.

Candida albicans is frequently co-isolated with the Gram-negative bacterium, *Pseudomonas aeruginosa*. *In vitro*, the interaction is complex, with both species influencing each other. Not only does the bacterium kill hyphal cells of *C. albicans* through physical interaction, it also affects *C. albicans* biofilm formation and morphogenesis, through various secreted factors and cell wall components. The present study sought to expand the current knowledge regarding the interaction between *C. albicans* and *P. aeruginosa*, using transcriptome analyses of early static biofilms. Under these conditions, a total of 2,537 open reading frames (approximately 40% of the

C. albicans transcriptome) was differentially regulated in the presence of *P. aeruginosa*. Upon deeper analyses it became evident that the response of *C. albicans* toward *P. aeruginosa* was dominated by a response to hypoxia, and included those associated with stress as well as iron and zinc homeostasis. These conditions may also lead to the observed differential regulation of genes associated with cell membrane synthesis, morphology, biofilm formation and phenotypic switching. Thus, *C. albicans* in polymicrobial biofilms with *P. aeruginosa* have unique transcriptional profiles that may influence commensalism as well as pathogenesis.

- 3 Fourie R, Albertyn J, Sebolai O, Gcilitshana O, Pohl CH. 2021. *Candida albicans SET3* plays a role in early biofilm formation, interaction with *Pseudomonas aeruginosa* and virulence in *Caenorhabditis elegans*. *Front Cell Infect Microbiol* 11:680732.

The yeast *Candida albicans* exhibits multiple morphologies dependent on environmental cues. *Candida albicans* biofilms are frequently polymicrobial, enabling interspecies interaction through proximity and contact. The interaction between

C. albicans and the bacterium, *Pseudomonas aeruginosa*, is antagonistic *in vitro*, with *P. aeruginosa* repressing the yeast-to-hyphal switch in *C. albicans*. Previous transcriptional analysis of *C. albicans* in polymicrobial biofilms with *P. aeruginosa* revealed

upregulation of genes involved in regulation of morphology and biofilm formation, including *SET3*, a component of the Set3/Hos2 histone deacetylase complex (Set3C). This prompted the question regarding the involvement of *SET3* in the interaction between *C. albicans* and *P. aeruginosa*, both *in vitro* and *in vivo*. We found that *SET3* may influence early

biofilm formation by *C. albicans* and the interaction between *C. albicans* and *P. aeruginosa*. In addition, although deletion of *SET3* did not alter the morphology of *C. albicans* in the presence of *P. aeruginosa*, it did cause a reduction in virulence in a *Caenorhabditis elegans* infection model, even in the presence of *P. aeruginosa*.

- 4 Mochochoko BM, Ezeokoli OT, Sebolai O, Albertyn J, Pohl CH. 2021. Role of the high-affinity reductive iron acquisition pathway of *Candida albicans* in prostaglandin E2 production, virulence, and interaction with *Pseudomonas aeruginosa*. *Medical Mycology* 59: 869–881.

Components of the iron reductive pathway of *Candida albicans* have been implicated in the production of prostaglandin E2 (PGE₂) and virulence. However, it is unknown whether other components of this pathway influence PGE₂. We investigated the role of the iron reductive pathway of *C. albicans* in biofilm formation, PGE₂ production, and virulence in *Caenorhabditis elegans*. Additionally, as the co-occurrence of *C. albicans* and *Pseudomonas aeruginosa* in host tissues is frequent and involves competition for host-associated iron, we examined the effects of this interaction. Deletion of multicopper oxidase gene, *FET99*, and iron permease genes, *FTH1* and *FTH2*, affected biofilm metabolic activity, and for

the *FTH2* mutant, also biofilm morphology. Deletion of *CCC1* (vacuolar iron transporter) and *CCC2* (P-type ATPase copper importer) also influenced biofilm morphology. For PGE₂ production, deletion of *FET99*, *FTH1*, *FTH2*, *CCC1*, and *CCC2* caused a significant reduction by monomicrobial biofilms, while *FTH2* deletion caused the highest reduction in polymicrobial biofilms. *URA3* positive mutants of *FET99* and *FTH2* demonstrated attenuated virulence in *C. elegans*, potentially due to the inability of mutants to form hyphae *in vivo*. Deductively, the role of the iron reductive pathway in PGE₂ synthesis is indirect, possibly due to their role in iron homeostasis.

- 5 Ogundeji AO, Mjokane N, Folorunso OS, Pohl CH, Nyaga MM, Sebolai OM. 2021. The Repurposing of acetylsalicylic acid as a photosensitiser to inactivate the growth of cryptococcal cells. *Pharmaceuticals* 14:404.

Photodynamic treatment (PDT) is often successful when used against aerobic microbes, given their natural susceptibility to oxidative damage. To this end, the current study aimed to explore the photodynamic action of acetylsalicylic acid (ASA; aspirin, which is commonly used to treat non-infectious ailments), when administered to respiring cryptococcal cells. The treatment of cryptococcal cells, i.e., exposure to 0.5 or 1 mM of ASA in the presence of ultraviolet light (UVL) for 10 min, resulted in a significant ($p < 0.05$) reduction in the growth of tested cells when compared to non-treated (non-Rx) cells, i.e., no ASA and no UVL. The treated cells were also characterised by diseased mitochondria, which is crucial for the survival of

respiring cells, as observed by a significant ($p < 0.05$) loss of mitochondrial membrane potential ($\Delta\Psi$ M) and significant ($p < 0.05$) accumulation of reactive oxygen species (ROS) when compared to non-Rx cells. Moreover, the photolytic products of acetylsalicylic acid altered the ultrastructural appearance of treated cells as well as limited the expression levels of the capsular-associated gene, *CAP64*, when compared to non-Rx cells. The results of the study highlight the potential use of ASA as a photosensitiser that is effective for controlling the growth of cryptococcal cells. Potentially, this treatment can also be used as an adjuvant, to complement and support the usage of current anti-microbial agents.

- 6 Madu UL, Ogundeji AO, Folorunso OS, Albertyn J, Pohl CH, Sebolai OM. 2021. The repurposing of the antimalaria drug, primaquine, as a photosensitizer to inactivate cryptococcal cells. *Photochem* 1(2):275-86.

Cryptococcal cells can manifest skin infections in immunocompromised persons. While it may be easy

to diagnose cryptococcal infection, treatment often fails due to the ineffectiveness of current antifungal

agents. To this end, the present study explored the repurposing of primaquine (PQ), as a photosensitizer. PDT was carried out using a germicidal ultraviolet (UV) lamp, which has a radiation output of approximately 625 μ W/cm² at a distance of 20 cm. When compared to the non-treated cells, the metabolic activity of crypto-coccal cells was significantly ($p < 0.05$) limited. The photolytic products of PQ were observed to alter the ultrastructure of treated cells. The latter was not incidental, as the same cells were also documented to lose their selective permeability.

Importantly, PDT also improved the efficiency of macrophages to kill internalized cryptococcal cells ($p < 0.05$) when compared to non-treated macrophages. Equally importantly, PDT was not detrimental to macrophages, as their metabolic activity was not significantly ($p > 0.05$) limited, even when exposed to 20x the MIC (determined for cryptococcal cells) and an exposure time that was 4x longer. Taken together, the results suggest PQ has the potential to control the growth of cryptococcal cells and limit their survival inside the macrophage.

- 7 Kankam G, Christians B, Maliehe M, Mjokane N, Ogundeji AO, Folorunso OS, Pohl CH, Sebolai OM. 2021. The first survey of cryptococcal cells in bird droppings across Bloemfontein, South Africa, *Veterinary World*, 14 (10): 2739-2744.

Background and Aim: Cryptococcal yeast cells are spread across different ecosystems through bird movement and are deposited in bird guano. These cells may be inhaled by humans and lead to cryptococcal pneumonia. In individuals with reduced immune T-cell populations, cells may disseminate to the brain and cause the often-deadly cryptococcal meningitis. In this study, we surveyed cryptococcal cells in bird droppings across the city of Bloemfontein, South Africa. **Materials and Methods:** We aseptically collected 120 bird dropping samples from 15 representative city sites. In the laboratory, samples were assessed with regards to location, weighed, and standardized to a mass of 1 g before suspension in 10 mL phosphate buffer saline. Samples were first screened using Calcofluor-white stain as it is a rapid technique for the detection of fungi via binding to cell wall components such as chitin. After this, positive

Calcofluor samples were serologically assayed for the cryptococcal antigen (CrAg). To confirm assay data, CrAg positive samples were then cultured on bird seed agar and resulting colonies were assessed using Indian ink. **Results:** We determined that 10/15 locations were positive for the CrAg. Pathogenic cells were identified on bird seed agar as brown colonies. When examined using microscopy, brown colony cells exhibited characteristic thick capsules representative of cryptococcal cells. **Conclusion:** This is the first proximate analysis showing the ecological distribution of cryptococcal cells in Bloemfontein. This is important as associated infections are acquired from the environment. Similarly, given the threat posed by cryptococcal cells to immunocompromised individuals, local authorities must initiate measures curbing the spread of these cells.

IV International Centre of Brewing and Distilling, Heriot–Watt University, Edinburgh, Scotland, and Canadian Institute of Fermentation Technology, Dalhousie University, Halifax, NS.
Communicated by Alex Speers <Alex.Speers@gmail.com>.

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 7010010>

Recently there has been an increased interest in characterising the rates of alcoholic fermentations. Sigmoidal models have been used to predict changes such as the rate of density decline. In this study, three published sigmoidal models were assessed and fit to industrial fermentation data. The first is the four-parameter logistic model described in the ASBC Yeast-14 method. The second model is a nested form of the four-parameter logistic function, adding an extra

parameter, creating the 5-parameter logistic equation., where an additional parameter was added to allow for asymmetry. The final model is a three-parameter logistic equation which describes the change in the Apparent Degree of Fermentation with time. The three models were compared by fitting them to industrial data from Australian and Canadian lagers, American and Scottish ales and Scotch Whisky fermentations. The model fits were then compared to

one another with a technique developed by Akaike and a nested F-test. The Akaike information criterion compares the models and accounts for both the goodness of fit, and the number of parameters in the model. Finally, consideration was given to the

establishment of prediction bands (that enclose the area that one can be 99% sure contains the true datapoints). Calculation of these bands was “challenging” but successful as the industrial fermentation data was heteroscedastic.

2 Kishnani P, Barr L, Speers RA. 2021. Evaluation of dimethyl sulfide thresholds. *J Amer Soc Brew Chem* DOI: 10.1080/03610470.2021.1945852

Dimethyl sulfide precursors (DMSP), present in both pilot plant floor and pneumatic malts, were used to produce craft beers and their levels were measured. The pneumatic malt was germinated at 14°C, and the floor malt was germinated at 19.6°C. GC analysis

revealed that, on average, the pneumatic malt contained 21.5µg DMSP/g malt, while the floor malt contained 7µg DMSP/g malt. Difficulties with DMS sensory analyses are discussed.

3 Speers, RA, Mishra A. 2021. Wort boil time and trub effects on fermentability. *J ASBC* 79:46-52.

The purpose of this research was to understand and evaluate the effect of high temperature treatment of wort at varying time periods (30, 45, 60, 90, and 120min) on wort fermentability. The effect of trub was also analyzed. Small-scale fermentations were conducted using a malt (malting from AC Metcalfe) free of premature yeast flocculation tendencies and a standard SMA yeast strain. The turbidity and apparent extract were measured at specific time intervals. The decline in extract was modeled with the ASBC Yeast-14 logistic equation predicting a sigmoidal curve. Turbidity measurements were modeled with a “tilted Gaussian” fit. Heat-treating the wort (at 100 or

121.1C) resulted in a significant decline in ADF ($p < 0.05$). As denoted by the midpoint of the fermentation, all fermentation runs, with and without trub, at lower wort boil durations fermented faster than longer or higher wort-boiling treatments. The decline in wort fermentability was highest upon treatment at wort temperature and pressure levels of 121.1C and 3.0atm. The amount of trub and the wort color formed after each heat treatment showed a gradual increase with heating duration. Free amino nitrogen levels of wort declined significantly with respect to both increase in wort boiling temperature and time intervals ($p < 0.001$).

4 Huismann M, Gormley F, Dzait D, Stewart K, Speers RA, Maskell DL. 2021b Unfilterable beer haze Part II: Identifying suspect cell wall proteins. *J Amer Soc Brew Chem* DOI: 10.1080/03610470.2021.1937461

The use of various diagnostic techniques has been previously utilized in the assessment of a commercially available India Pale Ale with cases of sporadically occurring unfilterable haze. The results from Part 1 suggested that β -glucans and proteins were the cause of the unfilterable haze and it was postulated that cell wall mannoproteins may also be a culprit of the unfilterable beer haze. In this follow-up study, proteins from high haze and low haze beer samples were precipitated and assessed using SDS-PAGE. Polyphenol interferences observed on the SDS-PAGE indicated that protein purification and targeted analysis was required. Proteins from high haze and low haze samples were fractionated and qualitatively identified

via LC-MS. A library was built from FASTA sequences of targeted yeast proteins to qualitatively analyze the high haze and low haze samples. The protein fractionation was successful at purifying and isolating proteins from high and low haze samples. Two protein peaks were observed in the high haze sample, while one protein peak was observed in the low haze sample. The targeted LC/MS analysis discovered the presence of yeast cell wall mannoproteins and flocculation proteins, particularly Flo1 and Flo9. Understanding the source of these hazes can provide an opportunity for brewers to mitigate against their formation by adjusting brewing and yeast management practices.

- 5 Huismann M, Gormley F, Dzait D, Speers RA, Maskell DL. 2021a. Unfilterable beer haze Part I: The investigation of an india pale ale haze. J Amer Soc Brew Chem <https://doi.org/10.1080/03610470.2021.1937460>

The nature of undesirable and unfilterable haze particles observed by craft breweries remains nebulous and presents a challenge when the aim is the production of bright beer. A commercial beer was studied in which the brewery had sporadically encountered unfilterable haze. In this study, it was hypothesized that unfilterable haze particles were formed due to increased concentrations of proteins, polyphenols, and/or beta-glucans. Samples of a high haze and low haze India Pale Ale were degassed and digested with enzymes amyloglucosidase, pepsin, and UltraFlo Max (Novozymes™). Additionally, the

protein, polyphenol, and beta-glucan content of each sample was measured. When comparing protein, polyphenol, and beta-glucan concentrations substantial differences between high haze and low haze protein concentrations were observed. Due to the unfilterable nature of these hazes, combined with experimental findings, it was hypothesized that yeast cell-wall proteins were responsible for this haze. Understanding of the source of these hazes offers brewers the opportunity to mitigate against their formation by adjusting brewing practices.

Presentations.

- 6 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.
- 7 Speers RA, Kilfoil G, Li Y, Onio A, Watts P. 2021. Further development of premature yeast methodology. Presented at the MBAA Conf. Cleveland, OH, Onsite: Thursday, October 28 - Saturday, October 30, 2021 Virtual: Nov. 3-5.
- 8 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.
- 9 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.
- 10 Speers RA. 2021. Episode 025 | Boil length and trub levels. <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).
- 11 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.

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

Recent publication.

- 1 Simbaña J, Portero-Barahona P, Carvajal Barriga EJ. 2021. Wild Ecuadorian *Saccharomyces cerevisiae* strains and their potential in the malt-based beverages industry. J Amer Soc Brew Chem DOI:10.1080/03610470.2021.1945366

Ecuador is a biodiverse country where a wide range of *Saccharomyces cerevisiae* yeast is present. Ecuador also holds a rich tradition of brewing corn-based beer. Genetics and domestication of *S.*

cerevisiae throughout thousands of years have resulted in a wide range of aromas and flavors produced during fermentation. A total of 94 yeast isolates were studied; RFLP analysis of the isolates was used for

grouping the strains according to their genotype. A group of 15 yeasts characterized by six different RFLP profiles was chosen. Fermentations were carried out at room temperature (~ 22 °C) to determine the fermented beers' chemical and organoleptic characteristics. Significant differences between the

yeast isolates were found by means of HPLC analysis and sensorial evaluation of the fermentation products. Moreover, fourteen out of fifteen isolates were found to be potentially useful in the production of different beer styles.

Book chapter in press.

- 2 Carvajal Barriga EJ, Portero-Barahona P, Narváez-Trujillo A. Chapter 13. Yeast diversity in underexplored regions for sustainable futures. In: Importance of Microbiology Education and Microbial Resource Management for sustainable futures. Elsevier. In press.

Ecuador is acknowledged as one of the megadiverse countries globally (Dupérré & Tapia, 2020), not only because of its latitude but also because of its large number of microhabitats created by the orography of Andes mountain chain that runs north to south of its territory. Ecuador is also one of the underexplored regions in the world in terms of microbial biodiversity. The current worldwide yeast species count has surpassed 2000 (*The Yeasts*, n.d.), of which many have metabolic features that might be useful in industry. Meanwhile, sampling, collecting, and conserving yeast species in Ecuador has not been extensive and primarily has been performed by the group belonging to the Neotropical Center for the Biomass Research (CNIB) at the Pontificia Universidad Católica del Ecuador. The Catholic University Yeast Collection in Quito (CLQCA) was founded in 2006 to preserve yeast biodiversity by collecting from selected substrates found in different ecosystems (CLQCA, n.d.). The data obtained from yeast isolates has positively contributed to explain yeast distribution, abundance, and adaptation of these microorganisms in a biodiverse country with four natural regions (Amazonia, Andes, Pacific Coast, and the Galapagos archipelago). Additionally, and of the same importance, the CLQCA has emphasized discovering and studying the metabolic capabilities of

Ecuadorian environmental yeast isolates for biotechnological applications such as probiotics, decontamination of pollutants, ancient yeast reanimation methods (Microbial Archaeology), screening of yeast metabolites for the industry, development and innovation of fermentation systems, second-generation ethanol research and development, and ancient fermented beverages recreation. Collaboration with yeast researchers' international networks has been of high importance for the progress of yeast projects, including the description of novel yeast species, organization of symposia and courses, organization of expeditions to pristine Ecuadorian natural regions, among other collaborative outcomes. This chapter aims to provide an overall view of the most relevant results of the projects carried out at the CLQCA. The search of sustainability by creating new opportunities for the industry and training for new entrepreneurs. This chapter also deals with the relevance of *ex situ* preservation of yeasts collected in natural environments and the biotechnological potential of Ecuadorian yeast species. Finally, the chapter demonstrates how a culture collection can find a niche as a supporter of the local economy by developing new technologies and services for the industry (Smith, 2003).

VI Laboratory of Yeast Genetics and Molecular Biology, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw, Poland. Communicated by Teresa Zoladek
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We, Teresa Zoladek with Joanna Kaminska, organized and guest edited the special issue of International Journal of Molecular Sciences entitled "Yeast Models and Molecular Mechanisms of Neurodegenerative Diseases". In this issue eight papers were published, including three [1-3] concerning studies of VPS13 proteins which show how using yeast model contributes to better

understanding of molecular mechanisms of human VPS13A-D-dependent diseases and helps to find new drug targets and potentially repurpose drugs for further study in higher eukaryotic model organisms. To continue, the special issue with the same title was open for submission in 2021 https://www.mdpi.com/journal/ijms/special_issues/Yeast_IJMS and already contains two VPS13-related papers [4,5].

We invite all yeast researchers to publish in our special issue the experimental and review articles concerning

studies related to neurodegenerative diseases.

The special issue articles are accompanied by the following editorial article.

- 1 Kaminska J, Zoladek T. 2021. yeast models and molecular mechanisms of neurodegenerative diseases. Int J Mol Sci 22(16):8775 - <https://doi.org/10.3390/ijms22168775>

The article gives a which short description of all achievements presented in this issue, including these related to VPS13. A relevant fragment follows:

“The *VPS13A-D* genes encode the four human VPS13A-D homologous proteins localized to different subsets of MCSs. This diverse localization could explain why different *VPS13* gene mutations are responsible for distinct neurological disorders. Moreover, VPS13A and VPS13D proteins link mitochondria to the ER and, therefore, are another example of proteins whose deficiencies cause mitochondrial dysfunction, leading to neurological diseases. VPS13 proteins are responsible for lipid transport between membranes. However, how this function is relevant to diseases is still unknown. Dziurdzik and Conibear, 2021 [1] showed the progress made from the study of the yeast Vps13 protein in understanding the determinants responsible for the proper localization of VPS13 proteins in a cell, especially recruitment to multiple MCSs. It is important to find the basis of VPS13 interaction with different organelles and know the precise function of each homologue at specific MCSs to understand the pathogenesis of VPS13-related diseases.

Soczewka et al. 2021 [2] and Wardaszka et al. 2021 [3] further characterized the importance of VPS13 proteins for cell physiology by studying the yeast *vps13* mutant devoid of the yeast Vps13 protein. A simple phenotype, i.e., the hypersensitivity of the *vps13* mutant to the commonly used detergent sodium

dodecyl sulphate (SDS), allowed the authors to use the power of yeast genetics to discover changes in copper homeostasis, in addition to the already described changes in calcium [6] and iron [7] homeostasis. Moreover, they showed several ways to alleviate the growth defect caused by the *vps13* mutant. The first way shown in this Special Issue is overexpression of the *RCN2* gene, encoding the Rcn2 protein, which causes partial downregulation of calcineurin activity, a key calcium-dependent phosphatase, by inhibiting only the subset of catalytic complexes [3]. The second way is the increase in the cellular level of copper ions via simple supplementation with copper salts, treatment with copper ionophores, or genetic manipulation, resulting in an increase in copper uptake [2]. This is an interesting finding since copper ion homeostasis has already been shown to be affected in Alzheimer’s and Parkinson’s diseases and copper ionophores are in clinical trials. These results point to calcineurin and copper homeostasis as potential therapeutic targets suitable for testing in higher eukaryotic models of VPS13-related diseases. [...] We hope this issue will reach a wide audience in the scientific community and boost further research to obtain new insights into the molecular pathogenesis of these diseases and the development of new therapeutic strategies.”

- [1] Dziurdzik SK, Conibear E. 2021. The Vps13 family of lipid transporters and its role at membrane contact sites. Int J Mol Sci 22(6):2905 - <https://doi.org/10.3390/ijms22062905>
- [2] Soczewka P, Tribouillard-Tanvier D, di Rago JP, Zoladek T, Kaminska J. 2021. Targeting copper homeostasis improves functioning of *vps13 δ* yeast mutant cells, a model of VPS13-related diseases. Int J Mol Sci 22(5):2248 - <https://doi.org/10.3390/ijms22052248>
- [3] Wardaszka P, Soczewka P, Sienko M, Zoladek T, Kaminska J. 2021. Partial inhibition of calcineurin activity by Rcn2 as a potential remedy for Vps13 deficiency. Int J Mol Sci 22(3):1193 - <https://doi.org/10.3390/ijms22031193>
- [4] Park JS, Hollingsworth NM and Neiman AM. 2021. Genetic Dissection of Vps13 Regulation in yeast using disease mutations from human orthologs. Int J Mol Sci 22(12):6200 - <https://doi.org/10.3390/ijms22126200>

- [5] Kolakowski D, Rzepnikowska W, Kaniak-Golik A, Zoladek T, Kaminska J. 2021. The GTPase Arf1 is a determinant of yeast Vps13 localization to the Golgi apparatus. *Int J Mol Sci.* 2021. 22(22):12274 - <https://doi.org/10.3390/ijms222212274>
- 2 Soczewka P, Kolakowski D, Smaczynska-de Rooij I, Rzepnikowska W, Ayscough KR, Kaminska J, Zoladek T. 2019. Yeast-model-based study identified myosin- and calcium-dependent calmodulin signalling as a potential target for drug intervention in chorea-acanthocytosis. *Dis Model Mech* 12(1): dmm036830 - <https://doi.org/10.1242/dmm.036830>
- 3 Soczewka P, Flis K, Tribouillard-Tanvier D, di Rago JP, Santos CN, Menezes R, Kaminska J, Zoladek T. 2020. Flavonoids as potential drugs for *VPS13*-dependent rare neurodegenerative diseases. *Genes* 11(7):828 - <https://doi.org/10.3390/genes11070828>

VII International Centre for Brewing and Distilling, Heriot Watt University, Riccarton, Edinburgh, Scotland, EH14 4AS. Communicated by Graham G. Stewart <profggstewart@aol.com>.

Recent publications.

- 1 Stewart GG. 2019. Brewer's yeast research – a keynote paper – part 1. *Brew Sci* 72:31-49.

This is the first part of a two-part article that considers the research activities associated with brewer's yeast strains. Initially, this part discusses factors that have directly influenced such activities as financing, qualified and experienced researcher availability, the advent of much technology /instrumentation and also ale/lager yeast similarities and differences, wort fermentation with an influence

on the uptake and metabolism of sugars and free amino nitrogen and the production of beer flavour metabolites during fermentation (metabolomics). Finally, stress effects on yeast with an emphasis on the fermentation of high gravity wort are discussed. In the second part of this paper, yeast flocculation, centrifugation, genetic manipulation and the spontaneous mutation of yeast strains are considered.

- 2 Stewart GG. 2019. Brewer's yeast research – a keynote paper – part 2. *Brew Sci* 72:94-108.

Following a general review of current developments in brewer's yeast species and strains in Part 1, Part 2 considers more specific aspects. Flocculation, centrifugation, genetic manipulation and spontaneous mutation aspects of a broad spectrum of brewer's yeast strains are reviewed in detail. In

particular, the complexities of the flocculation phenomenon as they are affected by genetic, physiological and environmental factors (nature-nature effects) are considered. The prevalent hypothesis, the lectin-like theory of flocculation, is discussed together with the genes that control this phenomenon.

- 3 Hill A, Stewart GG. 2019. Free amino nitrogen in brewing. *Fermentation* 2019, Pub. by MDPI AG, Basel, Switzerland. 5:1-14.

The role of nitrogenous components in malt and wort during the production of beer has long been recognized. The concentration and range of wort amino acids impact on ethanolic fermentation by yeast

and on the production of a range of flavour and aroma compounds in the final beer. This review summarizes research on Free Amino Nitrogen (FAN) within brewing, including various methods of analysis.

- 4 Stewart GG. 2019. Genetic manipulation of *Saccharomyces sp* that produce ethanol related metabolites /enzymes and biomass. Module in Food Science, Pub. by Elsevier, Boston, Mass., pp. 1-38.

The importance of molecular biology with *Saccharomyces cerevisiae* strains, and its closely related species, is emphasized in many relevant publications. *S. cerevisiae* was the first microorganism to be employed for the production of many foods and beverages. In 1986, the complete DNA

sequence of a haploid *S. cerevisiae* strain was reported. Six thousand genes were identified in this strain but five thousand of them are thought to be non-essential for the function of the yeast cell. As well as hybridization with *a* and α mating type cells, spontaneous mutation (leading to, for example,

respiratory deficient cells), other genetic manipulation techniques including spheroplast (protoplast) fusion and rare mating have been employed as a means to introduce foreign genetic material into the yeast's genome. Recombinant DNA (rDNA) presents the possibility of introducing additional DNA characteristics into yeast strains in a controlled

manner. It allows the transfer of genetic information between yeast strains and species and also between completely unrelated organisms. In addition, multiple gene editing with techniques such as CRISPR enables rapid strain engineering of both wild type yeasts and industrial yeasts such as alcohol producing cultures.

5 Stewart GG, Ryder DS. 2019. Sulfur metabolism during brewing. *Tech Q Master Brew Assoc Am* 56:39-46.

Sulfur metabolism plays an important role during the brewing process, particularly, but not exclusively, as part of wort fermentation and beer maturation. The critical sulfur compounds are: hydrogen sulphide, sulfur dioxide, dimethyl sulfide and thiols and all of

them contribute to beer flavour. In addition, sulfur-containing amino acids are critical for yeast metabolism. Hop components containing sulfur also contribute to beer character, particularly the light struck reaction.

6 Stewart GG. 2019. A brief history of brewing in Canada – a personal view. *Brewery History* 179:71-89.

I joined the Labatt Brewing Company in London, Ontario, Canada. This move from the United Kingdom to Canada enhanced my fascination with yeast that still exists today! Throughout my time at Labatt's, I was able to continue a focus on brewer's yeast research. This activity has been financially supported by: Labatt's, the University of Western Ontario and various funding bodies of the Canadian Federal Government. As well as discussing Canadian

history and its evolution into a Federation, this article primarily considers the formation, progress and technical development of the three major breweries in Canada (Molson, Carling O'Keefe and Labatt). However, because of the author's background, more attention has been devoted to Labatt. It is hoped that the readers of this article will obtain interesting information on the history of the Canadian brewing industry, particularly its technical development.

7 Stewart GG, Speers RA. 2019. Yeast nutrition and metabolism: effects on beer flavor. *Tech Q Master Brew Assoc Am* 56:76-89.

The primary metabolites of wort fermentation are ethanol, carbon dioxide and glycerol. They do not directly affect beer flavor but they collectively influence beer character. Beer flavor is largely (but not exclusively) influenced by other wort fermentation products (also referred to as secondary metabolites). Flavor products from wort fermentation have been progressively characterised during the past fifty years or so. These developments have been assisted by the genesis of novel analytical instrumentation and by enhanced knowledge of overall yeast metabolism

(both nature-nurture influences) during wort fermentation. This paper attempts to correlate the progress of fermentation that will influence beer flavour and its stability. The major focus here is a review considering the control of the metabolic production of: higher alcohols, esters, carbonyls, sulfur compounds and phenolics that contribute to beer flavor. In addition, because of its importance as a contemporary brewing process, the question of high gravity wort fermentation and its influence on beer flavor is considered.

8 Stewart GG. 2020. Stresses imposed on yeast during brewing fermentations and their effect on cellular activity. *Tech Q Master Brew Assoc Am* 57:1-8.

Brewer's yeast strains are exposed to a spectrum of threats and "insults" that cultures endure during wort fermentation. Culture storage (including acid washing) and its propagation can also stress yeast metabolic systems. Stresses are a plethora of parameters that a yeast culture tolerates during its propagation, storage, and throughout and, between,

wort fermentations. Stress can result in a number of effects on a yeast culture, particularly its wort fermentation efficiency. These also include: decreases in yeast viability and vitality, depletion of intracellular glycogen and increases in trehalose, excretion of proteinase A and other proteinases with effects on beer foam stability. Shear of cell wall components can also

occur. In addition, modification of a culture's flocculation characteristics can result, together with the formation of unfilterable mannan-protein beer

hazes. Also, increases in respiratory deficient (petite) and other yeast mutants have been noted.

- 9 Stewart GG, Anstruther AM. 2021. Yeast flocculation, sedimentation, and centrifugation. Tech Q Master Brew Assoc Am 58:143-153.

The importance of flocculation in order to crop a brewer's yeast culture at the end of primary wort fermentation, in order that the yeast culture can be reused during a subsequent fermentation, cannot be overstated. Modification to either a culture's flocculent or non-flocculent characteristics can affect (in a different manner) its overall wort fermentation performance. Flocculation is a yeast cell surface phenomenon and cell wall structure is critical and appropriate findings on this aspect are discussed here. There are many definitions of yeast flocculation but the one that has been employed for many years, almost

universally, would appear to be that: "flocculation is the phenomenon wherein yeast cells adhere in clumps and either sediment from the medium (usually wort) in which they are suspended or rise to the medium's surface". This definition excludes forms of cell chain formation which has been discussed separately in this review. Microamounts of calcium are necessary for the flocculation of brewer's yeast strains. The sedimentation performance of a brewer's yeast strain is often modified during repeated pitching in a brewing fermentation.

- 10 Russell I, Stewart GG, Kellershohn J (eds.). 2021. Whisky and Other Spirits – technology, production and marketing, 3rd Edition, Elsevier/Academic Press, Boston, USA. ISBN 978-0-12-822907-4.

The first two editions of this book, published in 2003 and 2014, met a need for a modern text covering the technology, production and marketing of whiskies internationally. The 3rd edition covers a much wider field of potable spirits (gin, vodka, rum, Korean soju

and saké) – an innovative category that continues to expand worldwide. Whilst all aspects of the processes are considered, yeast and fermentation receives particular attention!

VIII 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 Jacobus AP, Gross J, Evans JH, Ceccato-Antonini SR, Gombert AK. 2021. *Saccharomyces cerevisiae* strains used industrially for bioethanol production. Essays Biochem 65(2):147-161. doi: 10.1042/EBC20200160. PMID: 34156078.
- 2 Pendón MD, Madeira JV Jr, Romanin DE, Rumbo M, Gombert AK, Garrote GL. 2021. A biorefinery concept for the production of fuel ethanol, probiotic yeast, and whey protein from a by-product of the cheese industry. Appl Microbiol Biotechnol 105(9):3859-3871. doi: 10.1007/s00253-021-11278-y. Epub 2021 Apr 16. PMID: 33860834.
- 3 Rác HV, Mukhtar F, Imre A, Rádai Z, Gombert AK, Rátónyi T, Nagy J, Pócsi I, Pfliegler WP. 2021. How to characterize a strain? Clonal heterogeneity in industrial *Saccharomyces* influences both phenotypes and heterogeneity in phenotypes. Yeast 38(8):453-470. doi: 10.1002/yea.3562. Epub 2021 May 5. PMID: 33844327.
- 4 Rodrigues CIS, Wahl A, Gombert AK. 2021. Aerobic growth physiology of *Saccharomyces cerevisiae* on sucrose is strain-dependent. FEMS Yeast Res 21(3):foab021. doi: 10.1093/femsyr/foab021. PMID: 33826723.

Ph.D. Thesis recently defended.

- 5 Carla Inês Rodrigues Soares. Quantitative analysis of *Saccharomyces cerevisiae*'s growth and metabolism on sucrose. Delft University of Technology; promoters Andreas K. Gombert, Mark van Loosdrecht and Aljoscha Wahl.

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

Recent publications.

- 1 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, Péter 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. 2021. Nomenclatural issues concerning cultured yeasts and other fungi: why it is important to avoid unneeded name changes. *IMA Fungus* 12: 1-20.

The unambiguous application of fungal names is important to communicate scientific findings. Names are critical for (clinical) diagnostics, legal compliance, and regulatory controls, such as biosafety, food security, quarantine regulations, and industrial applications. Consequently, the stability of the taxonomic system and the traceability of nomenclatural changes is crucial for a broad range of users and taxonomists. The unambiguous application of names is assured by the preservation of nomenclatural history and the physical organisms representing a name. Fungi are extremely diverse in terms of ecology, lifestyle, and methods of study. Predominantly unicellular fungi known as yeasts are usually investigated as living cultures. Methods to characterize yeasts include physiological (growth) tests and experiments to induce a sexual morph; both methods require viable cultures. Thus, the preservation and availability of viable reference cultures are important, and cultures representing reference material are cited in species descriptions.

Historical surveys revealed drawbacks and inconsistencies between past practices and modern requirements as stated in the International Code of Nomenclature for Algae, Fungi, and Plants (ICNafp). Improper typification of yeasts is a common problem, resulting in a large number of invalid yeast species names. With this opinion letter, we address the problem that culturable micro-organisms, notably some fungi and algae, require specific provisions under the ICNafp. We use yeasts as a prominent example of fungi known from cultures. But viable type material is important not only for yeasts, but also for other cultivable Fungi that are characterized by particular morphological structures (a specific type of spores), growth properties, and secondary metabolites. We summarize potential proposals which, in our opinion, will improve the stability of fungal names, in particular by protecting those names for which the reference material can be traced back to the original isolate.

- 2 Sannino C, Borruso L, Mezzasoma A, Battistel D, Ponti S, Turchetti B, Buzzini P, Guglielmin M. 2021. Abiotic factors affecting the bacterial and fungal diversity of permafrost in a rock glacier in the Stelvio Pass (Italian Central Alps). *Appl Soil Ecol* 166:104079.

The impact of climate change in the European Alps has been roughly twice the global average, dramatically reducing permafrost extent and thickening of its active layer. Therefore, the study of the abiotic factors (i.e. chemical/physical parameters) affecting the microbial diversity inhabiting Alpine permafrost appears to be of dramatic relevance. Within the European Alps, the Stelvio area exhibits these effects in a particularly evident way, with

important consequences on microbial ecosystems. Therefore, microbial communities inhabiting a permafrost core collected in the Scorzuzo active rock glacier (Stelvio Pass, Italian Central Alps) were investigated along a depth gradient (410 to 524 cm from the surface). The taxonomic structures of bacterial and fungal communities were investigated via a next-generation sequencing (NGS) approach (Illumina MiSeq), targeting the bacterial V3-V4

regions of 16S rDNA and the fungal ITS2 region. Abiotic soil factors (grain size, electrical conductivity, ice/water content, pH, Loss-on-Ignition - LOI, total and organic carbon, nitrogen and phosphorous) were analysed. Richness and Shannon-H diversity indices were correlated to abiotic factors. Bacterial diversity was significantly ($p < 0.05$) correlated with LOI, while fungal diversity was significantly ($p < 0.05$) correlated with the depth gradient. The Constrained Analysis of Principal (CAP) coordinates were used to study the correlation between abiotic parameters and the taxonomic structure of bacterial and fungal communities. Among all tested variables, the depth gradient, water content, pH and LOI affected the taxonomic structure of bacterial communities (in particular, the abundance of bacterial amplicon sequence variants - ASVs - assigned to *Afipia* sp., *Chloroflexi*, *Gaiella* sp.,

Oryzihumus sp. and *Serratia* sp.), while fungal communities (ASVs assigned to *Naganishia* sp., *Rhodotorula* sp., Sordariomycetes and Taphrinales) were affected by the depth gradient. Co-occurrences (calculated by Pearson correlation coefficient) among microbial taxa (i.e. bacteria vs bacteria, bacteria vs fungi, fungi vs fungi) were investigated: the prevalence of significant ($p < 0.05$) positive co-occurrences was found, suggesting that the coexistence of different microbial taxa could play a crucial role in maintaining the ecological and taxonomic balance of both bacterial and fungal communities inhabiting the Alpine permafrost ecosystem. These findings suggest that the bacterial and fungal diversity of Alpine permafrost are affected in different ways by some abiotic factors.

- 3 Canini F, Geml J, Buzzini P, Turchetti B, Onofri S, D'Acqui LP, Ripa C, Zucconi L. 2021. Growth forms and functional guilds distribution of soil fungi in coastal versus inland sites of Victoria Land, Antarctica. *Biology* 10:320.

In Victoria Land, Antarctica, ice-free areas are restricted to coastal regions and dominate the landscape of the McMurdo Dry Valleys. These two environments are subjected to different pressures that determine the establishment of highly adapted fungal communities. Within the kingdom of fungi, filamentous, yeasts and meristematic/microcolonial growth forms on one side and different lifestyles on the other side may be considered adaptive strategies of particular interest in the frame of Antarctic constraints. In this optic, soil fungal communities from both coastal and Dry Valleys sites, already characterized

through ITS1 metabarcoding sequencing, have been compared to determine the different distribution of phyla, growth forms, and lifestyles. Though we did not find significant differences in the richness between the two environments, the communities were highly differentiated and Dry Valleys sites had a higher evenness compared to coastal ones. Additionally, the distribution of different growth forms and lifestyles were well differentiated, and their diversity and composition were likely influenced by soil abiotic parameters, among which soil granulometry, pH, P, and C contents were the potential main determinants.

- 4 Canini F, Geml J, D'Acqui LP, Buzzini P, Turchetti B, Onofri S, Ventura S, Zucconi L. 2021. Fungal diversity and functionality are driven by soil texture in Taylor Valley, Antarctica. *Fungal Ecol* 50:101041.

The McMurdo Dry Valleys surface is mainly constituted from unconsolidated permafrost. Despite the combination of cold and dry conditions, transiently wetted soils close to lake edges are hotspots of intense biological activity, that can support the surrounding soil ecosystems in such extreme environments. These soils host simple microbial communities that allow easy characterization of the parameters determining microbial establishment and diversification. Soil samples were collected close to three different glacial lakes (Lake Fryxell, Lake Hoare and Lake Joyce) located along a longitudinal gradient from the lower to the upper Taylor Valley. Fungal diversity and functionality of sampled soils were studied through

ITS1 metabarcoding sequencing. The correlation between the parameters describing fungal diversity (i.e. total richness, relative richness of dominant taxonomic and functional groups, and community composition) and the edaphic physicochemical parameters (i.e. pH, moisture, C, N, P, Na⁺, K⁺, Mg²⁺ and Ca²⁺, cation exchange capacity, and soil granulometry) was assessed. The fungal communities showed low richness (48 ± 32 OTUs per sample). Their composition was highly diversified even within different sites close to the same lake. The main parameters affecting the diversity and composition of fungal communities were soil texture, in turn influencing the retention of water and nutrients, and

physicochemical properties. This is of particular concern for the survival of these communities, given

the expected environmental changes due to global warming.

- 5 Rapoport A, Guzhova I, Bernetti L, Buzzini P, Kieliszek M, Kot AM. 2021. Carotenoids and some other pigments from fungi and yeasts. *Metabolites* 11:92.

Carotenoids are an essential group of compounds that may be obtained by microbiological synthesis. They are instrumental in various areas of industry, medicine, agriculture, and ecology. The increase of carotenoids' demand at the global market is now essential. At the moment, the production of natural carotenoids is more expensive than obtaining their synthetic forms, but several new approaches/directions on how to decrease this difference were developed during the last decades. This review briefly describes

the information accumulated until now about the beneficial effects of carotenoids on human health protection, their possible application in the treatments of various diseases, and their use in the food and feed industry. This review also describes some issues that are linked with biotechnological production of fungal and yeasts carotenoids, as well as new approaches /directions to make their biotechnological production more efficient.

- 6 Castellini M, Ubertini S, Barletta D, Baffo I, Buzzini P, Barbanera M. 2021. Techno-economic analysis of biodiesel production from microbial oil using cardoon stalks as carbon source. *Energies* 14:1473.

Today one of the most interesting ways to produce biodiesel is based on the use of oleaginous microorganisms, which can accumulate microbial oil with a composition similar to vegetable oils. In this paper, we present a thermo-chemical numerical model of the yeast biodiesel production process, considering cardoon stalks as raw material. The simulation is performed subdividing the process into the following sections: steam explosion pre-treatment, enzymatic hydrolysis, lipid production, lipid extraction, and alkali-catalyzed trans-esterification. Numerical results show that 406.4 t of biodiesel can be produced starting from 10,000 t of lignocellulosic biomass. An

economic analysis indicates a biodiesel production cost of 12.8 USD/kg, thus suggesting the need to increase the capacity plant and the lipid yield to make the project economically attractive. In this regard, a sensitivity analysis is also performed considering an ideal lipid yield of 22% and 100,000 t of lignocellulosic biomass. The biodiesel production costs related to these new scenarios are 7.88 and 5.91 USD/kg, respectively. The large capacity plant combined with a great lipid yield in the fermentation stage shows a biodiesel production cost of 3.63 USD/kg making the product competitive on the current market of biofuels by microbial oil.

- 7 Barbanera M, Castellini M, Tasselli G, Turchetti B, Cotana F, Buzzini P. 2021. Prediction of the environmental impacts of yeast biodiesel production from cardoon stalks at industrial scale. *Fuel* 283:118967.

The study aims to develop a full-scale plant to produce yeast biodiesel from cardoon stalks, based on the biorefinery concept, and to evaluate its environmental burden using a cradle-to-gate, scaled-up LCA approach. The results identified the following environmental hotspots all over the whole chain: the production of enzymes required in the hydrolysis of steam-exploded biomass and the heat and organic solvents required for lipid extraction and the subsequent distillation steps. The analysis of the LCA

normalization results revealed that the highest environmental burdens were associated with human carcinogenic toxicity (69.2%). A further interesting result was that the GHG emissions associated to yeast biodiesel are negative (-1.5 g CO₂ eq/MJ), due to the environmental credits generated by the co-products. Comparison with other biodiesel sources were also carried out showing that yeast biodiesel from cardoon stalks has the potential to become a competitive alternative from an environmental perspective.

Recent publications.

- 1 Caridi A. 2021. Selection of Calabrian strains of *Saccharomyces sensu stricto* for red wines. *Acta Alimentaria*, 50(4):565-573 - <https://doi.org/10.1556/066.2021.00119>

Phenolic compounds provide important quality attributes to red wines interacting with the organoleptic impact of wines. Yeast mannoproteins can interact with grape phenolic compounds, responsible for colour and antioxidant activity of wines. The aim of this work was to perform oenological characterisation and specific selection of Calabrian strains of *Saccharomyces sensu stricto*. Among the considered traits, the aptitude of the yeast

to preserve grape pigments and colour intensity was included. Among the best six yeast strains – Sc2731, Sc2742, Sc2756, Sc2773, Sc2774, and Sc2823 – strain Sc2742 exhibits the highest Folin–Ciocalteu index and strain Sc2774 the highest colour intensity. These two selected yeasts may be used as starter for the production of red wines in order to preserve grape pigments and colour intensity.

- 6 Caridi A, Romeo R, De Bruno A, Masaneo C, Poiana M. 2021. Long-term effects of different starter yeasts on colour and natural antioxidant power of red wines. *European Food Research and Technology*, 247(9):2391-2398 - <https://doi.org/10.1007/s00217-021-03800-3>.

Eighty-seven red wines produced from red grapes of the grape variety *Gaglioppo* from the Calabria region (vintage 2009) were analysed by standard methods after 4 and 120 months of ageing. So, a total of 29 selected starters of *Saccharomyces cerevisiae* - 3 wild type, 12 monoclonal cultures, and 14 hybrids - inoculated in triplicate were studied for their effects on colour and natural antioxidant power keeping of red

wine. Wine ageing decreased the red component, the colour intensity, and the DPPH values while the colour hue values increased. This research has shown that the evolution of wine ageing is affected in a very different way by the starter yeast used and has allowed choosing the best yeast strain useful to produce red wine able to endure very long-time ageing, until 10 years.

- 7 Caridi A. 2021. Physiological characterisation of Calabrian dairy yeasts and their possible use as adjunct cultures for cheese making. *Acta Alimentaria* 50(3):341-348. <https://doi.org/10.1556/066.2021.00001>.

Seventeen samples of Calabrian ewe's milk, ewe's cheese (*Pecorino del Poro*) made with raw milk, goat's milk, and goat's cheese (*Caprino d'Aspromonte*) made with raw milk were used to obtain 124 yeast isolates. The most abundant species was *Debaryomyces hansenii* (61.3%), followed by *Candida zeylanoides* (32.3%) and *Kluyveromyces marxianus*

(3.2%). The enzymatic profile of 25 selected yeast strains was determined. Lastly, they were studied for their interaction with eight dairy lactic acid bacteria – four coccal-shaped and four rod-shaped. The best strains may be used as adjunct cultures for cheese making.

- 8 Caridi A, Sidari R, Pulvirenti A, Blaiotta G, Ritieni A. 2021. Clonal selection of wine yeasts with differential adsorption activities towards phenolics and ochratoxin A. *Food Biotechnology*, *in press*. <https://doi.org/10.1080/08905436.2021.2006064>.

Recent publications.

- 1 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. 2021. Substrate, temperature, and geographical patterns among nearly 2,000 natural yeast isolates. *Yeast* epub - DOI: <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.

- 2 Lee SB, Tremaine M, Place M, Liu L, Pier A, Krause DJ, Xie D, Zhang Y, Landick R, Gasch AP, Hittinger CT, Sato TK. 2021. Crabtree/Warburg-like aerobic xylose fermentation by engineered *Saccharomyces cerevisiae*. *Metab Eng* 68:119-130. DOI: 10.1016/j.ymben.2021.09.008.

Bottlenecks in the efficient conversion of xylose into cost-effective biofuels have limited the widespread use of plant lignocellulose as a renewable feedstock. The yeast *Saccharomyces cerevisiae* ferments glucose into ethanol with such high metabolic flux that it ferments high concentrations of glucose aerobically, a trait called the Crabtree/Warburg Effect. In contrast to glucose, most engineered *S. cerevisiae* strains do not ferment xylose at economically viable rates and yields, and they require respiration to achieve sufficient xylose metabolic flux and energy return for growth aerobically. Here, we evolved respiration-deficient *S. cerevisiae* strains that can grow on and ferment xylose to ethanol aerobically, a trait analogous to the Crabtree/Warburg Effect for glucose. Through genome sequence comparisons and directed engineering, we determined that duplications of genes encoding engineered xylose metabolism enzymes, as

well as TKL1, a gene encoding a transketolase in the pentose phosphate pathway, were the causative genetic changes for the evolved phenotype. Reengineered duplications of these enzymes, in combination with deletion mutations in HOG1, ISU1, GRE3, and IRA2, increased the rates of aerobic and anaerobic xylose fermentation. Importantly, we found that these genetic modifications function in another genetic background and increase the rate and yield of xylose-to-ethanol conversion in industrially relevant switchgrass hydrolysate, indicating that these specific genetic modifications may enable the sustainable production of industrial biofuels from yeast. We propose a model for how key regulatory mutations prime yeast for aerobic xylose fermentation by lowering the threshold for overflow metabolism, allowing mutations to increase xylose flux and to redirect it into fermentation products.

- 3 Čadež N, Bellora N, Ulloa R, Tome M, Petković H, Groenewald M, Hittinger CT, Libkind D. 2021. *Hanseniaspora smithiae* sp. nov., a novel apiculate yeast species from Patagonian forests that lacks the typical genomic domestication signatures for fermentative environments. *Front Microbiol* 12:679894. DOI: 10.3389/fmicb.2021.679894.

During a survey of *Nothofagus* trees and their parasitic fungi in Andean Patagonia (Argentina), genetically distinct strains of *Hanseniaspora* were obtained from the sugar-containing stromata of parasitic *Cyttaria* spp. Phylogenetic analyses based on the single-gene sequences (encoding rRNA and actin) or on conserved, single-copy, orthologous genes from genome sequence assemblies revealed that these strains represent a new species closely related to *Hanseniaspora valbyensis*. Additionally, delimitation of this novel species was supported by genetic distance calculations using overall genome relatedness indices (OGRI) between the novel taxon and its closest relatives. To better understand the mode of speciation

in *Hanseniaspora*, we examined genes that were retained or lost in the novel species in comparison to its closest relatives. These analyses show that, during diversification, this novel species and its closest relatives, *H. valbyensis* and *Hanseniaspora jakobsenii*, lost mitochondrial and other genes involved in the generation of precursor metabolites and energy, which could explain their slower growth and higher ethanol yields under aerobic conditions. Similarly, *Hanseniaspora mollemarum* lost the ability to sporulate, along with genes that are involved in meiosis and mating. Based on these findings, a formal description of the novel yeast species *Hanseniaspora smithiae* sp. nov. is proposed, with CRUB 1602H as the holotype.

- 4 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, Péter 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. 2021. 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.

The abstract of this paper can be found under Dr. Buzzini's entry (IX).

- 5 Steenwyk JL, Phillips MA, Yang F, Date SS, Graham TR, Berman J, Hittinger CT, Rokas A. Under review. An orthologous gene coevolution network provides insight into eukaryotic cellular and genomic structure and function. *bioRxiv* under review. DOI: 10.1101/2021.07.09.451830

Orthologous gene coevolution—which refers to gene pairs whose evolutionary rates covary across speciation events—is often observed among functionally related genes. We present a comprehensive gene coevolution network inferred from the examination of nearly three million orthologous gene pairs from 332 budding yeast species spanning ~400 million years of eukaryotic evolution. Modules within the network provide insight into cellular and genomic structure and function, such as genes functioning in distinct cellular compartments and DNA replication. Examination of the phenotypic impact of network perturbation across 14 environmental conditions using deletion mutant data from the baker's yeast *Saccharomyces cerevisiae*

suggests that fitness in diverse environments is impacted by orthologous gene neighborhood and connectivity. By mapping the network onto the chromosomes of *S. cerevisiae* and the opportunistic human pathogen *Candida albicans*, which diverged ~235 million years ago, we discovered that coevolving orthologous genes are not clustered in either species; rather, they are most often located on different chromosomes or far apart on the same chromosome. The budding yeast coevolution network captures the hierarchy of eukaryotic cellular structure and function, provides a roadmap for genotype-to-phenotype discovery, and portrays the genome as an extensively linked ensemble of genes.

Recent publications.

- 1 Kachalkin AV, Abdullabekova DA, Magomedova ES, Yurkov AM. 2021. *Zygotorulaspora dagestanica* sp. nov., a novel ascomycetous yeast species associated with the Georgian honeysuckle (*Lonicera iberica* M. Bieb.) Int J Syst Evol Microbiol 71(4) - DOI: 10.1099/ijsem.0.004785

During an investigation of the yeast communities associated with wild fruit shrubs in Dagestan (Caucasus, Russia), four fermenting ascospore-producing yeast strains were isolated from leaves of the Georgian honeysuckle (*Lonicera iberica* M. Bieb.) and from soil underneath this plant. Phylogenetic analyses based on concatenated sequences of the ITS region and D1/D2 domains of the large subunit rRNA gene and concatenated sequences of the ribosomal DNA cystron, RPB2 and TEF1 genes showed that the isolated strains represented a new species of the genus

Zygotorulaspora. The new species was placed in the basal position to other species of the clade and close to *Zygotorulaspora mrakii*. Based on the results of phylogenetic analyses and the phenotypic characteristics of the four studied strains, a novel species is described, for which the name *Zygotorulaspora dagestanica* sp. nov. is proposed. The holotype is KBP Y-4591T, three metabolically inactive cryopreserved isotype cultures are DSM 100088, VKM Y-3060 and VKPM Y-4318. The MycoBank number is MB 838285.

- 2 Dmitrieva AS, Maksimova IA, Kachalkin AV, Markov AV. 2021. Age-related changes in the yeast component of the *Drosophila melanogaster* microbiome. Microbiology 90(2):229–236. DOI: 10.1134/S0026261721020028

The present work describes the quantitative and qualitative composition of the yeast component of the *D. melanogaster* microbiome in three laboratory lines, reared under different conditions, at four developmental stages: late larvae and adults aged 1, 7, and 14 days after eclosion. In all three lines, the total yeast abundance changed similarly with the age of insects, with the highest and lowest yeast counts in 7- and 1-day adults, respectively. In the fly lines reared on

moderately unfavorable substrates supplemented with 2 and 4% NaCl, the abundance and species diversity of yeasts at all four developmental stages was higher than in the flies reared on a standard (favorable) food substrate. Our results indicate the inconstancy of the yeast component of the *D. melanogaster* microbiome and its regular changes with the insect's age, which must be taken into account when studying the relationships between symbiotic yeasts and their hosts.

- 3 Kachalkin AV, Glushakova AM, Venzhik AS. 2021. Presence of clinically significant endophytic yeasts in agricultural crops: monitoring and ecological safety assessment. IOP Conf. Series: Earth and Environmental Science 723(4):042005. DOI: 10.1088/1755-1315/723/4/042005

Endophytic yeasts from internal tissues of 54 names of fruits, nuts and vegetables from 36 countries were studied. In total, 74 species of yeasts were isolated from analysed agricultural crops. The study revealed high values of occurrence and abundance of

clinically significant yeasts *Candida parapsilosis* and *Meyerozyma guilliermondii* in some internal tissues that may pose a potential threat to human health, primarily for immunocompromised individuals.

- 4 Abdullabekova DA, Magomedova ES, Magomedov GG, Kachalkin AV. 2021. Yeasts as an element of ampelocenosis soil biodiversity in an arid climate. Arid Ecosystems 11(3):299–303. DOI: 10.1134/S2079096121030021

The results of a study of the light chestnut soil of an ampelocenosis located in a zone with a semidesert-steppe regime and a low hydrothermal coefficient are

presented. The study of the taxonomic composition of yeasts, which can act as a model group of microorganisms in the study of soil-microbiological

processes, has shown the ability of this soil to play the role of a biological reservoir for storage and distribution in the natural cycle of various physiological groups of yeasts. The accumulation of allochthonous

yeasts, as well as other groups of microorganisms, influencing the biodiversity of the ampelocenosis soil, expands the potential for the participation of mycobiota in soil processes.

- 5 Crous PW et al. 2021. Fungal Planet description sheets: 1182–1283. *Persoonia* 46:313–528. DOI: 10.3767/persoonia.2021.46.11

Novel species of yeasts described in this study:

Metschnikowia taurica Kachalkin, A.M. Glushakova & M.A. Tomashevskaya, sp. nov. from fruits of *Ziziphus jujube*;

Teunia lichenophila Kachalkin, M.A. Tomashevskaya & T.A. Pankratov, sp. nov. as endophyte from lichen *Cladonia rangiferina*

- 6 Zueva AI, Kachalkin AV, Maksimova IA, Nguyen Van Thinh, Tiunov AV. 2021. Properties of the yeast communities associated with termites of a tropical monsoon forest: Cat Tien National Park, Vietnam. *Microbiology* 90(4):489–499. DOI: 10.1134/S0026261721040172

Yeast populations were investigated for the following substrates associated with termitaria of five termite species occurring in the Cat Tien National Park (south Vietnam): nest walls, fungal gardens (if present), and termite gut and body surface, as well as for the litter and upper soil horizons near the termitaria. The isolated fungi with yeast type of growth were assigned to 39 species, 10 of which have not been described at the time of this study. The yeast communities developing in termite guts and on their

body surface exhibited low species diversity. The yeasts isolated from termite guts and body surface were characterized by a relatively broad spectrum of morphological and physiological properties (24 to 33 parameters out of the 34 ones in the standard description). The most common properties were the presence of mycelial or pseudomycelial growth, as well as ability to ferment glucose, use plant glycosides (salicin) and simple products of wood degradation (xylose and cellobiose) as growth substrates.

- 7 Kuznetsova TA, Vecherskii MV, Khayrullin DR, Stepankov AA, Maximova IA, Kachalkin AV, Ushakova NA. 2021. Dramatic effect of the black soldier fly larvae on fungal community of a compost. *J Sci Food Agric* - DOI: 10.1002/jsfa.11601

Black soldier fly larvae (BSFL) offer very good prospects for the production of cheap and high-quality dietary protein. This insect is able to consume low-quality substrates, including food waste. The purpose of this study was to investigate the fungal community of the compost produced by BSF larvae reared on a food waste substrate. It was found that the primary mycobiome of the food waste substrate that was used consisted of 19 families, represented mainly by phytopathogenic and endophytic genera. Larva incubation

led to the complete elimination of all mycelial fungi from the resulting compost. The final mycobiome consisted of only two yeast species, *Pichia kudriavzevii* and *Diutina rugosa*, with a total abundance of 1.2×10^7 CFU g⁻¹. The rearing of BSFL led to the complete elimination of mycelial fungi from its feed substrate. The final compost lacked harmful fungi, including molds. This information may be crucial for BSF compost utilization.

XIII 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 <d.p.navarro@ibv.uio.no> and <david.peris@campusviu.es>.

New paper.

- 1 Bendixsen, P, Peris D, Stelkens R. 2021. Patterns of genomics instability in interspecific yeast hybrids with diverse ancestries. *Frontiers Fungal Biol* 2:52

The genomes of hybrids often show substantial deviations from the features of the parent genomes, including genomic instabilities characterized by chromosomal rearrangements, gains, and losses. This plastic genomic architecture generates phenotypic diversity, potentially giving hybrids access to new ecological niches. It is however unclear if there are any generalizable patterns and predictability in the type and prevalence of genomic variation and instability across hybrids with different genetic and ecological backgrounds. Here, we analyzed the genomic architecture of 204 interspecific *Saccharomyces* yeast hybrids isolated from natural, industrial fermentation, clinical, and laboratory environments. Synchronous mapping to all eight putative parental species showed significant variation in read depth indicating frequent aneuploidy, affecting 44% of all hybrid genomes and particularly smaller chromosomes. Early generation hybrids with largely

equal genomic content from both parent species were more likely to contain aneuploidies than introgressed genomes with an older hybridization history, which presumably stabilized the genome. Shared k-mer analysis showed that the degree of genomic diversity and variability varied among hybrids with different parent species. Interestingly, more genetically distant crosses produced more similar hybrid genomes, which may be a result of stronger negative epistasis at larger genomic divergence, putting constraints on hybridization outcomes. Mitochondrial genomes were typically inherited from the species also contributing the majority nuclear genome, but there were clear exceptions to this rule. Together, we find reliable genomic predictors of instability in hybrids, but also report interesting cross- and environment-specific idiosyncrasies. Our results are an important step in understanding the factors shaping divergent hybrid genomes and their role in adaptive evolution.

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Ph.D. theses.

- 1 Jule Brandenburg (2021). Lipid production from lignocellulosic material by oleaginous yeasts. Swedish University of Agricultural Science, Uppsala, Sweden. Doctoral Thesis No. 2021: 30.

<https://pub.epsilon.slu.se/23277/>

Oleaginous yeasts are a potential lipid source for production of fuels, chemicals and food or feed and use of lignocellulosic biomass as feedstock is considered a sustainable approach. Pre-treatment of lignocellulose is required to release the major carbon sources, glucose, xylose and other sugars for lipid production by oleaginous yeasts, but also releases inhibitory compounds. Aim of this thesis was to examine the potential for producing lipids from different lignocellulosic substrates using oleaginous yeasts and to develop analytical methods for monitoring the kinetics of lipid accumulation, as a basis for further investigations of physiological differences in oleaginous yeasts on different substrates. Investigations of 29 different oleaginous

yeast strains revealed considerable differences in xylose utilisation capacity, even among very closely related strains. Some strains were very efficient in accumulating lipids from all carbon sources in lignocellulose hydrolysate, others showed no or only weak growth on xylose and in one case intracellular lipid degradation during consumption of xylose was observed. Further investigation demonstrated that it is possible to combine furfural production from hemicellulose and microbial lipid or ethanol production from the cellulose fraction of wheat straw material. An investigation of lipid production from birch wood hemicellulose containing high amounts of xylose and acetic acid revealed that by establishing a pH-regulated feeding strategy, acetic acid could be

utilised as an additional carbon source and no growth inhibition was observed. Target parameters when studying lipid-accumulating yeasts are intracellular lipid content and lipid profile. However, classical extraction-based analytical methods are time- and work intensive. Therefore, a non-invasive method based on high-throughput Fourier transform infrared

(FTIR) spectroscopy was established. Overall, large diversity among oleaginous yeasts was revealed, especially when converting xylose. Promising strains for lipid production from different substrates were identified, providing a baseline for further studies on the physiology of oleaginous yeasts and on biotechnological production of microbial lipids.

- 2 Mikolaj Chmielarz. 2021. Conversion of lignocellulose and crude glycerol to lipids by oleaginous yeasts. Physiology and diversity. Swedish University of Agricultural Science, Uppsala, Sweden. Doctoral Thesis No. 2021:53 <https://pub.epsilon.slu.se/24912/>

Oleaginous yeasts are organisms capable of accumulating lipids. Some of them can grow on various substrates considered to be too toxic for many other microorganisms. Crude glycerol (CG) and hemicellulose hydrolysate (HH) are two examples of such substrates. Lipid quantification by extraction is a time-consuming process which requires usage of organic solvents and strong acids when applied on yeasts. To address this, we developed a new method using Fourier-transform near infra-red (FT-NIR) spectroscopy to quantify lipids within yeast cells. The resulting model for *Rhodotorula toruloides* had a R^2 of 98% and a 5% error in prediction when compared with the traditional lipid extraction method. The method was used to follow lipid formation kinetics in subsequent experiments. Out of 27 tested oleaginous yeast strains, less than half could grow on crude

glycerol. Two strains, *R. toruloides* CBS14 and *Rhodotorula glutinis* CBS3044 grew well on this substrate, and were even activated when it was supplemented with hemicellulose hydrolysate (producing up to 12.5 g/L lipids). RNA sequencing in *R. toruloides* CBS14 revealed increased transcription of genes related to energy metabolism, mitochondrial enzymes and genes involved in protein synthesis. There were only little differences in genes related to glycerol metabolic pathways. Probably, cells grown in CGHH have a more efficient energy metabolism and thus, more ATP to build up biomass and lipids, and to take up substrate from the medium. The new method for lipid quantification and the identified regulated genes can be the basis for further manipulations of yeast metabolism to reach sustainable microbial oil production from residual substrates.

Recent publications.

- 3 Martín-Hernandez GC, Müller B, Chmielarz M, Brandt C, Hölzer M, Viehweger A, Passoth V. 2021. Chromosome-level genome assembly and transcriptome-based annotation of the oleaginous yeast *Rhodotorula toruloides* CBS 14. *Genomics* 113, 4022-4026. <https://doi.org/10.1016/j.ygeno.2021.10.006>
- 4 Sanya DRA, Onésime D, Passoth V, Maiti MK, Chattopadhyay A, Khot MB. 2021. Yeasts of the *Blastobotrys* genus are promising platforms for lipid- based fuels and oleochemicals production. *Appl Microbiol Biotechnol* 105, 4879-4897, <https://doi.org/10.1007/s00253-021-11354-3>
- 5 Brandenburg J, Blomqvist J, Shapaval V, Kohler A, Sampels S, Sandgren M, Passoth V. 2021. Oleaginous yeasts respond differently to carbon sources present in lignocellulose hydrolysate. *Biotechnol Biofuels* 14, 124. <https://doi.org/10.1186/s13068-021-01974-2>
- 6 Chmielarz M, Blomqvist J, Sampels S, Sandgren M, Passoth V 2021. Microbial lipid production from crude glycerol and hemicellulosic hydrolysate with oleaginous yeasts. *Biotechnol Biofuels* 12, 65. <https://doi.org/10.1186/s13068-021-01916-y>
- 7 Laurent J, Struyf N, Bautil A, Bakeeva A, Chmielarz M, Lylyy M, Herrera-Malaver B, Passoth V, Verstrepen KJ, Courtin CM. 2021. The potential of *Kluyveromyces marxianus* to produce low-FODMAP straight-dough and sourdough bread: a pilot study. *Food Bioprocess Technol.* <https://doi.org/10.1007/s11947-021-02681-4>

I have received the visit of Dr. Kyria Bundy-Mills, Curator of the Phaff Yeast Culture Collection at the University of California, Davis. The purpose was to plan for the forthcoming move of my research collection to Davis, where it will be merged with the Phaff collection. Many of the new accessions will be added to the public catalogue, making it possible to conduct extensive biogeographic studies on certain

species for which large numbers of isolates have been preserved, along with geographic coordinates, barcode sequences, and growth test responses. Dr. Bundy-Mills obtained a substantial grant from the National Science Foundation in support of the move of some 5000 cryogenically stored cultures and the forthcoming management of the strains.

I am delighted to report that my last graduate student, Dong Kyung Lee, successfully defended his M.Sc. thesis recently.

- 1 Lee DK. 2021. *Metschnikowia* mitochondria. M.Sc. thesis, Department of Biology, University of Western Ontario, London, Ontario, Canada

Mitochondrial genomes are known for their diverse characteristics and are an attractive model to study genome evolution. Draft nuclear genomes of 71 *Metschnikowia* yeast strains are publicly available but their mitochondrial genome assemblies are incomplete, thereby making genome studies difficult. To remediate this shortcoming, complete mitochondrial genomes of 71 *Metschnikowia* strains were assembled from the draft nuclear genomes. *Metschnikowia* mitochondrial genomes exhibit an unprecedented amount of diversity, particularly with respect to the

frequency and distribution of introns, which is often reflected upon overall genome size variations. Additionally, loss of synteny between strains of the same species further strengthens the notion that mitochondrial genomes evolve differently from their host genomes. Diversities shown from multiple genome characteristics explored in this thesis therefore highlight the importance of mitochondrial genomes for studying evolution and diversity of genomes that were often neglected.

The abstract of a preliminary report appeared in the June 2020 issue of the Yeast Newsletter. The complete citation is given here.

- 2 Lee DK, Hsiang T, Lachance MA, Smith DR. The strange mitochondrial genomes of *Metschnikowia* yeasts. *Current Biology*. 2020 Jul 20;30(14):R800-1.

Other papers.

- 3 Barros KO, Souza RM, Palladino F, Cadete RM, Santos ARO, Goes-Neto A, Berkov A, Zilli JE, Vital MJS, Lachance MA, Rosa CA. 2021. *Cyberlindnera dasilvae* sp. nov., a xylitol-producing yeast species isolated from rotting wood and frass of cerambycid larva. *Int J Syst Evol Microbiol* 71(9). DOI 10.1099/ijsem.0.004986

Six yeast isolates were obtained from rotting wood samples in Brazil and frass of a cerambycid beetle larva in French Guiana. Sequence analysis of the ITS-5.8S region and the D1/D2 domains of the large subunit rRNA gene showed that the isolates represent a novel species of *Cyberlindnera*. This novel species is related to *Cyberlindnera japonica*, *Cyberlindnera xylosilytica*, *Candida easanensis* and *Candida maesa*. It is heterothallic and produces asci with two or four hat-shaped ascospores. The name *Cyberlindnera dasilvae* sp. nov. is proposed to accommodate the

novel species. The holotype of *Cy. dasilvae* is CBS 16129T and the designated paratype is CBS 16584. The MycoBank number is 838252. All isolates of *Cy. dasilvae* were able to convert xylose into xylitol with maximum xylitol production within 60 and 72 h. The isolates produced xylitol with values ranging from 12.61 to 31.79 g l⁻¹ in yeast extract-peptone-xylose medium with 5% xylose. When the isolates were tested in sugarcane bagasse hydrolysate containing around 35–38 g l⁻¹ d-xylose, isolate UFMG-CM-Y519 showed maximum xylitol production.

- 4 Thompson SRL, Lee DK, Lachance MA, Smith DR. 2021. Mutational effects of mobile introns on the mitochondrial genomes of *Metschnikowia* yeasts. *Frontiers in Genetics*. DOI: 10.3389/fgene.2021.785218.

It has been argued that DNA repair by homologous recombination in the context of endonuclease-mediated cleavage can cause mutations. To better understand this phenomenon, we examined homologous recombination following endonuclease cleavage in a native genomic context: the movement of self-splicing introns in the mitochondrial genomes of *Metschnikowia* yeasts. Self-splicing mitochondrial introns are mobile elements, which can copy and paste themselves at specific insertion sites in mitochondrial DNA using a homing endonuclease in conjunction with homologous recombination. Here, we explore the mutational effects of self-splicing introns by comparing sequence variation within the intron-rich *cox1* and *cob* genes from 71 strains (belonging to 40

species) from the yeast genus *Metschnikowia*. We observed a higher density of single nucleotide polymorphisms around self-splicing-intron insertion sites. Given what is currently known about the movement of organelle introns, it is likely that their mutational effects result from the high binding affinity of endonucleases and their interference with repair machinery during homologous recombination (or, alternatively, via gene conversion occurring during the intron insertion process). These findings suggest that there are fitness costs to harbouring self-splicing, mobile introns and will help us better understand the risks associated with modern biotechnologies that use endonuclease-mediated homologous recombination, such as CRISPR-Cas9 gene editing.

- 5 Santos ARO, Aires A, Pontes A, Silva M, Brito PH, Groenewald M, Melo CGS, Lachance MA, Sampaio JP, Rosa CA. 2021. *Phaffia brasiliensis* sp. nov., a yeast species isolated from soil in a Cerrado - Atlantic Rain Forest ecotone site in Brazil. *Int J Syst Evol Microbiol* (accepted September 2021).
- 6 Maciel NOP, Santos ARO, Felix CR, Landell NF, Pagani DM, Pimenta RS, Morais PB, Angchuan J, Wongpanit K, Srisuk N, Lachance MA, Rosa CA. 2021. *Wickerhamiella martinezcruziae* f.a., sp. nov., a yeast species isolated from tropical habitats. *Int J Syst Evol Microbiol* (accepted September 2021).

Recent Meetings

Some highlights of ICY15, Vienna, Austria

The fourth International Symposium on Yeasts, as it was then called, was held in 1974, in Vienna, Austria, under the oversight of Dr. H. Klaushofer. As a mere graduate student, attending a meeting in Europe was out of the question. I was instead entrusted with the custody of my doctoral mentor's house during his absence, which came with a number of benefits, including the use of an Austin Healy 3000. Forty-seven years later, my dream of visiting the world capital of classical music was again thwarted, this time by a puny virus. No matter, as 21st century technology and a wondrous organizing team, led by Prof. Diethard Mattanovich, put together an excellent 'virtual' 15th International Congress on Yeasts. The online platform offered the usual blend of plenary and concurrent sessions, while the social program featured a yeast-shaped park, complete with quiet areas, poster session rooms, and importantly, a pub, where colleagues and friends could meet over whatever drink was appropriate for their respective

time zones. Here are some of my own high-lights.

Participants in the first ever ISY/ICY quiz competed for their knowledge of yeast trivia. Congratulations go to the *Latinos* team, who scored 27 over 30 questions. I am proud to report achieving third position with my team, *Priti*, consisting of Priti Regmi, Neža Čadež, Heide-Marie Daniel, and me, with a score of 25, but achieved not quite as fast as the *SPO* team, but faster than the *Novo* team.

Part of the success of the conference is owed to a slate of excellent speakers. My special salutations go to Jef Boeke, who provided a lucid update on genome restructuring in *S. cerevisiae*, Chris Hittinger, in part for his 1000 genome project, but above all for so wisely recognizing *Saccharomyces* as the PALE BLUE DOT of yeast biology, and Judith Berman, for her endearing account of antifungal tolerance in *Candida albicans*. There were many other highlights, but I single these out for not just their science, but the human quality that they reflected.

Finally, my heart goes to the Beer Workshop. This tour-de-force started with the amazing story of how Diego Libkind shifted his interest from *Phaffia* to *Saccharomyces*, drawing in the talent of Zé Paulo Sampaio and Chris Hittinger to assemble evidence that *S. eubayanus* is the missing wild progenitor of lager yeast. The project attracted the attention of colleagues worldwide, notably from brewmasters at the iconic Heineken brewery. This story illustrates the importance of skill, insight, collaboration, serendipity, and the love of science. Heineken's Willem Van Waesberghe and Viktor Boer discussed the challenges of brewing with a new yeast and marketing the

resulting product, important aspects that are often overlooked in yeast symposia. Beer, of course, is a major contributor to yeast scholarship, and so it was entirely appropriate for Charles Moritz, from the Mattanovich lab, to recount the brewing of the ICY VIENNA LAGER which would have been offered for tasting, had we convened in Vienna as originally planned. He also explained how a *Komagataella* species was engineered to become Crabtree-positive and used to brew a beer, something that would have been pass up as a preposterous proposition at the 1974 Vienna Symposium.

André Lachance

**International Commission on Yeasts (ICY)
Mycology and Eukaryote Microbiology (MEM) Division
International Union of Microbiological Societies (IUMS)
ICY Commissioners Meeting, Tuesday, August 24, 2021
15th International Congress on Yeasts (ICY15) held online
Minutes of Meeting**

Present (75): Hiroshi Takagi (ICY Chair; Japan), Diethard Mattanovich (ICY15 Chair; Austria), Diego Libkind (Argentina), Sakkie Pretorius (Australia), Vladimir Jiranek (Australia), Brigitte Gasser (Austria), Heide-Marie Daniel (Belgium), Johan Thevelein (Belgium), Patrick Fickers (Belgium), Ana Clara Schenberg (Brazil), Rosane Freitas Schwan (Brazil), Leda Cristina Mendonça-Hagler (Brazil), Marc-André Lachance (Canada), Vivien Measday (Canada), María Angélica Ganga (Chile), Feng-Yan Bai (China), Huiqiang Lou (China), Li-Lin Du (China), Vladimir Mrsa (Croatia), Hana Sychrova (Czech Republic), Lene Jespersen (Denmark), Birgitte Regenberg (Denmark), Merja Penttila (Finland), Jean-Marie Francois (France), Sylvie Dequin (France), Andrey Yurkov (Germany), Doris Rauhut (Germany), Anna Maraz (Hungary), Peter Gabor (Hungary), Matthias Sipiczki (Hungary), Jyoti Prakash Tamang (India), Rajendra Prasad (India), John Morrissey (Ireland), Kenneth H Wolfe (Ireland), Pietro Buzzini (Italy), Lisa Granchi (Italy), Patrizia Romano (Italy), Akihiko Kondo (Japan), Masako Takashima (Japan), Alexander Rapoport (Latvia), Anne Gschaedler (Mexico), Ricardo Vázquez-Juárez (Mexico), Ida van der Klei (the Netherlands), Teun Boekhaut (the Netherlands), Adrianna Skoneczna (Poland), Grzegorz Bartosz (Poland), Teresa Zoladek (Poland), Jose Paulo Sampaio (Portugal), Ji-Sook Hahn (South Korea), Aleksey Kachalkin (Russia), Elena Naumova (Russia),

Ee-Sin Chen (Singapore), Milan Čertík (Slovakia), Neza Cadez (Slovenia), Petrovič Uroš (Slovenia), Peter Raspor (Slovenia), Florian Franz Bauer (South Africa), Koos Albertyn (South Africa), Evodia Setati (South Africa), Amparo Querol (Spain), Ramón González (Spain), Jens Nielsen (Sweden), Volkmar Passoth (Sweden), Charoen Charoenchai (Thailand), Nitnipa Soontorngun (Thailand), Hüseyin Erten (Turkey), Graeme Walker (UK), Daniela Delneri (UK), Tom Ellis (UK), Steve Oliver (UK), Andriy Sibirny (Ukraine), Francisco Carrau (Uruguay), Chris Todd Hittinger (USA), Kyria Boundy-Mills (USA), Sally Ann Meyer (USA).

Apologies (10): Charles Abbas (ICY Vice-Chair; USA), Ana Clara Schenberg (Brazil), Liliana Godoy (Chile), Javier Carvajal Carvajal (Ecuador), Patrícia Lappe Oliveras (Mexico), Jack T. Pronk (the Netherlands), Hyun Ah Kang (South Korea), Graham Stewart (UK), Graham Stewart (UK), Mykhailo Gonchar (Ukraine).

Meeting Agenda

Chair's Opening Remarks: Dr. Hiroshi Takagi welcomed the 75 delegates to the online meeting. He thanked Dr. Diethard Mattanovich and the Organizing Committees for their excellent job regarding the joint virtual event 'ICY15 and ICYGMB30' which is a well balanced and well organized scientific and cultural

program. Dr. Takagi also presented the meeting agenda and confirmed the minutes of the previous meeting held in Antalya, Turkey (October 22, 2019).

Tribute to our Colleagues: Dr. Takagi informed of sad news that our honorable Commissioner, Dr. Alexander (Lex) Scheffers, Professor Emeritus of Delft University of Technology, the Netherlands, passed away May 12, 2021. Dr. Scheffers was a very active member of ICY and a solid and reliable person as an excellent yeast scientist, particularly in yeast physiology and metabolism. He was also the first Editor-in-Chief of FEMS Yeast Research, and his passion for yeast research, managerial skills, and energetic activity played a key role in establishing FEMS Yeast Research as a successful community journal. In memory of his many achievements, ICY and FEMS honored him during ICY15 by awarding a best presentation prize in the field of yeast physiology, sponsored by FEMS. In addition, Dr. Takagi announced that another distinguished yeast scientist, Dr. Stefan Hohmann, who was not an ICY Commissioner, passed away in early July, 2021. Dr. Hohmann was Professor at Gothenburg University and Chalmers University of Technology, Sweden. He was one of the European pioneers of yeast molecular biology and also chaired the International Conference on Yeast Genetics and Molecular Biology (ICYGMB) in 2003 and 2019. Commissioners mourned their death by paying a one minute's silence tribute. Dr. Takagi also introduced that Dr. Jack Pronk has kindly prepared a short obituary for Dr. Scheffers, which contains a photograph provided by the Scheffers family and is accompanied by a page dedicated to his 2003 FEMS Special Merits Award. Finally, Dr. Takagi asked Dr. Rob Samson, the secretary-general of IUMS, to upload the contents to the special site "Gallery of colleagues in yeast research who passed away" in our official webpage.

Introduction of new commissioners: Dr. Takagi introduced three new ICY Commissioners. Dr. Birgitte Regenberg is an Associate Professor in the Department of Biology, University of Copenhagen, Denmark, and is an expert on biofilm formation, genetics, and genomics of *S. cerevisiae*. She recently pioneered work on extra-chromosomal circular DNA. She was nominated by Dr. Lene Jespersen. Dr. Daniela Delneri is Professor in the Faculty of Biology, Medicine and Health at the University of Manchester, UK, and is an expert on yeast genomics, evolution, and environmental biology, and works on the role of genome rearrangements in yeast species. Dr. Tom Ellis

is Professor in the Department of Bioengineering at Imperial College London, UK, and is an expert on the fields of synthetic biology and biotechnology. He has led the UK's efforts on the Yeast 2.0 Project. They were nominated by Drs. Steve Oliver, Graeme Walker, and Ian Roberts. Each candidate provided his/her CV with a list of publications and two Letters of Recommendation from relevant National or International Societies and from current members of ICY. These documents were spread electronically among Commissioners, who could express their attitude to the candidates. According to the nomination processes, Commissioners voted (Pros/Cons) last year by email and the three candidates were unanimously elected as new members of ICY (Dr. Regenberg got support from 62 Commissioners and Drs. Delneri and Ellis obtained support from 48 Commissioners).

Overview of ICY Activities as Chair: For Dr. Takagi, this is his last meeting as ICY Chair since 2016 for the past five years. He provided a brief overview of ICY activities under this tenure as Chair.

Donation of the ICY14 Surplus Funds: Dr. Takagi, Chair of the ICY14 Organizing Committee, donated the surplus funds of ICY14 to forthcoming meetings (ISSY33, International Conference on Non-conventional Yeasts, ISSY34, ISSY35 and ICY15).

Management of five Commissioners' meetings: Dr. Takagi managed in total 5 Commissioners' meetings with the ISSY/ICY organizers, Dr. John Morrissey at ISSY33 held in Cork, Ireland, June, 2017 (Present: 38, Apologies: 37), Dr. Andriy Sibirny at the International Conference on "Non-conventional Yeasts" held in Rzeszów, Poland, May, 2018 (Present: 23), Dr. Diego Libkind at ISSY34 held in Bariloche, Argentina, October, 2018 (Present: 35, Apologies: 36), Dr. Hüseyin Erten at ISSY35 held in Antalya, Turkey, October, 2019 (Present: 35, Apologies: 26), and Dr. Diethard Mattanovich at ICY15 held online, August 2021 (Present: 75, Apologies: 10)
<https://www.iums.org/index.php/previous-icy-meetings>
<https://www.iums.org/index.php/minutes-of-icy-meeting>

Election of 16 New Commissioners: Dr. Takagi gave final approval to 16 nominees in total as new Commissioners at various meetings, based on the results of pre-voting by email: Drs. Brigitte Gasser (Austria), Ramón González (Spain) and Evodia Setati (S. Africa) in 2017, Drs. Masako Takashima (Japan), Vivien Measday (Canada) and Chen Ee Sin (Singapore) in May, 2018, Drs. Andrey Yurkov

(Germany) and Kenneth H. Wolfe (Ireland) in October, 2018. Drs. Milan Čertík (Slovakia), Adrianna V. Skoneczna (Poland), Christopher Todd Hittinger (USA), Aleksey Kachalkin (Russia) and Francisco Carrau (Uruguay) in 2019, Drs. Birgitte Regenbergh (Denmark), and Daniela Delneri and Tom Ellis (UK) in 2021.

<https://www.iums.org/index.php/commissioners>

Nomination Processes and Membership Criteria: Dr. Takagi reconfirmed the nomination processes and accepted the criteria of ICY membership provisionally at the meeting held in 2017. These nomination processes and membership criteria have been included in the ICY Statutes.

<https://www.iums.org/index.php/statutes-of-icy>

Tribute to Ten Colleagues: Dr. Takagi informed ICY of the passing of 10 prominent yeast scientists over the past 7 years. The Commissioners mourned their death by paying one minute of silence. In order to honor their contribution to the community, the Commissioners dedicated some memorial addresses or lectures in the programs of ISSY/ICY meetings.

<https://www.iums.org/index.php/gallery-of-past-yeast-researchers>

Launch of ICY Website: Dr. Takagi opened the ICY website by linking to the IUMS homepage with the aid of Dr. Rob Samson, Secretary-General of IUMS, who has kindly set up and updated free of charge. Now, this website includes the history and founders of ICY, a gallery of yeast researchers who passed away, statutes, minutes of ICY meetings, previous ICY meetings, a list of Commissioners, and a link to the Yeast Newsletter.

<https://www.iums.org/index.php/home-icy>

Other Business/Activities: ICY has been recognized under the umbrella of IUMS and one of five International Commissions within Mycology and Eukaryotic Microbiology (MEM) Division. At the past IUMS meeting in Montreal (2014), there was a suggested change to the naming of the Mycology Division to encompass other eukaryotic systems. After discussion, Dr. Takagi wrapped their opinions that it is good for ICY to be under the umbrella of IUMS in terms of beneficial exchange of science and personnel with other microbial societies. In response to an inquiry by the IUMS Executive Board, Dr. Takagi completed the annual report that is the IUMS MEM ComCof's (Committees, Commissions and Federations) reports for Directors meeting in the past three years. Dr. Teun Boekhout introduced "The

Yeasts" project and "The Yeasts Foundation", which was endorsed by ICY and IUMS. Also, Dr. Patrizia Romano (Italy) introduced the book "Yeasts in the production of wine", co-edited by Dr. Romano and Dr. Graham Fleet, which will be dedicated to Dr. Fleet. Moreover, Dr. Takagi introduced a Japanese style toast and celebration to the Commissioners, "Kampai" and "Banzai", with a Japanese traditional spirit, Awamori, brewed with his lovely yeast strains. Dr. Takagi thanked the Commissioners for their cooperation. The Commissioners also expressed their gratitude to Dr. Takagi.

Voting for New Chair Nominees: In accordance to our tradition, the organizer of the general congress (ICY), Dr. Diethard Mattanovich, is a candidate for new Chair. After several nomination statements for him including those of Drs. Brigitte Gasser, Jens Nielsen and Andriy Sybirny, the Commissioners voted for Dr. Mattanovich and he has been elected with unanimity as new ICY Chair until ICY16 in 2024. The Commissioners expressed their congratulations to Dr. Diethard Mattanovich.

Updates for Upcoming ISSY Meetings: Each organizer provided a progress report on meeting preparation. Dr. Vivien Measday reported current progress in the preparation for ISSY36 to be held in Vancouver, Canada (July 12-16, 2022). Dr. Vladimir Jiranek also presented an update on ISSY37 planned for Adelaide, Australia (November, 2023). Regarding ISSY36, Commissioners actively exchanged various opinions on how to run the meeting (on site, online, or hybrid style). Based on the current situation of the COVID-19 pandemic, it is now quite difficult to judge, but the Commissioners will decide preferably no later than January 2022.

Final Presentation for ICY16 (2024): At the previous meeting in Turkey (2019), Dr. Mathabatha Evodia Setati, representing Dr. Florian Franz Bauer, presented the outline of ICY16 in 2024 including the venue (Cape Town, South Africa) with an advertising brochure. After some questions and comments by the Commissioners, proposals for ICY16 (host country and chair) were unanimously accepted. At this meeting, Dr. Bauer gave a final presentation on the outline of ICY16. Dr. Takagi proposed approval (but NOT vote) for final confirmation of the host country and chair. Dr. Bauer was officially approved as Chair of ICY16 (2024).

Suggestions for Future ISSY: Regarding ISSY38 (2025), Dr. Takagi proposed at the previous meeting in Turkey (2019) that Northern Europe is the best candidate region, such as Denmark or Sweden, as a host country and asked Drs. Lene Jespersen, Volkmar Passoth, and Jens Nielsen to organize ISSY38. It was also discussed that there may be other countries who wish to host ISSY38. However, due to the COVID-19 pandemic that forced upcoming three meetings to shift to the following year, this matter is currently left unresolved. Dr. Takagi also showed the list of candidate countries and regions for future ISSY/ICY

meetings after ISSY38 in terms of the geographical balance and the presence of active yeast community. Considering the preparation period, the Commissioners need to decide the potential venue, date and Chair for ISSY38 (2025) and ISSY39 (2026) at the next meeting in Vancouver (2022).

Chair's Closing Remarks: On behalf of ICY, Dr. Hiroshi Takagi expressed gratitude once again to Drs. Diethard Mattanovich and Brigitte Gasser, who kindly arranged this online meeting. The ICY meeting was adjourned.

Minutes presented by Dr. Hiroshi Takagi, ICY Chair (ICY14 in 2016 ~ ICY15 in 2021), and Dr. Diethard Mattanovich, ICY Chair (ICY15 in 2021 ~ ICY16 in 2024)

Future Meeting

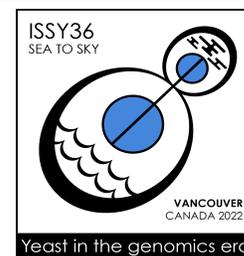
ISSY36 – Sea to Sky

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.

More details will be posted at <https://issy36.com>

Please contact Vivien Measday for sponsorship inquiries: <vmeasday@mail.ubc.ca>

Fifty Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

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

January 1972

Volume XX, Number 2

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University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol, BS18 9AF, England. Communicated by R. Davenport.

1. Spring Metschnikowia isolates have been obtained from several habitats within an English vineyard. This is the first time that this ascomycetous stage has been reported in England. Strains have been tentatively identified as M. pulcherrima and M. reukaufii but further tests are being carried out as these particular yeasts are morphologically somewhat different from other published descriptions.

Chonnam National University, Department of Biology, Chonnam, Korea. Communicated by M. S. Park.

This paper was presented on November 7, 1970, at the 14th Korean Biological Society, Seoul, Korea.

Studies on the Wild Yeasts of Korea - with Emphasis on Natural Habitats

ABSTRACT

This research is based on the presumption that cultivated yeast originates from wild yeast, and that the former can be isolated from the latter, which is widely scattered in the natural world. Accordingly, research on the geographical and ecological distribution of yeast is extremely useful.

In this study, an attempt is made to investigate and analyse the locality, and to clarify the growth cycle of yeast with reference to the seasons and the ecological niche.

It has also been found that Drosophila is inclined to follow the smell of fruit, the slime flux of trees, and mushroom gills in quest of food and a place to inhabit - and are necessarily drawn to yeast that has high capacity for fermentation.

The slime flux of trees and the gills of mushrooms were found to be suitable places for Drosophila to lay eggs. Some yeast from Drosophila crops showed the same pattern as that of the yeast from slime flux.

Georgia State University, 33 Gilmer Street, S.E., Atlanta, Georgia 30303.
Communicated by D. G. Ahearn.

A limited number of copies of "Recent Trends in Yeast Research (D. Ahearn, ed.) Spectrum, Monograph Series in the Arts and Sciences, Georgia State University. 206 pp. (1970)" are still available at \$4.50 per copy.

Institute of Medical Microbiology, University of Aarhus, 8000 Aarhus C, Denmark. Communicated by A. Stenderup.

The work here continues on yeast DNA. Our main interests have been on species in the genera Torulopsis and Candida. GC contents, genome sizes, and amounts of repetitive DNA are currently being investigated. Other studies include mitochondrial DNA and immunological research on the pathogenesis of yeast infections.

Several yeast friends were able to visit the institute in recent months either before or after some European meetings. These included Dr. H. J. Phaff (California), Dr. R. Mayorga (Guatemala), Dr. J. F. T. Spencer (Canada) and Dr. David Yarrow (Delft).

In January Dr. Sally Ann Meyer will complete a year's stay at the institute. She has been working with DNA from Candida and Torulopsis species. She tells us that she has enjoyed her year in Denmark very much.

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439. Communicated by F. Schlenk.

Earlier studies in this Laboratory on the influences of sulfur amino acid supplement on the digestibility of cell walls by Helix pomatia enzymes have been continued by Dr. K. A. Killick. Her results are summarized in a paper, Modification of the Composition and Structure of the Yeast Cell Wall by Culture in the Presence of Sulfur Amino Acids. K. A. Killick, J. Bacteriol. 106, 931-7 (1971).

Hiroshima University, Department of Fermentation Technology, Faculty of Engineering, Sendamachi 3, Hiroshima, Japan 730. Communicated by Noboru Kawakami.

Below follow abstracts of two recent publications:

DIGESTION OF LIVING YEAST CELLS WITH PHYSARUM POLYCEPHALUM

Noboru Kawakami

[Bot. Mag. Tokyo 84:35-40 (February 25, 1971)]

Abstract

On a mixed culture of Physarum polycephalum and Candida utilis, yeast cells ingested in the living plasmodium are digested, and remnants of the cell wall are found in the plasmodium in addition to cells in an almost intact form with intracellular highly dense substances. When growing yeast cells are treated with an extract from plasmodia the cell wall and intracellular substances are also disintegrated. The digestion, however, did not advance to produce spheroplasts or protoplasts under the condition employed in this experiment.

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

Sequential use of 2-deoxyglucose and snail gut enzyme for spheroplast preparation in wild strain and respiratory deficient mutants of a "petite negative" yeast Schizosaccharomyces pombe 972h⁻
F. Foury and A. Goffeau

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

COMPOSITION OF WHISKY FLAVOUR
H. Suomalainen and L. Nykänen

Process Biochemistry 5 (1970), 13-18

Investigations on the flavour of whisky have shown that the aroma fraction consists of aldehydes, fusel alcohols, esters and fatty acids. The composition of the aroma group in different brands of whisky has been reviewed and the effects of the components upon the flavour of whisky are compared. The kinds of aroma components formed in the fermentation do not seem to depend decisively upon the raw material. To date, investigations concerned with the aroma composition of different alcoholic beverages indicate that the same components always appear in the amounts that are quantitatively largest. Nevertheless, the qualitative similarity of the aroma composition, along with the ability of the yeast to produce the same aroma compounds even in nitrogen-free sugar fermentation, gives the yeast a central role in the formation of different aroma components.

International Yeast Meetings

1. The following resolution, proposed by the IAMS Commission on Yeasts and Yeast-like Micro-organisms, was adopted by the participants of the First Specialized Symposium on Yeasts, held at Smolenice Castle, June 1-4, 1972.
 1. The participants of the First Specialized Symposium on Yeasts at Smolenice Castle, June 1-4, 1972, wish to express the most sincere thanks to their Czechoslovak Hosts for having organized an extremely profitable and pleasant meeting.

Yeast Genetics Stock Center, Donner Laboratory, University of California, Berkeley, California 95720.

We are pleased to announce the establishment of a Yeast Genetics Stock Center supported by the National Science Foundation. We will maintain and make available stocks representing most of the commonly studied genetic loci in Saccharomyces cerevisiae. Additionally, wild type, polyploid, and other special stocks will be available. The stock list, currently under preparation, will be available upon request and will also be published in a future issue of the Yeast News Letter. We invite correspondence concerning the deposition of stocks that you believe should be available through the stock center. We expect investigators will continue to maintain stocks pertaining directly to their own research and will deposit only those stocks that are of general utility.

S. Fogel, R. Mortimer and J. Bassel

The work in the laboratory of H. J. Phaff, University of California at Davis, is continuing. Dr. A. Martini has returned to the University of Perugia, Italy after a two-year stay in our laboratory. Dr. Steven Douglas (Ph.D. from the Biochemistry Department at Davis) and Dr. Leda Christina Mendonça (from the Institute of Microbiology, Rio de Janeiro, Brazil) have joined our group and they are continuing the work on DNA base composition and homology of yeasts. Mr. Graham Fleet, graduate student from Australia, is completing his work on lytic enzymes of Schizosaccharomyces and on the cell wall composition of species of that genus. A more detailed progress report will be communicated in the Spring issue of the Yeast News Letter.
