Yeast

A Newsletter for Persons Interested in Yeast

ISSN 0513-5222

Official Publication of the International Commission on Yeasts of the International Union of Microbiological Societies (IUMS)

DECEMBER 2016 **Volume LXV, Number II**

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

Editorial

Nobel Prize in Physiology and Medicine awarded to Prof. Yoshinori Ohsumi

Prof. Yoshinori Ohsumi, Tokyo Institute of Technology, has been awarded the 2016 Nobel Prize in Physiology or Medicine for his discoveries of mechanisms for autophagy, using yeast as a model system. Prof. Ohsumi, who was a keynote speaker at the 14th International Congress on Yeasts, is seen here in the company of Prof. Hiroshi Takagi, Chair of the International Commission on Yeasts, during the congress.

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

MA Lachance, Editor

I Japan Collection of Microorganisms, RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan. Communicated by Masako Takashima <masako@jcm.riken.jp>.

JCM determined draft genome sequences of various strains of fungi and related species (ca. 120 species) and made the genome sequence data available in public databases and our own homepage - http://www.jcm.riken.jp/cgi-bin/nbrp/nbrp_list.cgi. The genome sequencing project was performed in collaboration with the Genome Network Analysis Support Facility (GeNAS) at the RIKEN Center for Life Science Technologies and was supported by the FY2014 Genome Information Upgrading Program of the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan. For most of the strains, data of automated gene prediction and functional annotation are also opened to the public through our homepage. In publications, please refer to both the JCM strain number and the genome accession number of the strain used in the study, and state that the genome data was determined by JCM and GeNAS through the Genome Information Upgrading Program of NBRP in an appropriate section reporting the use thereof. We appreciate your feedback such as publication information and updating of the genome data. The sequenced microbial strains and their genome DNAs are available from RIKEN BRC-JCM and RIKEN BRC-DNA Bank, respectively.

Recent papers related to the genome sequencing project.

1 Sriswasdi S, Takashima M, Manabe R, Ohkuma M, Sugita T, Iwasaki W. 2016. Global deceleration of gene evolution following recent genome hybridizations in fungi. Genome Res 26:1081-1090.

2 Cho O, Ichikawa T, Kurakado S, Takashima M, Manabe R, Ohkuma M, Sugita T. 2016. Draft genome sequence of the causative antigen of summer-type hypersensitivity pneumonitis, *Trichosporon domesticum* JCM 9580. Genome Announc 4:e00651-16.

3 Masuya H, Manabe R, Ohkuma M, Endoh R. 2016. Draft genome sequence of *Raffaelea quercivora* JCM 11526, a Japanese oak wilt pathogen associated with the platypodid beetle, *Platypus quercivorus*. Genome Announc 4: e00755-16.

4 Takashima M, Manabe R-I, Iwasaki W, Ohyama A, Ohkuma M, Sugita T. 2015. Selection of orthologous genes for construction of a highly resolved phylogenetic tree and clarification of the phylogeny of Trichosporonales species. PLoS ONE 10:e0131217.

II Biology Department, Brooklyn College, Brooklyn, New York 11210. Communicated by Nasim A. Khan <nasim.khan4@verizon.net>.

Letter to the Editor

An update on the molecular weight and substrate specificity of maltase in *Saccharomyces cerevisiae*

The molecular weight of purified maltase (E.C. 3.2.1.20) was determined by Khan and Eaton (1967) as 68,500 daltons by gel filtration on Sephadex G-100. Later publications have confirmed the molecular weight of maltase using other techniques. For example the direct nucleotide sequence has resulted in a value of 68,094 and the protein sequencing gave a value of 68,300 and 68,600 (Bio-CYC data base collection). The enzyme maltase from yeast is a monomer and shows strict substrate specificty. Maltase is specific for the hydrolysis of maltose and sucrose, but not for isomaltose and α-methylglucoside. However, α-methylglucoside is an inducer of both maltase and isomaltase. When yeast cells are grown in yeast-extract broth with α-methylglucoside as a carbon source, both α-glucosidases (maltase and α-methyl-glucosidase) are induced. Similarly cells grown on maltose induce maltase as well as isomaltase. It is important to note however, the two α-glucosidases are induced approximately in 3:1 ratio respectively. A recent paper by Yamamoto et. al. (2004) has shown Val216 in the active site decides the substrate specificity of alpha-glucosidase in *Saccharomyces cerevisiae*.

References:

1 Khan NA & Eaton NR. 1967. Purification and characterization of maltase and α-methyl-glucosidase from yeast. Biochim Biophys Acta 146:173-180.

2 Yamamoto K et al. 2004. Eur. J. Biochem 271:3414-3420.

III Laboratory of Genetics, Genome Center of Wisconsin, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, J.F. Crow Institute for the Study of Evolution, University of Wisconsin, Madison, WI 53706, USA. Communicated by Chris Todd Hittinger <cthittinger@wisc.edu>.

Recent publications.

1 McIlwain SJ, Peris D, Sardi M, Moskvin OV, Zhan F, Myers K, Riley NM, Buzzell A, Parreiras L, Ong IM, Landick R, Coon JJ, Gasch AP, Sato TK, Hittinger CT. 2016. Genome sequence and analysis of a stress-tolerant, wild-derived strain of *Saccharomyces cerevisiae* used in biofuels research. G3 (Bethesda) 6:1757-1766.

The genome sequences of more than 100 strains of the yeast *Saccharomyces cerevisiae* have been published. Unfortunately, most of these genome assemblies contain dozens to hundreds of gaps at repetitive sequences, including transposable elements, tRNAs, and subtelomeric regions, which is where novel genes generally reside. Relatively few strains have been chosen for genome sequencing based on their biofuel production potential, leaving an additional knowledge gap. Here we describe the nearly complete genome sequence of GLBRCY22-3 (Y22-3), a strain of *S. cerevisiae* derived from the stress-tolerant wild strain NRRL YB-210 and subsequently engineered for xylose metabolism. After benchmarking several genome assembly approaches, we developed a pipeline to integrate Pacific Biosciences (PacBio) and Illumina sequencing data and achieved one of the highest quality genome assemblies for any *S. cerevisiae* strain. Specifically, the contig N50 is 693 kbp, and the sequences of most chromosomes, the mitochondrial genome, and 2-micron plasmid are complete. Our annotation predicts 92 genes that are not present in the reference genome of the laboratory strain S288c, over 70% of which were expressed. We predicted functions for 43 of these genes, 28 of which were previously uncharacterized and unnamed. Remarkably, many of these genes are predicted to be involved in stress tolerance and carbon metabolism and are shared with a Brazilian bioethanol production strain, even though the strains differ dramatically at most genetic loci. The Y22-3 genome sequence provides an exceptionally high-quality resource for basic and applied research in bioenergy and genetics.

2 Lopes MR, Morais CG, Kominek J, Cadete RM, Soares MA, Uetanabaro APT, Fonseca C, Lachance MA, Hittinger CT, Rosa CA. 2016. Genomic analysis and D-xylose fermentation of three novel *Spathaspora* species: *Spathaspora girioi* sp. nov., *Spathaspora hagerdaliae* f. a., sp. nov., and *Spathaspora gorwiae* f. a., sp. nov. FEMS Yeast Res 16: fow044.

Three novel D-xylose-fermenting yeast species of *Spathaspora* clade were recovered from rotting wood in regions of the Atlantic Rainforest ecosystem in Brazil. Differentiation of new species was based on analyses of the gene encoding the D1/D2 sequences of large subunit of rRNA and on 642 conserved, single-copy, orthologous genes from genome sequence assemblies from the newly described species and 15 closely-related Debaryomycetaceae/ Metschnikowiaceae species. *Spathaspora girioi* sp. nov. produced unconjugated asci with a single elongated ascospore with curved ends; ascospore formation was not observed for the other two species. The three novel species ferment D-xylose with different efficiencies. *Spathaspora hagerdaliae* sp. nov. and *Spathaspora girioi* sp. nov. showed xylose reductase (XR) activity strictly dependent on NADPH, whereas *Spathaspora gorwiae* sp. nov. had XR activity that used both NADH and NADPH as co-factors. The genes that encode enzymes involved in D-xylose metabolism (xylose reductase, xylitol dehydrogenase, and xylulokinase) were also identified for these novel species. The type strains are *Spathaspora girioi* sp. nov. UFMG-CM-Y302^T (= CBS 13476), *Spathaspora hagerdaliae* f.a., sp. nov. UFMG-CM-Y303^T (= CBS 13475), and *Spathaspora gorwiae* f.a., sp. nov. UFMG-CM-Y312^T (= CBS 13472).

3 Wisecaver JH, Alexander WG, King SB, Hittinger CT, Rokas A. 2016. Dynamic evolution of nitric oxide detoxifying flavohemoglobins, a family of single-protein metabolic modules in bacteria and eukaryotes. Mol Biol Evol 33:1979-87.

Due to their functional independence, proteins that comprise standalone metabolic units, which we name single-protein metabolic modules, may be particularly prone to gene duplication (GD) and horizontal gene transfer (HGT). Flavohemoglobins (flavoHbs) are prime examples of single-protein metabolic modules, detoxifying nitric oxide (NO), a ubiquitous toxin whose antimicrobial properties many life forms exploit, to nitrate, a common source of nitrogen for organisms. FlavoHbs appear widespread in bacteria and have been identified in a handful of microbial eukaryotes, but how the distribution of this ecologically and biomedically important protein family evolved remains unknown. Reconstruction of the evolutionary history of 3,318 flavoHb protein sequences covering the family's known diversity showed evidence of recurrent HGT at multiple evolutionary scales including intra-bacterial HGT, as well as HGT from bacteria to eukaryotes. One of the most striking examples of HGT is the acquisition of a flavoHb by the dandruff- and eczema-causing fungus *Malassezia* from *Corynebacterium* Actinobacteria, a transfer that growth experiments show is capable of mediating NO resistance in fungi. Other flavoHbs arose via GD; for example, many filamentous fungi possess two flavoHbs that are differentially targeted to the cytosol and mitochondria, likely conferring protection against external and internal sources of NO, respectively. Because single-protein metabolic modules such as flavoHb function independently, readily undergo GD and HGT, and are frequently involved in organismal defense and competition, we suggest that they represent "plug-and-play" proteins for ecological arms races.

4 Peris D, Langdon QK, Moriarty RV, Sylvester K, Bontrager M, Charron G, Leducq JB, Landry CR, Libkind D, Hittinger CT. 2016. Complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. PLoS Genet 12: e1006155.

Lager-style beers constitute the vast majority of the beer market, and yet, the genetic origin of the yeast strains that brew them has been shrouded in mystery and controversy. Unlike ale-style beers, which are generally brewed with *Saccharomyces cerevisiae*, lagers are brewed at colder temperatures with allopolyploid hybrids of *Saccharomyces eubayanus* × *S. cerevisiae*. Since the discovery of *S. eubayanus* in 2011, additional strains have been isolated from South America, North America, Australasia, and Asia, but only interspecies hybrids have been isolated in Europe. Here, using genome sequence data, we examine the relationships of these wild *S. eubayanus* strains to each other and to domesticated lager strains. Our results support the existence of a relatively low-diversity (π = 0.00197) lineage of *S. eubayanus* whose distribution stretches across the Holarctic ecozone and includes wild isolates from Tibet, new wild isolates from North America, and the *S. eubayanus* parents of lager yeasts. This Holarctic lineage is closely related to a population with higher diversity (π = 0.00275) that has been found primarily in South America but includes some widely distributed isolates. A second diverse South American population (π = 0.00354) and two early-diverging Asian subspecies are more distantly related. We further show that no single wild strain from the Holarctic lineage is the sole closest relative of lager yeasts. Instead, different parts of the genome portray different phylogenetic signals and ancestry, likely due to outcrossing and incomplete lineage sorting. Indeed, standing genetic variation within this wild Holarctic lineage of *S. eubayanus* is responsible for genetic variation still segregating among modern lager-brewing hybrids. We conclude that the relationships among wild strains of *S. eubayanus* and their domesticated hybrids reflect complex biogeographical and genetic processes.

5 Riley R, Haridas S, Wolfe KH, Lopes MR, Hittinger CT, Göker M, Salamov AA, Wisecaver JH, Long TM, Calvey CH, Aerts AL, Barry KW, Choi C, Clum A, Coughlan AY, Deshpande S, Douglass AP, Hanson SJ, Klenk HP, LaButti KM, Lapidus A, Lindquist EA, Lipzen AM, Meier-Kolthoff JP, Ohm RA, Otillar RP, Pangilinan JL, Peng Y, Rokas A, Rosa CA, Scheuner C, Sibirny AA, Slot JC, Stielow JB, Sun H, Kurtzman CP, Blackwell M, Grigoriev IV, Jeffries TW. 2016. Comparative genomics of biotechnologically important yeasts. Proc Natl Acad Sci USA 113: 9882-9887.

Ascomycete yeasts are metabolically diverse, with great potential for biotechnology. Here, we report the comparative genome analysis of 29 taxonomically and biotechnologically important yeasts, including 16 newly sequenced. We identify a genetic code change, CUG-Ala, in *Pachysolen tannophilus* in the clade sister to the known CUG-Ser clade. Our well-resolved yeast phylogeny shows that some traits, such as

methylotrophy, are restricted to single clades, whereas others, such as l-rhamnose utilization, have patchy phylogenetic distributions. Gene clusters, with variable organization and distribution, encode many pathways of interest. Genomics can predict some biochemical traits precisely, but the genomic basis of others, such as xylose utilization, remains unresolved. Our data also provide insight into early evolution of ascomycetes. We document the loss of H3K9me2/3 heterochromatin, the origin of ascomycete mating-type switching, and panascomycete synteny at the MAT locus. These data and analyses will facilitate the engineering of efficient biosynthetic and degradative pathways and gateways for genomic manipulation.

6 Zhou X, Peris D, Kominek J, Kurtzman CP, Hittinger CT, Rokas A. 2016. *in silico* Whole Genome Sequencer & Analyzer (iWGS): a computational pipeline to guide the design and analysis of *de novo* genome sequencing studies. G3 (Bethesda) 6:3655-3662.

The availability of genomes across the tree of life is highly biased toward vertebrates, pathogens, human disease models, and organisms with relatively small and simple genomes. Recent progress in genomics has enabled the *de novo* decoding of the genome of virtually any organism, greatly expanding its potential for understanding the biology and evolution of the full spectrum of biodiversity. The increasing diversity of sequencing technologies, assays, and *de novo* assembly algorithms have augmented the complexity of *de novo* genome sequencing projects in nonmodel organisms. To reduce the costs and challenges in *de novo* genome sequencing projects and streamline their experimental design and analysis, we developed iWGS (*in silico* Whole Genome Sequencer and Analyzer), an automated pipeline for guiding the choice of appropriate sequencing strategy and assembly protocols. iWGS seamlessly integrates the four key steps of a *de novo* genome sequencing project: data generation (through simulation), data quality control, *de novo* assembly, and assembly evaluation and validation. The last three steps can also be applied to the analysis of real data. iWGS is designed to enable the user to have great flexibility in testing the range of experimental designs available for genome sequencing projects, and supports all major sequencing technologies and popular assembly tools. Three case studies illustrate how iWGS can guide the design of *de novo* genome sequencing projects, and evaluate the performance of a wide variety of user-specified sequencing strategies and assembly protocols on genomes of differing architectures. iWGS, along with a detailed documentation, is freely available at https://github.com/zhouxiaofan1983/iWGS.

7 Sato TK, Tremaine M, Parreiras LS, Hebert AS, Myers KS, Higbee AJ, Sardi M, McIlwain SJ, Ong IM, Breuer RJ, Narasimhan RA, McGee MA, Dickinson Q, La Reau A, Xie D, Tian M, Piotrowski JS, Reed JL, Zhang Y, Coon JJ, Hittinger CT, Gasch AP, Landick R. 2016. Directed evolution reveals unexpected epistatic interactions that alter metabolic regulation and enable anaerobic xylose use by *Saccharomyces cerevisiae*. PLoS Genet 12:e1006372.

The inability of native *Saccharomyces cerevisiae* to convert xylose from plant biomass into biofuels remains a major challenge for the production of renewable bioenergy. Despite extensive knowledge of the regulatory networks controlling carbon metabolism in yeast, little is known about how to reprogram *S. cerevisiae* to ferment xylose at rates comparable to glucose. Here we combined genome sequencing, proteomic profiling, and metabolomic analyses to identify and characterize the responsible mutations in a series of evolved strains capable of metabolizing xylose aerobically or anaerobically. We report that rapid xylose conversion by engineered and evolved *S. cerevisiae* strains depends upon epistatic interactions among genes encoding a xylose reductase (*GRE3*), a component of MAP Kinase (MAPK) signaling (*HOG1*), a regulator of Protein Kinase A (PKA) signaling (*IRA2*), and a scaffolding protein for mitochondrial iron-sulfur (Fe-S) cluster biogenesis (*ISU1*). Interestingly, the mutation in *IRA2* only impacted anaerobic xylose consumption and required the loss of *ISU1* function, indicating a previously unknown connection between PKA signaling, Fe-S cluster biogenesis, and anaerobiosis. Proteomic and metabolomic comparisons revealed that the xylose-metabolizing mutant strains exhibit altered metabolic pathways relative to the parental strain when grown in xylose. Further analyses revealed that interacting mutations in *HOG1* and *ISU1* unexpectedly elevated mitochondrial respiratory proteins and enabled rapid aerobic respiration of xylose and other non-fermentable carbon substrates. Our findings suggest a surprising connection between Fe-S cluster biogenesis and signaling that facilitates aerobic respiration and

anaerobic fermentation of xylose, underscoring how much remains unknown about the eukaryotic signaling systems that regulate carbon metabolism.

8 Kuang MC, Hutchins PD, Russell JD, Coon JJ, Hittinger CT. 2016. Ongoing resolution of duplicate gene functions shapes the diversification of a metabolic network. eLife 5:e19027.

The evolutionary mechanisms leading to duplicate gene retention are well understood, but the long-term impacts of paralog differentiation on the regulation of metabolism remain underappreciated. Here we experimentally dissect the functions of two pairs of ancient paralogs of the *GAL*actose sugar utilization network in two yeast species. We show that the *Saccharomyces uvarum* network is more active, even as over-induction is prevented by a second co-repressor that the model yeast *Saccharomyces cerevisiae* lacks. Surprisingly, removal of this repression system leads to a strong growth arrest, likely due to overly rapid galactose catabolism and metabolic overload. Alternative sugars, such as fructose, circumvent metabolic control systems and exacerbate this phenotype. We further show that *S. cerevisiae* experiences homologous metabolic constraints that are subtler due to how the paralogs have diversified. These results show how the functional differentiation of paralogs continues to shape regulatory network architectures and metabolic strategies long after initial preservation.

9 Bellora N, Moliné M, David-Palma M, Coelho MA, Hittinger CT, Sampaio JP, Gonçalves P, Libkind D. 2016. Comparative genomics provides new insights into the diversity, physiology, and sexuality of the only industrially exploited tremellomycete: *Phaffia rhodozyma*. BMC Genomics 17: 901.

BACKGROUND: The class Tremellomycetes (Agaricomycotina) encompasses more than 380 fungi. Although there are a few edible *Tremella* spp., the only species with current biotechnological use is the astaxanthin-producing yeast *Phaffia rhodozyma* (Cystofilobasidiales). Besides astaxanthin, a carotenoid pigment with potent antioxidant activity and great value for aquaculture and pharmaceutical industries, *P. rhodozyma* possesses multiple exceptional traits of fundamental and applied interest. The aim of this study was to obtain, and analyze two new genome sequences of representative strains from the northern (CBS 7918T, the type strain) and southern hemispheres (CRUB 1149) and compre them to a previously published genome sequence (strain CBS 6938). Photoprotection and antioxidant related genes, as well as genes involved in sexual reproduction were analyzed. RESULTS: Both genomes had ca. 19 Mb and 6000 protein coding genes, similar to CBS 6938. Compared to other fungal genomes *P. rhodozyma* strains and other Cystofilobasidiales have the highest number of intron-containing genes and highest number of introns per gene. The Patagonian strain showed 4.4 % of nucleotide sequence divergence compared to the European strains which differed from each other by only 0.073 %. All known genes related to the synthesis of astaxanthin were annotated. A hitherto unknown gene cluster potentially responsible for photoprotection (mycosporines) was found in the newly sequenced *P. rhodozyma* strains but was absent in the non-mycosporinogenic strain CBS 6938. A broad battery of enzymes that act as scavengers of free radical oxygen species were detected, including catalases and superoxide dismutases (SODs). Additionally, genes involved in sexual reproduction were found and annotated. CONCLUSIONS: A draft genome sequence of the type strain of *P. rhodozyma* is now available, and comparison with that of the Patagonian population suggests the latter deserves to be assigned to a distinct variety. An unexpected genetic trait regarding high occurrence of introns in *P. rhodozyma* and other Cystofilobasidiales was revealed. New genomic insights into fungal homothallism were also provided. The genetic basis of several additional photoprotective and antioxidant strategies were described, indicating that *P. rhodozyma* is one of the fungi most well-equipped to cope with environmental oxidative stress, a factor that has probably contributed to shaping its genome.

10 Shen XX, Zhou X, Kominek J, Kurtzman CP, Hittinger CT, Rokas A. 2016. Reconstructing the backbone of the Saccharomycotina yeast phylogeny using genome-scale data. G3 (Bethesda) epub: 10.1534/g3.116.034744.

Understanding the phylogenetic relationships among the yeasts of the subphylum Saccharomycotina is a prerequisite for understanding the evolution of their metabolisms and ecological lifestyles. In the last

two decades, the use of rDNA and multi-locus data sets has greatly advanced our understanding of the yeast phylogeny, but many deep relationships remain unsupported. In contrast, phylogenomic analyses have involved relatively few taxa and lineages that were often selected with limited considerations for covering the breadth of yeast biodiversity. Here we used genome sequence data from 86 publicly available yeast genomes representing 9 of the 11 known major lineages and 10 non-yeast fungal outgroups to generate a 1,233-gene, 96-taxon data matrix. Species phylogenies reconstructed using two different methods (concatenation and coalescence) and two data matrices (amino acids or the first two codon positions) yielded identical and highly supported relationships between the 9 major lineages. Aside from the lineage comprised by the family Pichiaceae, all other lineages were monophyletic. Most interrelationships among yeast species were robust across the two methods and data matrices. However, 8 of the 93 internodes conflicted between analyses or data sets, including the placements of: the clade defined by species that have reassigned the CUG codon to encode serine, instead of leucine; the clade defined by a whole genome duplication; and the species *Ascoidea rubescens*. These phylogenomic analyses provide a robust roadmap for future comparative work across the yeast subphylum in the disciplines of taxonomy, molecular genetics, evolutionary biology, ecology, and biotechnology. To further this end, we have also provided a BLAST server to query the 86 Saccharomycotina genomes, which can be found at http://y1000plus.org/blast.

IV VTT Technical Research Centre of Finland Ltd, Tietotie 2, Espoo, P.O. Box 1000, FI-02044, Finland. Communicated by Brian Gibson <brian.gibson@vtt.fi>.

Recent publications.

1 Biz A, Sugai-Guérios MH, Kuivanen J, Maaheimo H, Krieger N, Mitchell DA, Richard P. 2016. The introduction of the fungal d-galacturonate pathway enables the consumption of d-galacturonic acid by *Saccharomyces cerevisiae*. Microbial Cell Factories 15:144.

Pectin-rich wastes, such as citrus pulp and sugar beet pulp, are produced in considerable amounts by the juice and sugar industry and could be used as raw materials for biorefineries. One possible process in such biorefineries is the hydrolysis of these wastes and the subsequent production of ethanol. However, the ethanol-producing organism of choice, *Saccharomyces cerevisiae*, is not able to catabolize d-galacturonic acid, which represents a considerable amount of the sugars in the hydrolysate, namely, 18 % (w/w) from citrus pulp and 16 % (w/w) sugar beet pulp. In the current work, we describe the construction of a strain of *S. cerevisiae* in which the five genes of the fungal reductive pathway for d-galacturonic acid catabolism were integrated into the yeast chromosomes: *gaa*A, *gaa*C and *gaa*D from *Aspergillus niger* and *lgd*1 from *Trichoderma reesei,* and the recently described d-galacturonic acid transporter protein, *gat*1, from *Neurospora crassa*. This strain metabolized d-galacturonic acid in a medium containing d-fructose as co-substrate. This work is the first demonstration of the expression of a functional heterologous pathway for d-galacturonic acid catabolism in *Saccharomyces cerevisiae*. It is a preliminary step for engineering a yeast strain for the fermentation of pectin-rich substrates to ethanol.

2 Magalhães F, Vidgren V, Ruohonen L & Gibson B. 2016. Sugar utilization by Group 1 strains of the hybrid lager yeast *Saccharomyces pastorianus*. FEMS Yeast Res 16 (5)

Brewer's wort is a challenging environment for yeast as it contains predominantly *α*-glucoside sugars. There exist two subgroups of the lager yeast *Saccharomyces pastorianus* which differ in sugar utilisation. We performed wort ferment-ations and compared representative strains from both groups with respect to their ability to transport and ferment maltose and maltotriose. Additionally, we mapped the transporters *MALx1*, *AGT1*, *MPHx* and *MTT1* by Southern blotting. Contrary to previous observations, group I com-prises a diverse set of strains, with varying ability to transport and ferment maltotriose. Of the eight group I strains, three efficiently utilised maltotriose, a property enabled by the presence of transmembrane transporters *SeAGT1* and *MTT1*. A58, a variant of the group I type strain (CBS1513) performed particularly well, taking up maltotriose at a higher rate than maltose and retaining significant transport activity at temperatures as low as 0∘C. Analysis of trans-porter distribution in this strain

revealed an increased copy number of the *MTT1* gene, which encodes the only permease known with higher affinity for maltotriose than maltose and low temperature dependence for transport. We propose that much of the variation in lager yeast fermentation behaviour is determined by the presence or absence of specific transmembrane transporters.

3 Djordjević V, Willaert R, Gibson B & Nedović V. 2016. Immobilized yeast cells and secondary metabolites. In: *Fungal Metabolites*, J.-M. Mérillon, K.G. Ramawat (eds.). Springer International Publishing, Switzerland - doi: 10.1007/978-3-319-19456-1_33-1.

The use of immobilized cell technology (ICT) is viewed as a promising biotechnological tool to achieve high volumetric productivities of yeast fermentation in bioindustry of alcoholic beverages. During this process a huge number of organic compounds are being formed as yeast secondary metabolites, among which volatile compounds, such as higher alcohols, esters and vicinal diketones are the most important flavoring compounds. The objective of this chapter is to summarize the knowledge on the origin of the flavor-active and non-volatile compounds synthesized by yeast and to describe how the composition of the medium, culture strain, process conditions (temperature, aeration etc.), bioreactor design, and other critical parameters influence the metabolic activities of yeast cultures. Despite the technological and economic advantages provided by ICT, commercialization of this technology experienced only limited success, mainly due to unpredictable effect of immobilization on yeast physiology. This chapter is an attempt to rationalize and make some conclusions about the impact of cell immobilization on yeast metabolism collected from empirical experiences in production of alcoholic beverages. The knowledge addressing this issue may be of particular benefit to the nascent bioflavor industry.

4 Piirainen M, Boer H, de Ruijter JC, Frey AD. 2016. A dual approach for improving homogeneity of a human-type N-glycan structure in *Saccharomyces cerevisiae*. Glycoconjugate J 33:189-199.

N-glycosylation is an important feature of therapeutic and other industrially relevant proteins, and engineering of the N-glycosylation pathway provides opportunities for developing alternative, non-mammalian glycoprotein expression systems. Among yeasts, *Saccharomyces cerevisiae* is the most established host organism used in therapeutic protein production and therefore an interesting host for glycoengineering. In this work, we present further improvements in the humanization of the N-glycans in a recently developed *S. cerevisiae* strain. In this strain, a tailored trimannosyl lipid-linked oligosaccharide is formed and transferred to the protein, followed by complex-type glycan formation by Golgi apparatus-targeted human N-acetylglucosamine transferases. We improved the glycan pattern of the glycoengineered strain both in terms of glycoform homogeneity and the efficiency of complex-type glycosylation. Most of the interfering structures present in the glycoengineered strain were eliminated by deletion of the MNN1 gene. The relative abundance of the complex-type target glycan was increased by the expression of a UDP-N-acetylglucosamine transporter from *Kluyveromyces lactis*, indicating that the import of UDP-N-acetylglucosamine into the Golgi apparatus is a limiting factor for efficient complex-type N-glycosylation in *S. cerevisiae*. By a combination of the MNN1 deletion and the expression of a UDP-N-acetylglucosamine transporter, a strain forming complex-type glycans with a significantly improved homogeneity was obtained. Our results represent a further step towards obtaining humanized glycoproteins with a high homogeneity in *S. cerevisiae*.

5 Kuivanen J, Sugai-Guérios MH, Arvas M, Richard P. 2016. A novel pathway for fungal D-glucuronate catabolism contains an L-idonate forming 2-keto-L-gulonate reductase. Sci Rep 6:26329.

For the catabolism of D-glucuronate, different pathways are used by different life forms. The pathways in bacteria and animals are established, however, a fungal pathway has not been described. In this communication, we describe an enzyme that is essential for D-glucuronate catabolism in the filamentous fungus *Aspergillus niger*. The enzyme has an NADH dependent 2-keto-L-gulonate reductase activity forming L-idonate. The deletion of the corresponding gene, the gluC, results in a phenotype of no growth on D-glucuronate. The open reading frame of the *A. niger* 2-keto-L-gulonate reductase was expressed as an active protein in the yeast *Saccharomyces cerevisiae*. A histidine tagged protein

was purified and it was demonstrated that the enzyme converts 2-keto-L-gulonate to L-idonate and, in the reverse direction, L-idonate to 2-keto-L-gulonate using the NAD(H) as cofactors. Such an L-idonate forming 2-keto-L-gulonate dehydrogenase has not been described previously. In addition, the finding indicates that the catabolic D-glucuronate pathway in *A. niger* differs fundamentally from the other known D-glucuronate pathways.

V Yeast Molecular Genetics Laboratory, Institute of Molecular Biology "Acad. Rumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev str., 1113 Sofia, Bulgaria. Communicated by George Miloshev <miloshev@bio21.bas.bg> - http://www.chromatinepigenetics.com

Abstracts of recent publications.

1 Vasileva B, Georgieva M, Staneva D, Zagorchev P and Miloshev G. 2016. Chromatin modulates cellular response to UV light during the process of chronological aging. Comptes rendus de l'Academie bulgare des Sciences, in press.

All processes that involve the molecule of DNA are mainly regulated by chromatin remodelling complexes (CRCs). CRCs interact with chromatin, remodel its structure and thus allow access of transcription, repair and/or replication factors to DNA. The yeast *Saccharomyces cerevisiae* is a preferred model organism for studies regarding chromatin structure and is a brilliant model organism in Biology of ageing. An important part of CRCs is the family of actin-related proteins (Arp's). It has been shown recently that Arp4p, the actin-related protein 4 homologue in *S. cerevisiae*, mediates the interaction between the CRCs and the linker histone and thus influences chromatin structure and dynamics. The aim of this study is to reveal the significance of chromatin-remodelling complexes for cellular response to UV stress during chronological ageing. The results show the importance of chromatin organization for the preservation of genome stability during cellular ageing, and moreover, the role of the linker histone in the mediation of this cellular response to UV light irradiation.

2 Georgieva M, Zagorchev P, Miloshev G. 2015. Random, double- and single-strand DNA breaks can be differentiated in the method of Comet assay by the shape of the comet image. Electrophoresis 36:2553-60.

Comet assay is an invaluable tool in DNA research. It is widely used to detect DNA damage as an indicator of exposure to genotoxic stress. A canonical set of parameters and specialized software programs exist for Comet assay data quantification and analysis. None of them so far has proven its potential to employ a computer-based algorithm for assessment of the shape of the comet as an indicator of the exact mechanism by which the studied genotoxins cut in the molecule of DNA. Here, we present 14 unique measurements of the comet image based on the comet morphology. Their mathematical derivation and statistical analysis allowed precise description of the shape of the comet image which in turn discriminated the cause of genotoxic stress. This algorithm led to the development of the "CometShape" software which allowed easy discrimination among different genotoxins depending on the type of DNA damage they induce.

VI Department of AGRARIA, "*Mediterranea*" University of Reggio Calabria, Via Feo di Vito, I-89122 Reggio Calabria, Italy. Communicated by Andrea Caridi <acaridi@unirc.it>.

Recent publications.

1 Caridi A, De Bruno A, De Salvo E, Piscopo A, Poiana M, Sidari R. 2016. Selected yeasts to enhance phenolic content and quality in red wine from low pigmented grapes. Eur Food Res Technol - DOI 10.1007/s00217-016-2750-9

The aim of this work was to enhance - by yeast activity - the quality of red wine produced from black grapes of the Calabrian *Gaglioppo* variety, used as a model for grapes with reduced synthesis of

anthocyanins. Six selected strains of *Saccharomyces cerevisiae* were usedto control winemaking trials. Among the wines, there are significant differences, due to the wine starter used. The following technological parameters were significantly different from strain to strain: total acidity, alcoholic degree, tartaric, malic, lactic, and acetic acid, and free and total SO_2; moreover, the following phenolic parameters were significantly different from strain to strain: A420, A520, A620, colour intensity, colour hue, Folin-Ciocalteu index, percentage of DPPH inactivation, total anthocyanins, total polyphenols (A280), total tannins, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside. From a sensory standpoint, significant differences were observed among wine samples during a short bottle aging. Data validate the main role that wine yeast selection plays in enhancing the quality of red wine from low pigmented grape.

2 Sidari R, Martorana A, De Bruno A, Piscopo A, Poiana M, Caridi A 2016. Preliminary study by molecular methods on yeast population of differently fermented green table olives of the cultivar *Nocellara messinese*. 14th International Congress on Yeasts, poster P082, Book of Abstract p.303, Hyogo (Japan), 11-15 September 2016.

INTRODUCTION: Main microorganisms involved in table olive fermentations are lactic acid bacteria and yeasts. During fermentation the presence and the dominance of microorganisms are affected by factors such as pH and salt concentration. Aim of the present study was to investigate the yeast population in fermentations with brine at different concentration of NaCl, also artificially acidified. Materials and METHODS: Vats containing olives of *Nocellara messinese* cultivar were filled with brines formulated as follow: a) 8% NaCl (w/v), b) 8% NaCl acidified to pH 4.3, c) 5% NaCl for 20 days and then brought to 8% NaCl, d) 5% NaCl for 20 days, then brought to 8% NaCl and acidified to pH 4.3. Fermentations were carried out at room temperature for 8 months. Yeasts were isolated on YPD medium throughout the fermentation time. The yeast identifications were performed by PCR-RFLP of the internal transcribed spacer region; DNA was extracted from the different olive trials. RESULTS AND DISCUSSION: A total of 100 yeasts was isolated; they belong mainly to the species *Kluyveromyces marxianus*, *Pichia kudriavzevii*, and *Wycherhamomyces anomalus*. These species were confirmed by culture-independent method that allowed tracing the yeast population. Throughout the 8 months, different evolution of the yeast population was observed; this is mainly related to the technological parameters used in the fermentations. The main yeast technological traits will be studied; the correlation between the yeast population and the brine formulation will be useful to improve the quality and the shelflife of the fermented olives.

3 Martorana A, Caridi A, Sidari R. 2016. Preliminary study by molecular methods on lactic acid bacteria and yeasts of Calabrian sourdoughs. The Food Factor I Barcelona Conference, Book of Abstract p.134, Barcelona (Spain), 2-4 November 2016.

Sourdough is widely utilized in the production of traditional bread. Lactic acid bacteria (LAB) and yeasts coexist establishing a dynamic equilibrium that determines the peculiarity of this product as well as its long-term storage. Aim of the present study was to investigate LAB and yeast population of Calabrian sourdoughs. Four sourdoughs collected from bakeries located in Catanzaro (PF1, PF2, and PF4) and in Reggio Calabria (PF5) were tested for pH and total titratable acidity (TTA). For the microbiological analyses, each sample was homogenized, serially diluted, and plated in MRS and SDB for LAB and in YPD for yeasts. DNA was extracted from representative colonies both of LAB and yeasts and subjected to amplification using specific primers [1, 2]. The LAB and the yeasts identification was performed by PCR-ARDRA [3] and by PCR-RFLP [2] comparing the profile isolates to those of reference strains by International collections. Among the sourdoughs, PF1 showed the lowest pH (3.93) and the highest TTA (9.60 ml of NaOH 0.1 N) while the opposite was observed in PF2 (pH: 5.59 and TTA: 2 ml of NaOH 0.1 N). The highest count for LAB was recorded in PF1, PF4, and PF5 (10^7 CFU/g) and for yeasts in PF1 and PF2 (10^6 CFU/g). In PF1 and PF5, a dominance of *Lactobacillus sanfranciscensis* was observed; in PF2 additionally was observed *Pediococcus acidilactici* while in PF4 the obtained profiles did not match any of the reference strains considered in this contribution. Concerning the yeasts, *Saccharomyces cerevisiae* was detected in all the sourdoughs. LAB and yeasts will be tested for

technological features to select the best autochthonous strains to use as starter in sourdough production.

References

1 Young JPW, Downer HL, Eardly BD. 1991. Phylogeny of the phototrophic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. J Bacteriol 173(7):2271–2277.

2 Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A. 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J Syst Bacteriol 49:329–337.

3 Aquilanti L, Silvestri G, Zannini E, Osimani A, Santarelli S, Clementi F. 2007. Phenotypic, genotypic and technological characterization of predominant lactic acid bacteria in Pecorino cheese from central Italy. J Appl Microbiol 103:948–960.

VII Bioprocess & Metabolic Engineering Lab (LEMeB), University of Campinas, Campinas SP, Brazil. Communicated by Andreas K. Gombert <gombert@unicamp.br>.

Recent publications.

1 Beato FB, Bergdahl B, Rosa CA, Forster J, Gombert AK. 2016. Physiology of *Saccharomyces cerevisiae* strains isolated from Brazilian biomes: new insights into biodiversity and industrial applications. FEMS Yeast Res 16(7) - DOI: 10.1093/femsyr/fow076

2 Gombert AK, Madeira JV Jr, Cerdán ME, González-Siso MI. 2016. *Kluyveromyces marxianus* as a host for heterologous protein synthesis. Appl Microbiol Biotechnol 100(14):6193-6208.

3 Moreira TC, da Silva VM, Gombert AK, da Cunha RL. 2016. Stabilization mechanisms of oil-in-water emulsions by *Saccharomyces cerevisiae*. Colloids Surf B Biointerfaces 143:399-405.

VIII Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands. Communicated by Ferry Hagen <F.Hagen@cwz.nl>.

Recent publications.

1 Nyazika TK, Hagen F, Machiridza T, Kutepa M, Masanganise F, Hendrickx M, Boekhout T, Magombei-Majinjiwa T, Siziba N, Chin'ombe N, Mateveke K, Meis J, Robertson VJ. 2016. *Cryptococcus neoformans* population diversity and clinical outcomes of HIV-associated cryptococcal meningitis patients in Zimbabwe. J Med Microbiol - doi: 10.1099/jmm.0.000354.

HIV and cryptococcal meningitis coinfection are a major public health problem in most developing countries. *Cryptococcus neoformans sensu stricto* is responsible for the majority of HIV-associated cryptococcosis cases in sub-Saharan Africa. Despite the available information, little is known about cryptococcal population diversity and its association with clinical outcomes in patients with HIV-associated cryptococcal meningitis in sub-Saharan Africa. In a prospective cohort we investigated the prevalence and clinical outcome of *C. neoformans sensu stricto* meningitis among HIV-infected patients in Harare, Zimbabwe and compared the genotypic diversity of the isolates with those collected from other parts of Africa. Molecular typing was done using amplified fragment length polymorphism genotyping and microsatellite typing. The majority of patients with HIV-associated *C. neoformans sensu stricto* meningitis in this cohort were males (n=33/55; 60.0%). The predominant *C. neoformans sensu stricto* genotype among the Zimbabwean isolates was genotype AFLP1/VNI (n=40; 72.7%), followed by AFLP1A/VNB/VNII (n=8; 14.6%) and AFLP1B/VNII was the least isolated (n=7; 12.7%). Most of the isolates were mating-type α (n=51; 92.7%) and only 4 (7.3%) were mating-type a. Overall in-hospital mortality was 55.6% (n=30) and no difference between infecting genotype and clinical outcome of patient (P=0.73) or CD4+ counts (P=0.79) was observed. Zimbabwean *C. neoformans sensu stricto* genotypes demonstrated a high level of genetic diversity by microsatellite typing and 51 genotypes within the main molecular types AFLP1/VNI, AFLP1A/VNB/VNII and AFLP1B/VNII were identified. This study demonstrate that *C. neoformans sensu stricto* in Zimbabwe has a high level of genetic diversity when compared to other regional isolates.

2 Chiriac A, Mares M, Mihaila D, Solovan C, Moldovan C, Stolnicu S, Hagen F. 2016. Case report: Primary cutaneous cryptococcosis during infliximab therapy. Dermatol Ther - doi: 10.1111/dth.12405.

3 Nyazika TK, Herkert PF, Hagen F, Mateveke K, Robertson VJ, Meis JF. 2016. In vitro antifungal susceptibility profiles of *Cryptococcus* species isolated from HIV-associated cryptococcal meningitis patients in Zimbabwe. Diagn Microbiol Infect Dis 86(3):289-292.

Cryptococcus neoformans is the leading cause of cryptococcosis in HIV-infected subjects worldwide. Treatment of cryptococcosis is based on amphotericin B, flucytosine, and fluconazole. In Zimbabwe, little is known about antifungal susceptibility of *Cryptococcus*. Sixty-eight genotyped *Cryptococcus* isolates were tested for antifungal profiles. Amphotericin B, isavuconazole, and voriconazole showed higher activity than other triazoles. Fluconazole and flucytosine were less effective, with geometric mean MICs of 2.24 and 2.67mg/L for *C. neoformans* AFLP1/VNI, 1.38 and 1.53mg/L for *C. neoformans* AFLP1A/VNB/VNII and AFLP1B/VNII, and 1.85 and 0.68mg/L for *Cryptococcus tetragattii*, respectively. A significant difference between flucytosine geometric mean MICs of *C. neoformans* and *C. tetragattii* was observed (P=0.0002). The majority of isolates (n=66/68; 97.1%) had a wild-type MIC phenotype of all antifungal agents. This study demonstrates a favorable situation with respect to the tested antifungals agents. Continued surveillance of antifungal suscept ibility profiles is important due to the high burden of cryptococcosis in Africa.

4 Chen M, Al-Hatmi AM, Chen Y, Ying Y, Fang W, Xu J, Hagen F, Hong N, Boekhout T, Liao W, Pan W. 2016. Cryptococcosis and tuberculosis co-infection in mainland China. Emerg Microbes Infect 5(9):e98.

5 Fang W, Chen M, Liu J, Hagen F, Ms A, Al-Hatmi, Zhang P, Guo Y, Boekhout T, Deng D, Xu J, Pan W, Liao W. 2016. Cryptococcal meningitis in systemic lupus erythematosus patients: pooled analysis and systematic review. Emerg Microbes Infect 5(9):e95.

6 Herkert PF, Hagen F, de Oliveira Salvador GL, Gomes RR, Ferreira MS, Vicente VA, Muro MD, Pinheiro RL, Meis JF, Queiroz-Telles F. 2016. Molecular characterisation and antifungal susceptibility of clinical *Cryptococcus deuterogattii* (AFLP6/VGII) isolates from Southern Brazil. Eur J Clin Microbiol Infect Dis 35(11):1803-1810.

Cryptococcosis, caused by *Cryptococcus gattii sensu lato*, is an emerging disease that was initially found in (sub)tropical regions but recently expanded to temperate regions. *Cryptococcus gattii s.l.* infections are mostly encountered in healthy individuals, frequently affecting both lungs and the central nervous system (CNS). Usually, *C. gattii s.l.* is less susceptible to antifungal compounds than its counterpart, *C. neoformans s.l.* We studied 18 clinical *C. gattii s.l.* isolates with amplified fragment length polymorphism (AFLP) fingerprinting, mating-typing, multi-locus sequence typing (MLST) and antifungal susceptibility testing. All isolates were *C. deuterogattii* (genotype AFLP6/VGII), 14 were mating-type α and four were type a. Amphotericin B, itraconazole, voriconazole, posaconazole and isavuconazole showed high activity, with minimum inhibitory concentration (MIC) ranges of 0.063-0.25, 0.031-0.25, 0.031-0.25, 0.031-0.25 and <0.016-0.25 μg mL-1, respectively. Fluconazole and flucytosine had high geometric mean MICs of 2.07 and 3.7 μg mL-1, respectively. Most cases occurred in immuno-competent patients (n = 10; 55.6 %) and CNS involvement was the most common clinical presentation (n = 14; 77.8 %). Three patients (16.7 %) showed sequelae, hyperreflexia, dysarthria, diadochokinesia, anosmia and upper limb weakness. In conclusion, all infections were caused by *C. deuterogattii* (AFLP6/VGII) and the majority of patients were immunocompetent, with the CNS as the most affected site. All antifungal drugs had high in vitro activity against *C. deuterogattii* isolates, except fluconazole and flucytosine.

7 Calvo B, Melo AS, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F, Meis JF, Colombo AL. 2016. First report of *Candida auris* in America: Clinical and microbiological aspects of 18 episodes of candidemia. J Infect. 73(4):369-374.

OBJECTIVES: Characterization of a hospital outbreak of *Candida auris* candidemia that involved 18 critically ill patients in Venezuela. METHODS: Bloodstream isolates of *C. auris* obtained from 18 patients admitted at a medical center in Maracaibo, between March, 2012 and July, 2013 were included. Species identification was confirmed by ITS rDNA sequencing. Isolates were subsequently typed by amplified fragment length polymorphism fingerprinting (AFLP). Susceptibility testing was performed according to CLSI. Clinical data were collected from all cases by using a standard clinical form. RESULTS: A total of 13 critically ill pediatric and 5 adult patients, with a median age of 26 days, were included. All were previously exposed to antibiotics and multiple invasive medical procedures. Clinical management included prompt catheter removal and antifungal therapy. Thirteen patients (72%) survived up to 30 days after onset of candidemia. AFLP fingerprinting of all *C. auris* isolates suggested a clonal outbreak. The isolates were considered resistant to azoles, but susceptible to anidulafungin and 50% of isolates exhibited amphotericin B MIC values of >1 µg/ml. CONCLUSIONS: The study demonstrated that *C. auris* is a multiresistant yeast pathogen that can be a source of health-care associated infections in tertiary care hospitals with a high potential for nosocomial horizontal transmission.

8 Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, Colombo AL, Hagen F, Meis JF, Chowdhary A. 2016. Identification and typing of the emerging pathogen *Candida auris* by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses 59(8):535-8.

Candida auris is an emerging antifungal resistant yeast species causing nosocomial and invasive infections, emphasising the need of improved diagnostics and epidemiological typing methods. We show that MALDI-TOF VITEK-MS followed by amplified length polymorphisms allows for accurate species identification and subsequent epidemiological characterisation of strains encountered during potential outbreaks.

9 Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, Ryan L, Shackleton J, Trimlett R, Meis JF, Armstrong-Jones D, Fisher MC. 2016. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. Antimicrob Resist Infect Contr - doi: 10.1186/s13756-016-0132-5

BACKGROUND: *Candida auris* is a globally emerging multidrug resistant fungal pathogen causing nosocomial transmission. We report an ongoing outbreak of *C. auris* in a London cardio-thoracic center between April 2015 and July 2016. This is the first report of *C. auris* in Europe and the largest outbreak so far. We describe the identification, investigation and implementation of control measures. METHODS: Data on *C. auris* case demographics, environmental screening, implementation of infection prevention/control measures, and antifungal susceptibility of patient isolates were prospectively recorded then analysed retrospectively. Speciation of *C. auris* was performed by MALDI-TOF and typing of outbreak isolates performed by amplified fragment length polymorphism (AFLP). RESULTS: This report describes an ongoing outbreak of 50 *C. auris* cases over the first 16 month (April 2015 to July 2016) within a single Hospital Trust in London. A total of 44 % (n = 22/50) patients developed possible or proven *C. auris* infection with a candidaemia rate of 18 % (n = 9/50). Environmental sampling showed persistent presence of the yeast around bed space areas. Implementation of strict infection and prevention control measures included: isolation of cases and their contacts, wearing of personal protective clothing by health care workers, screening of patients on affected wards, skin decontamination with chlorhexidine, environmental cleaning with chorine based reagents and hydrogen peroxide vapour. Genotyping with AFLP demonstrated that *C. auris* isolates from the same geographic region clustered. CONCLUSION: This ongoing outbreak with genotypically closely related *C. auris* highlights the importance of appropriate species identification and rapid detection of cases in order to contain hospital acquired transmission.

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Recent publication.

1 Vadkertiová R, Molnárová J, Lux A, Vaculík M, Lišková D, 2016. Yeasts associated with an abandoned mining area in Pernek and their tolerance to different chemical elements. Folia Microbiol 61:199–207.

Four plants, *Cirsium arvense* (creeping thistle), Equisetum arvense (field horsetail), Oxalis acetosella (wood sorrel) and *Phragmites australis* (common reed), which grew in an abandoned Sb-mining area in Pernek (Malé Karpaty Mts., Slovakia), were investigated for the yeast species. Yeasts were isolated from both the leaves of the plants and the soil adjacent to the plants. In total, 65 yeast cultures, belonging to 11 ascomycetous and 5 basidiomycetous yeast species, were isolated. The species most frequently isolated from both the soil and leaf samples were *Trichosporon porosum*, *Galactomyces candidus* and *Candida solani*, whereas *Aureobasidium pullulans, Candida tsuchiyae* and *Sporidiobolus metaroseus* were isolated exclusively from the plant leaves. All the yeast species isolated were tested for their tolerance to two heavy metals (Cd, Zn) and three metalloids (As, Sb and Si). The yeasts isolated from both the leaves and soils exhibited a high tolerance level to both As and Sb, present in elevated concentrations at the locality. Among the yeast species tested, Cryptococcus musci, a close relative to Cryptococcus humicola, was the species most tolerant to all the chemical elements tested, with the exception of Si. It grew in the presence of 200 mmol/L Zn, 200 mmol/L Cd, 60 mmol/L As and 50 mmol/L Sb, and therefore, it can be considered as a multi-tolerant species. Some of the yeast species were tolerant to the individual chemical elements. The yeast-like species *Trichosporon laibachii* exhibited the highest tolerance to Si of all yeasts tested, and *Cryptococcus flavescens* and *Lindnera saturnus* showed the same tolerance as *Cryptococcus musci* to Zn and As, respectively. The majority of the yeasts showed a notably low tolerance to Cd (not exceeded 0.5 mmol/L), which was present in small amounts in the soil. However, *Candida solani*, isolated from the soil, exhibited a higher tolerance to Cd (20 mmol/L) than to As (2 mmol/L).

2 Breierová E, Čertík M, Márová I. 2016. Removal of copper (II) ions from aqua growth medium by red yeast. Materials Science Forum 851:3-7.

Copper is a natural fungicide and is the active component of various pesticides. We detected uptake of higher concentration of the copper ions and responses to this stress in combination with presence hydrogen peroxide as a source of free radicals were studied on the three red yeast strains of species *Rhodotorula glutinis* (two strains) and *Sporobolomyces roseus* (one strain). The maximum Cu sorption was observed at the cells of strain *Rhodotorula glutinis* CCY 20- 2-33 (25,18 mg/g dry weight) and at their exopolymers which accumulated the amount 10.22 mg/g dry weight. The remaining copper was sorbed onto the fibrillar part of cell wall (3.75 mg/g dry weight). The presence peroxide (oxidative stress) in cultivation medium decreased of the toleration of yeasts to Cu^{2+} ions and cells were able to take up less of about 17 % (from 3mM on 2,5 mM), although total uptake was lower about 11.01- 15.96 %. We found that the strains of *Rhodotorula glutinis* are able to uptake about 44 % more copper ions (25.18 - 24.32 mg/g dry weight) in compared with strain of *Sporobolomyces roseus* (16.92 dry weight). However, the addition of peroxide into the cultivation medium the addition of affecting trade changes by reduce of the ability to uptake Cu2+ ions. The exopolymers and fibrillar part of cell wall these yeast were used as biopolymers with high sorption ability for metals.

3 Karelin AA, Tsvetkov YuE, Paulovičová E, Paulovičová L and Nifantiev NE. 2015. Blockwise synthesis of a pentasaccharide structurally related to the mannan fragment from the *Candida albicans* cell wall corresponding to the antigenic factor 6. Russian Chemical Bulletin, International Edition 64:2942-2948.

A spacer-armed pentasaccharide structurally related to a fragment of the mannan from the cell wall of the fungus *Candida albicans* and corresponding to the antigenic factor 6 has been synthesized. This

compound, comprising two -β(1→2)- and three -β(1→2)- linked mannose residues, was prepared by glycosylation of a selectively protected -α(1→2)- di-mannoside bearing an a glycone spacer and a free OH group at atom C(2′) with a -β(1→2)- trimannoside glycosyl donor. The successful synthesis evidences that large -β (1→2) - oligomannoside donor blocks can be used for the preparation of oligosaccharides including extended sequences of repeating -β(1→2)- linked mannose residues.

4 Paulovičová E, Paulovičová L, Pilišiová R, Jančinová V, Yashunsky DV, Karelin AA, Tsvetkov YE, Nifantiev NE. 2016. The evaluation of β-(1→3)-nonaglucoside as an anti-*Candida albicans* immune response inducer. Cellular Microbiol 18:1294–1307.

Synthetically prepared bovine serum albumin (BSA) conjugate of linear β-(1→3)-nonaglucoside ligand (G9) has been applied as a biological response immunomodulator in vivo and ex vivo. Active immunization of Balb/c mice revealed effective induction of specific humoral responses in comparison with *Candida* β-D-glucan and *Candida* whole cells. Induced post-vaccination serum exhibited a growthinhibition effect on the multi-azole-resistant clinical strain *Candida albicans* CCY 29-3-164 in experimental mucocutaneous infection ex vivo. Evaluation of immune cell proliferation and the cytotoxic potential of the G9-ligand has revealed its bioavailability and an immunostimulative effect in vaccination sensitized Balb/c mice splenocytes and RAW 264.7 macrophages.

5 Karelin A.A., Tsvetkov Y.E., Paulovičová E., Paulovičová L., Nifantiev N.E. A blockwise approach to the synthesis of (1→2)-linked oligosaccharides corresponding to fragments of the acid- stable β-mannan from the *Candida albicans* cell wall. Eur. J. Org. Chem. 2016, 1173–1181

Oligomannosides comprising short β-(1→2)-oligomannosyl blocks attached through a glycoside bond to an α-(1→2)-linked oligomannoside are a constituent of side chains of the mannan from the *Candida* cell wall. This type of oligomannoside is known as an acid-stable β -mannan and corresponds to the antigenic factor 6. In this communication, we report a convergent blockwise synthesis of four spacer-armed oligomannosides of this type including from 1 to 4 β-mannosyl and 2–3 α-mannosyl residues. β-(1→2)-Oligomannosyl thioglycosides and sulfoxides containing up to four mannose units were employed for introduction of β -oligomannoside sequences. The efficiency of β-mannosylation with mono- and oligomannoside donors was shown to depend more on the structure of the reaction partners than on the applied activation protocol. The oligomannosides obtained will be used to prepare various molecular tools for investigation of the *Candida* cell wall.

6 Radošinská J, Mezešová L, Okruhlicová L, Frimmel K, Breierová E, Barteková M, Vrbiar N. 2016. Effect of yeast biomass with high content of carotenoids on erythrocyte deformability, NO production and Na,K-ATPase activity in healthy and LPS treated rats. Clin Hemorheol Microcircul Preprint 1-10.

Measurements of red blood cell (RBC) deformability together with estimation of NO-synthase activity and Na,K-ATPase activity were used for characterization of RBC functionality in rats subjected to single dose of Escherichia coli lipopolysaccharides (LPS) at a dose of 1 mg/kg. We hypothesized that LPS might initiate a malfunction of RBC. We also investigated the potential effect of carotenoids (10 mg/kg/day) produced in red yeast biomass of *Rhodotorula glutinis* on RBC in LPS-challenged rats. LPS significantly reduced the deformability of RBC (by 14%) together with decrease of NO-synthase activity by 20%. Daily supplementation of carotenoids for 10 days attenuated the LPS-induced injury, as observed by 22% increase of RBC deformability and 23% increase of NO-synthase activity. The activity of Na,K-ATPase was also improved probably due to increased number of active enzyme molecules as indicated by 66% enhancement of Vmax value, hence maintaining the activity of erythrocyte Na,K-ATPase to the level even higher as compared with healthy control animals. It may be concluded that administration of yeast biomass with high content of carotenoids resulted in advanced function of erythrocytes as concerns their ability to squeeze through narrow capillaries of the circulation, better intrinsic production of NO and improvement of intracellular homeostasis of sodium.

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Recent publications.

1 Blackwell M, Kurtzman CP. 2016. Social wasps promote social behavior in *Saccharomyces* spp. Proc Natl Acad Sci USA 113(8):1971-1973.

2 Dien BS, Zhu JY, Slininger PJ, Kurtzman CP, Moser BR, O'Bryan PJ, Gleisner R, Cotta MA. 2016. Conversion of SPORL pretreated Douglas fir forest residues into microbial lipids with oleaginous yeasts. RSC Adv 6:20695–20705

Douglas fir is the dominant commercial tree grown in the United States. In this study Douglas fir residue was converted to single cell oils (SCO) using oleaginous yeasts. Monosaccharides were extracted from the woody biomass by pretreating with sulfite and dilute sulfuric acid (SPORL process) and hydrolyzing using commercial cellulases. A new SPORL process that uses pH profiling was compared to the traditional method. Both processes yielded 77 g l^{-1} concentration of sugars. The SPORL generated sugars were evaluated for conversion to SCO using yeasts *Lipomyces tetrasporus* and *Yarrowia lipolytica* in batch cultures containing SPORL sugars diluted to 60% v/v supplemented with nitrogen at an appropriate C : N ratio of 75 : 1. An extended lag phase was observed for both yeasts, which was eliminated by including SPORL sugars diluted to 40% v/v in the seed cultures for acclimation. The maximum lipid concentrations were 3.18–5.13 g l^{-1}. This corresponded to yields of 0.06–0.17 g lipid per g beginning sugars and productivities of 0.99–1.42 g l^{-1} d^{-1}. Lipid concentrations for *L. tetrasporus* were further amplified by using two schemes incorporating multiple batch cultures. In the first, the yeast was grown in 40% v/v SPORL sugars and the entire contents of this fermentation transferred to undiluted SPORL sugars not supplemented with nitrogen. The result was the production of 13.4 g l^{-1} lipids within 3 days. This corresponds to a yield of 0.174 g g^{-1} and a productivity of 4.47 g l^{-1} d^{-1}. The second approach was to thrice transfer the yeast cells in 60% v/v SPORL sugars supplemented with limited nitrogen to promote further lipid formation. The end result was 18.1 g l^{-1} of lipids with a process yield and productivity of 0.104 g g^{-1} and 1.29 g l^{-1} d^{-1}, respectively. This is the first report that the authors are aware of demonstrating the feasibility of converting unconditioned woody biomass to single cell oil.

3 Dien BS, Slininger PJ, Kurtzman CP, Moser BR, O'Bryan PJ. 2016. Identification of superior lipid producing *Lipomyces* and *Myxozyma* yeasts. AIMS Environ Sci. 3(1):1-20.

Oleaginous yeasts are of interest for production of single cell oils from sugars. Eighteen members of the *Lipomyces* and *Myxozyma* clade were screened for lipid production when cultured on 10%w/v glucose. The highest ranking yeasts included *L. tetrasporus* (21 g/L), *L. spencer-martinsiae* (19.6 g/L), and *L. lipofer* (16.7 g/L). By contrast, *Rhodosporidium toruloides*, which was included as a positive control, produced 16.7 g/L. The *L. tetrasporus* and *L. lipofer* were further characterized for growth and lipid production on sugars present in biomass hydrolysates. These included L-arabinose, xylose, and an equal glucose and xylose mixture. *L. tetrasporus* had lipid titers of 16.3–20.8 g/L and *L. lipofer* 12.5–17.0 g/L. When both strains were grown on an equal mixture of glucose and xylose, xylose was consumed immediately following glucose. Lipid contents for the yeasts consisted primarily of C18:1 and C16:0, which makes them a promising source of lipids for fuel applications.

4 Hittinger C.T., Rokas A., Bai F.Y., Boekhout T., Gonçalves P., Jeffries T.W., Kominek J., Lachance M.A., Libkind D., Rosa C.A., Sampaio J.P., Kurtzman C.P. 2015. Genomics and the making of yeast biodiversity. Curr Opin Genet Dev 35:100-109.

Yeasts are unicellular fungi that do not form fruiting bodies. Although the yeast lifestyle has evolved multiple times, most known species belong to the subphylum Saccharomycotina (syn. Hemiascomycota, hereafter yeasts). This diverse group includes the premier eukaryotic model system, Saccharomyces cerevisiae; the common human commensal and opportunistic pathogen, *Candida albicans*; and over

1000 other known species (with more continuing to be discovered). Yeasts are found in every biome and continent and are more genetically diverse than angiosperms or chordates. Ease of culture, simple life cycles, and small genomes (~10-20Mbp) have made yeasts exceptional models for molecular genetics, biotechnology, and evolutionary genomics. Here we discuss recent developments in understanding the genomic underpinnings of the making of yeast biodiversity, comparing and contrasting natural and human-associated evolutionary processes. Only a tiny fraction of yeast biodiversity and metabolic capabilities has been tapped by industry and science. Expanding the taxonomic breadth of deep genomic investigations will further illuminate how genome function evolves to encode their diverse metabolisms and ecologies.

5 Kurtzman CP. 2015. Identification of food and beverage spoilage yeasts from DNA sequence analyses. Int J Food Microbiol 213:71-78.

Detection, identification and classification of yeasts have undergone major changes in the last decade and a half following application of gene sequence analyses and genome comparisons. Development of a database (barcode) of easily determined DNA sequences from domains 1 and 2 (D1/D2) of the nuclear large subunit rRNA gene and from ITS now permits many laboratories to identify species quickly and accurately, thus replacing the laborious and often inaccurate phenotypic tests previously used. Phylogenetic analysis of gene sequences has resulted in a major revision of yeast systematics resulting in redefinition of nearly all genera. This new understanding of species relationships has prompted a change of rules for naming and classifying yeasts and other fungi, and these new rules are presented in the recently implemented International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). The use of molecular methods for species identification and the impact of Code changes on classification will be discussed, especially in the context of food and beverage spoilage yeasts.

6 Kurtzman CP. 2016. Description of *Groenewaldozyma* gen. nov. for placement of *Candida auringiensis*, *Candida salmanticensis* and *Candida tartarivorans*. Antonie van Leeuwenhoek. 109(7):1041-1045.

DNA sequence analyses have demonstrated that species of the polyphyletic anamorphic ascomycete genus *Candida* may be members of described teleomorphic genera, members of the *Candida tropicalis* clade upon which the genus *Candida* is circumscribed, or members of isolated clades that represent undescribed genera. From phylogenetic analysis of gene sequences from nuclear large subunit rRNA, mitochondrial small subunit rRNA and cytochrome oxidase II, *Candida auringiensis* (NRRL Y-17674(T), CBS 6913(T)), *Candida salmanticensis* (NRRL Y-17090(T), CBS 5121(T)), and *Candida tartarivorans* (NRRL Y-27291(T), CBS 7955(T)) were shown to be members of an isolated clade and are proposed for reclassification in the genus *Groenewaldozyma* gen. nov. (MycoBank MB 815817). Neighbouring taxa include species of the *Wickerhamiella* clade and *Candida blankii*.

7 Kurtzman CP, Robnett CJ, Blackwell M. 2016. Description of *Teunomyces* gen. nov. for the *Candida kruisii* clade, *Suhomyces* gen. nov. for the *Candida tanzawaensis* clade and *Suhomyces kilbournensis* sp. nov. FEMS Yeast Res. 16(5) - doi: 10.1093/femsyr/fow041.

DNA sequence analysis has shown that species of the *Candida kruisii* clade and species of the *Candida tanzawaensis* clade represent phylogenetically circumscribed genera, which are described as *Teunomyces* gen. nov., type species *T. kruisii*, and *Suhomyces* gen. nov., type species *S. tanzawaensis*. Many of the species are distributed worldwide and they are often isolated from fungus-feeding insects and their habitats. Included is the description of *Suhomyces kilbournensis* (type strain NRRL Y-17864, CBS 14276), a species found almost exclusively on maize kernels (*Zea mays*) in Illinois, USA.

8 Kurtzman CP, Price NP, Ray KJ, Kuo TM. 2016. Fermentative production of sophorolipids from soybean and other vegetable oils. U.S. Patent 9,382,566 B1. July 5, 2016.

Open chain sophorolipids may be produced by fermentation with *Candida* sp. NRRL Y-27208 or

C. riodocensis. Dimers and trimers of sophorolipids are also produced. The sorphorolipids are produced by inoculating a fermentation medium comprising a carbon source and a lipid, with *Candida riodocensis* or *Candida* species NRRL Y-27208, and incubating under aerobic conditions and for a period of time effective to produce an open chain sophorolipid in the medium. The sophorolipids may be subsequently recovered from the fermentation medium.

9 Riley R, Haridas S, Wolfe KH, Lopes MR, Hittinger CT, Göker M, Salamov AA, Wisecaver JH, Long TM, Calvey CH, Aerts AL, Barry KW, Choi C, Clum A, Coughlan AY, Deshpande S, Douglass AP, Hanson SJ, Klenk HP, LaButti KM, Lapidus A, Lindquist EA, Lipzen AM, Meier-Kolthoff JP, Ohm RA, Otillar RP, Pangilinan JL, Peng Y, Rokas A, Rosa CA, Scheuner C, Sibirny AA, Slot JC, Stielow JB, Sun H, Kurtzman CP, Blackwell M, Grigoriev IV, Jeffries TW. 2016. Comparative genomics of biotechnologically important yeasts. Proc Natl Acad Sci U S A. 113(35):9882-9887.

Ascomycete yeasts are metabolically diverse, with great potential for biotechnology. Here, we report the comparative genome analysis of 29 taxonomically and biotechnologically important yeasts, including 16 newly sequenced. We identify a genetic code change, CUG-Ala, in *Pachysolen tannophilus* in the clade sister to the known CUG-Ser clade. Our well-resolved yeast phylogeny shows that some traits, such as methylotrophy, are restricted to single clades, whereas others, such as L-rhamnose utilization, have patchy phylogenetic distributions. Gene clusters, with variable organization and distribution, encode many pathways of interest. Genomics can predict some biochemical traits precisely, but the genomic basis of others, such as xylose utilization, remains unresolved. Our data also provide insight into early evolution of ascomycetes. We document the loss of H3K9me2/3 heterochromatin, the origin of ascomycete mating-type switching, and panascomycete synteny at the MAT locus. These data and analyses will facilitate the engineering of efficient biosynthetic and degradative pathways and gateways for genomic manipulation.

10 Shen XX, Zhou X, Kominek J, Kurtzman CP, Hittinger CT, Rokas A. 2016. Reconstructing the backbone of the Saccharomycotina yeast phylogeny using genome-scale data. G3 (Bethesda) doi: 10.1534/g3.116.034744.

Understanding the phylogenetic relationships among the yeasts of the subphylum Saccharomycotina is a prerequisite for understanding the evolution of their metabolisms and ecological lifestyles. In the last two decades, the use of rDNA and multi-locus data sets has greatly advanced our understanding of the yeast phylogeny, but many deep relationships remain unsupported. In contrast, phylogenomic analyses have involved relatively few taxa and lineages that were often selected with limited considerations for covering the breadth of yeast biodiversity. Here we used genome sequence data from 86 publicly available yeast genomes representing 9 of the 11 known major lineages and 10 non-yeast fungal outgroups to generate a 1,233-gene, 96-taxon data matrix. Species phylogenies reconstructed using two different methods (concatenation and coalescence) and two data matrices (amino acids or the first two codon positions) yielded identical and highly supported relationships between the 9 major lineages. Aside from the lineage comprised by the family Pichiaceae, all other lineages were monophyletic. Most interrelationships among yeast species were robust across the two methods and data matrices. However, 8 of the 93 internodes conflicted between analyses or data sets, including the placements of: the clade defined by species that have reassigned the CUG codon to encode serine, instead of leucine; the clade defined by a whole genome duplication; and the species *Ascoidea rubescens*. These phylogenomic analyses provide a robust roadmap for future comparative work across the yeast subphylum in the disciplines of taxonomy, molecular genetics, evolutionary biology, ecology, and biotechnology. To further this end, we have also provided a BLAST server to query the 86 Saccharomycotina genomes, which can be found at http://y1000plus.org/blast.

11 Slininger PJ, Dien BS, Kurtzman CP, Moser BR, Bakota EL, Thompson SR, O'Bryan PJ, Cotta MA, Balan V, Jin M, Sousa L da C, Dale BE. 2016. Comparative lipid production by oleaginous yeasts in hydrolyzates of lignocellulosic biomass and process strategy for high titers. Biotechnol Bioeng 113(8):1676-1690.

Oleaginous yeasts can convert sugars to lipids with fatty acid profiles similar to those of vegetable oils, making them attractive for production of biodiesel. Lignocellulosic biomass is an attractive source of sugars for yeast lipid production because it is abundant, potentially low cost, and renewable. However, lignocellulosic hydrolyzates are laden with byproducts which inhibit microbial growth and metabolism. With the goal of identifying oleaginous yeast strains able to convert plant biomass to lipids, we screened 32 strains from the ARS Culture Collection, Peoria, IL to identify four robust strains able to produce high lipid concentrations from both acid and base-pretreated biomass. The screening was arranged in two tiers using undetoxified enzyme hydrolyzates of ammonia fiber expansion (AFEX)-pretreated cornstover as the primary screening medium and acid-pretreated switch grass as the secondary screening medium applied to strains passing the primary screen. Hydrolyzates were prepared at ~18-20% solids loading to provide ~110 g/L sugars at ~56:39:5 mass ratio glucose:xylose:arabinose. A two stage process boosting the molar C:N ratio from 60 to well above 400 in undetoxified switchgrass hydrolyzate was optimized with respect to nitrogen source, C:N, and carbon loading. Using this process three strains were able to consume acetic acid and nearly all available sugars to accumulate 50-65% of cell biomass as lipid (w/w), to produce 25-30 g/L lipid at 0.12-0.22 g/L/h and 0.13-0.15 g/g or 39-45% of the theoretical yield at pH 6 and 7, a performance unprecedented in lignocellulosic hydrolyzates. Three of the top strains have not previously been reported for the bioconversion of lignocellulose to lipids. The successful identification and development of top-performing lipid-producing yeast in lignocellulose hydrolyzates is expected to advance the economic feasibility of high quality biodiesel and jet fuels from renewable biomass, expanding the market potential for lignocellulose-derived fuels beyond ethanol for automobiles to the entire U.S. transportation market.

12 Zhou X, Peris D, Kominek J, Kurtzman CP, Hittinger CT, Rokas A. 2016. *In silico* Whole Genome Sequencer & Analyzer (iWGS): a computational pipeline to guide the design and analysis of de novo genome sequencing studies. G3 (Bethesda) - doi: 10.1534/g3.116.034249.

The availability of genomes across the tree of life is highly biased toward vertebrates, pathogens, human disease models, and organisms with relatively small and simple genomes. Recent progress in genomics has enabled the de novo decoding of the genome of virtually any organism, greatly expanding its potential for understanding the biology and evolution of the full spectrum of biodiversity. The increasing diversity of sequencing technologies, assays, and de novo assembly algorithms have augmented the complexity of de novo genome sequencing projects in non-model organisms. To reduce the costs and challenges in de novo genome sequencing projects and streamline their experimental design and analysis, we developed iWGS (in silico Whole Genome Sequencer and Analyzer), an automated pipeline for guiding the choice of appropriate sequencing strategy and assembly protocols. iWGS seamlessly integrates the four key steps of a de novo genome sequencing project: data generation (through simulation), data quality control, de novo assembly, and assembly evaluation and validation. The last three steps can also be applied to the analysis of real data. iWGS is designed to enable the user to have great flexibility in testing the range of experimental designs available for genome sequencing projects, and supports all major sequencing technologies and popular assembly tools. Three case studies illustrate how iWGS can guide the design of de novo genome sequencing projects and evaluate the performance of a wide variety of user-specified sequencing strategies and assembly protocols on genomes of differing architectures. iWGS, along with a detailed documentation, is freely available at https://github.com/zhouxiaofan1983/iWGS.

XI Biotechnologies laboratory, UFR Biosciences, University Félix Houphouet-Boigny, 22 bp 582, Abidjan 22, Ivory Coast and Yeast Molecular Genetics Laboratory, UMR5240 Microbiologie, Adaptation et Pathogénie, University Lyon 1, 10 rue Raphaël Dubois, F-69622 Villeurbanne, France. Communicated by Pr Marc Lemaire <marc.lemaire.bio@univ-lyon1.fr>
http://map.univ-lyon1.fr/spip.php?article261&lang=en

Recent publication.

1 Samagaci L, Ouattara H, Niamke S and Lemaire M. 2016. *Pichia kudrazevii* and *Candida nitrativorans* are the most well-adapted and relevant yeasts species fermenting cocoa in Agneby-Tiassa, an local Ivorian cocoa producing region . Food Intl Res doi: 10.1016/j.foodres.2016.10.007

Cocoa bean fermentation is a spontaneous and still poorly controlled process that very often leads to end-prod- ucts of heterogeneous quality. This fermentation is driven by a succession of complex microbial communities where yeasts play key roles during the first stages of the process. In this study, we identified and analyzed the growth dynamics of yeasts involved in cocoa bean fermentation from the Ivorian region called Agneby-Tiassa. The results showed that *Pichia kudriazevii* and *Candida nitrativorans* were the dominant yeasts in fermented cocoa from Agneby-Tiassa. Five other species, namely *Candida tropicalis, Candida intermedia, Candida nitrativorans, H. uva-rum* and *H. guilliermondii*, were also found but in smaller numbers. Additionally, intraspecies diversity was determined by examining the length polymorphism of the genetic marker ITS1-5.8S-ITS2 and its PCR-RFLP analysis. We showed that the dominant species, *P. kudriazevii* and *C. nitrativorans*, are well adapted to environmental conditions specific to cocoa bean fermentation as they are resistant to high temperatures (40 °C) and high ethanol concentrations (20%). Moreover, *P. kudriazevii* and *C. nitrativorans* species exhibited pectin-hydrolysing enzymatic activ-ities suggesting a key role in the degradation of cocoa bean pulp during fermentation. Furthermore, these pectinolytic activities occurred in acidic growth conditions (pH 3–5), which correspond to the pH conditions of the early steps of cocoa fermentation. Taken together, these results show that *P. kudriazevii* and *C. nitrativorans* are key players in cocoa bean fermentation in the Agneby-Tiassa region and they are promising candidates for developing starter cocktails that could be used to improve the overall efficiency of cocoa fermentation in Ivory Coast.

XII Microorganisms Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran. Communicated by S. Nasr <shaghayegh2963@yahoo.com.

Recent publication.

1 Nasr S, Nguyen HDT, Reza M, Soudi SASF, Sipiczki M. 2016. *Wickerhamomyces orientalis* f.a., sp. nov.: an ascomycetous yeast species belonging to the *Wickerhamomyces* clade. Int J Syst Evol Microbiol 66:2534–2539.

Five closely related yeast strains were isolated from soil in Kharg Island, Persian Gulf, Iran, and from fallen fruits in Galle, Sri Lanka, during separate projects. Morphologically, the strains produced white-coloured yeast colonies, with cells that were ovoid to ellipsoidal, making branched, true hyphae and pseudohyphae. Ascospore formation was not observed. Biochemically, the strains were able to ferment D-glucose and weakly ferment D-galactose. The strains could use a wide variety of carbon sources except methanol and hexadecane. Phylogenetic analyses using combined sequences of the small ribosomal subunit and the D1/D2 domains of the LSU, as well as the internal transcribed spacer regions, suggested that these strains belong to the *Wickerhamomyces* clade and that together they form one strongly supported phylogenetic clade. Differences in their sequences, biochemistry and morphology suggest they are representatives of distinct species of the genus *Wickerhamomyces*. Therefore, the name *Wickerham-omyces orientalis* f.a., sp. nov. is proposed to accommodate these novel strains; the type strain is IBRC-M 30103T (=CBS 13306T). The MycoBank number is MB 807323.

XIII State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIgenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

We were glad to accept in our lab Taiwanese colleagues Lee Ching-Fu (National Tsing Hua University) and Wang Pin-Han (Tonghai University) during short visit in June 2016. Many thanks to Dr. Ching-Fu Lee for hosting us in his lab in November 2015 and November 2016.

Dr. V.I. Kondratieva has retired after 40 years of scientific activity in our lab. She fruitfully participated in many genetic yeast projects, viz. on *Arthroascus*, *Eremothecium*, *Kluyveromyces*, *Komagataella*, *Ogataea*, *Pichia/Hansenula*, *Saccharomyces*, *Schizosaccharomyces*, *Williopsis*, and *Zygowilliopsis*.

The following are papers for 2016 or in press.

1 Naumov GI, Kondratieva VI, Meshcheryakova EV, Naumova ES. 2016. Taxonomic genetics of methylotrophic yeast genus *Komagataella*: new biological species *K. kurtzmanii.* Russian J Genetics. 52(4):378-382. © Pleiades Publishing, Ltd.

2 Naumov GI, Shalamitskiy MYu, Naumova ES. 2016. New family of pectinase genes *PGU1b–PGU3b* of the pectinolytic yeast *Saccharomyces bayanus* var. *uvarum*. Doklady Biochem Biophys 467: 89–91. © Pleiades Publishing, Ltd.

Using yeast genome databases and literature data, we have conducted a phylogenetic analysis of pectinase *PGU* genes from *Saccharomyces* strains assigned to the biological species *S. arboricola, S. bayanus* (var. *uvarum*), *S. cariocanus, S. cerevisiae, S. kudriavzevii, S. mikatae, S. paradoxus*, and hybrid taxon *S. pastorianus* (syn. S. *carlsbergensis*). Single *PGU* genes were observed in all *Saccharomyces* species, except *S. bayanus*. The superfamily of divergent *PGU* genes has been documented in *S. bayanus* var. *uvarum* for the first time. Chromosomal localization of new *PGU1b*, *PGU2b*, and *PGU3b* genes in the yeast *S. bayanus* var. *uvarum* has been determined by molecular karyotyping and Southern hybridization.

3 Shalamitskiy MYu., Naumov GI. 2016. Identification and polymorphism of pectinase genes *PGU* in the *Saccharomyces bayanus* complex. Russian J Genetics. 52(5): 535–538. © Pleiades Publishing, Ltd.

Pectinase (endo-polygalacturonase) is the key enzyme splitting plant pectin. The corresponding single gene *PGU1* is documented for the yeast *S. cerevisiae*. On the basis of phylogenetic analysis of the *PGU* nucleotide sequence available in the GenBank, a family of divergent *PGU* genes is found in the species complex *S. bayanus*: *S. bayanus* var. *uvarum*, *S. eubayanus*, and hybrid taxon *S. pastorianus*. The *PGU* genes have different chromosome localization.

4 Naumov GI, Shalamitskiy MYu, Martynenko NN, Naumova ES. 2016. Molecular phylogeny of pectinase genes *PGU* in the yeast genus *Saccharomyces.* Microbiology (Moscow). 85(6):734–743. © Pleiades Publishing, Ltd.

Using yeast genome databases and literature data, we have conducted a phylogenetic analysis of pectinase *PGU* genes from 112 *Saccharomyces* strains assigned to the biological species *S. arboricola*, *S. bayanus* (var. *uvarum*), *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus* and hybrid taxon *S. pastorianus* (syn. *S. carlsbergensis*). The superfamily of divergent *PGU* genes has been found. Within the *Saccharomyces* species, identity of *PGU* gene nucleotide sequences was 98.8–100% for *S. cerevisiae*, 86.1–95.7% for *S. bayanus* (var. *uvarum*), 94–98.3% for *S. kudriavzevii* and 96.8–100% for *S. paradoxus/S. cariocanus.* Nevertheless, natural interspecific transfer of *PGU* gene from *S. cerevisiae* to *S. bayanus* and from *S. paradoxus* to *S. cerevisiae* can occur. For the first time, a family of polymeric *PGU1b, PGU2b, PGU3b* and *PGU4b* genes is documented for the yeast *S. bayanus* var. *uvarum* important for winemaking.

5 Naumov GI. 2017. Genetic polymorphism of sherry *Saccharomyces cerevisiae* yeasts. Microbiology (Moscow). 86 (1) (in press).

6 Naumova ES, Sadykova AZh., Michailova YuV, Naumov GI. 2017. Polymorphism of lactose genes in the dairy yeasts *Kluyveromyces marxianus*, potential probiotic microorganisms. Microbiology (Moscow) (in press).

Using molecular karyotyping and Southern blot hybridization we studied chromosomal polymorphism of *LAC* genes controlling fermentation of lactose in *Kluyveromyces marxianus* strains isolated from various dairy products and natural sources in Russia and CIS countries. Profound polymorphism of karyotype patterns and accumulation of *LAC* genes were observed in dairy *K. marxianus* yeasts. *K. marxianus* strains isolated from dairy products intensively fermented lactose at 37ºC after one day of fermentation, while non-dairy strains showed delayed fermentation or did not ferment lactose at all. Based on the fermentation tests, we selected twelve *K. marxianus* strains, which are of interest as potential probiotic microorganisms suitable for further molecular genetic studies and breeding.

7 Naumova ES., Lee CFu, Kondratieva VI, Sadykova AZh, Naumov GI. 2017. Molecular genetic polymorphism of soil yeasts of the genus *Williopsis* from Taiwan Island. Russian J Genetics (in press).

Comparative molecular genetic study of *Williopsis* yeasts isolated in different world regions revealed some peculiarities of species content in Taiwan. Some *Williopsis* strains may represent novel species. In Taiwan, four of the five known *Williopsis* species were documented: *W. saturnus, W. suaveolens, W. mrakii* and *W. subsufficiens*. The *W. saturnus* yeasts predominate in Taiwanese soils, while *W. suaveolens* is more frequently isolated in Europe.

8 Naumov GI, Shalamitskiy MYu, Naumova ES. 2016. Identification and polymorphism of pectinase genes *PGU* in the *Saccharomyces bayanus* complex. 14th International Congress on Yeasts, 11-15 September 2016, Awaji, Japan, P. 294.

9 Naumova ES, Boundy-Mills K, Lee CF, Naumov GI. 2016. Molecular genetic differentiation of species in the methanol assimilating genus *Komagataella.* 14th International Congress on Yeasts, 11-15 September 2016, Awaji, Japan, P. 260.

10 Sadykova AZh. 2016. Genetic bases for selection of fermenting *Saccharomyces* and *Kluyveromyces* yeasts. Ph.D. Thesis, GosNIIgenetika, Moscow.

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Recent publication.

1 Prillinger H & Lopandic K. 2015. Yeast-types of the Basidiomycota using cell wall sugars and ribosomal DNA sequences. Stapfia 103:3-18.

The cell wall carbohydrate composition was correlated with the large subunit rDNA (D1/D2) based phylogeny to estimate its significance in evolution of the Basidiomycota. The majority of investigated isolates showed three main cell wall sugar types: the *Microbotryum*-, the *Ustilago*- and the *Tremella*-type. The *Microbotryum*-type (mannose-glucose-galactose-fucose) corresponded with the subphylum Pucciniomycotina, the *Ustilago*-type (glucose-mannose-galactose) with the Ustilaginomycotina and the *Tremella*-type (glucose-mannose-xylose) with the Agaricomycotina. However, in a number of isolates additional carbohydrates were also identified. A sporadical appearance of rhamnose and xylose within the Microbotryomycetes of the Pucciniomycotina, or glucose-mannose pattern within the Agarico-mycetes, and galactose or fucose within the Tremellomycetes of the Agari-comycotina indicated that the cell wall carbohydrate composition charac-terised rather classes or subclasses than subphyla. The appearance of rhamnose, that is also present in the cell walls of the Taphrinales of the Ascomycota, may indicate a basal

position of the Pucciniomycotina in the evolution of the Basidiomycota. This result is in conflict with the D1/D2 based phylogeny, which suggested that the Ustilaginomycotina occupy a basal position in the neighborjoining tree. The occurrence of the glucose-mannose pattern of the *Saccharomyces*-type of the ascomycetous yeasts and in the highest evolved basidiomycetous yeast isolates (Agaricaceae) from two *Cyphomyrmex* species suggests that the Saccharomycotina are basal in the phylogenetic tree of the Ascomycota. Based on the presence of teliospores, some teleomorphs with CoQ 8 and ribosomal DNA sequences a new class within the Agaricomycotina, the Cystofilobasidiomycetes, was introduced for the *Cystofilobasidium* and *Mrakia* species.

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The following papers have been recently published or are in press.

1 Sena LM, Morais CG, Lopes MR, Santos RO, Uetanabaro AP, Morais PB, Vital MJ, de Morais MA Jr, Lachance MA, Rosa CA. 2017. D-xylose fermentation, xylitol production and xylanase activities by seven new species of *Sugiyamaella.* Antonie van Leeuwenhoek - doi: 10.1007/s10482-016-0775-5

Sixteen yeast isolates identified as belonging to the genus *Sugiyamaella* were studied in relation to D-xylose fermentation, xylitol production, and xylanase activities. The yeasts were recovered from rotting wood and sugarcane bagasse samples in different Brazilian regions. Sequence analyses of the internal transcribed spacer (ITS) region and the D1/D2 domains of large subunit rRNA gene showed that these isolates belong to seven new species. The species are described here as *Sugiyamaella ayubii* f.a., sp. nov. (UFMG-CM-Y607^T = CBS 14108^T), *Sugiyamaella bahiana* f.a., sp. nov. (UFMG-CM-Y304^T = CBS 13474^T), *Sugiyamaella bonitensis* f.a., sp. nov. (UFMG-CM-Y608^T = CBS 14270^T), *Sugiyamaella carassensis* f.a., sp. nov. (UFMG-CM-Y606^T = CBS 14107^T), *Sugiyamaella ligni* f.a., sp. nov. (UFMG-CM-Y295^T = CBS 13482^T), *Sugiyamaella valenteae* f.a., sp. nov. (UFMG-CM-Y609^T = CBS 14109^T) and *Sugiyamaella xylolytica* f.a., sp. nov. (UFMG-CM-Y348^T = CBS 13493^T). Strains of the described species *S. boreocaroliniensis, S. lignohabitans, S. novakii* and *S. xylanicola*, isolated from rotting wood of Brazilian ecosystems, were also compared for traits relevant to xylose metabolism. *S. valenteae* sp. nov., *S. xylolytica* sp. nov., *S. bahiana* sp. nov., *S. bonitensis* sp. nov., *S. boreocarolinensis, S. lignohabitans* and *S. xylanicola* were able to ferment D-xylose to ethanol. Xylitol production was observed for all *Sugiyamaella* species studied, except for *S. ayubii* sp. nov. All species studied showed xylanolytic activity, with *S. xylanicola, S. lignohabitans* and *S. valenteae* sp. nov. having the highest values. Our results suggest these *Sugiyamaella* species have good potential for biotechnological applications.

2 Cadete RM, Melo-Cheab MA, Viana AL, Oliveira ES, Fonseca C, Rosa CA. 2016. The Yeast *Scheffersomyces amazonensis* is an efficient xylitol producer. World J Microbiol Biotechnol 32:207.

This study assessed the efficiency of *Scheffersomyces amazonensis* UFMG-CM-Y493^T, cultured in xylose-supplemented medium (YPX) and rice hull hydrolysate (RHH), to convert xylose to xylitol under moderate and severe oxygen limitation. The highest xylitol yields of 0.75 and 1.04 g.g^{-1} in YPX and RHH, respectively, were obtained under severe oxygen limitation. However, volumetric productivity in RHH was ninefold decrease than that in YPX medium. The xylose reductase (XR) and xylitol dehydrogenase (XDH) activities in the YPX cultures were strictly dependent on NADPH and NAD^+ respectively, and were approximately 10% higher under severe oxygen limitation than under moderate oxygen limitation. This higher *Sc. amazonensis* xylitol production observed under severe oxygen limitation can be attributed to the higher XR activity and shortage of the NAD^+ needed by XDH. These results suggest that *Sc. amazonensis* UFMG-CM-Y493^T is one of the greatest xylitol producers described to date and reveal its potential use in the biotechnological production of xylitol.

3 Pereira CB, Pereira de Sá N, Borelli BM, Rosa CA, Barbeira PJ, Cota BB, Johann S. 2016. Antifungal activity of ecicosanoic acids isolated from the endophytic fungus *Mycosphaerella* sp. against *Cryptococcus neoformans* and *C. gattii.* Microb Pathog 100: 205-212.

The antifungal effects of two eicosanoic acids, 2-amino-3,4-dihydroxy-2-25-(hydroxymethyl)-14-oxo-6,12-eicosenoic acid (compound 1) and myriocin (compound 2), isolated from *Mycosphaerella* sp. were evaluated against *Cryptococcus neoformans* and *C. gattii*. The compounds displayed antifungal activities against several isolates of *C. neoformans* and *C. gattii*, with minimal inhibitory concentration (MIC) values ranging from 0.49 to 7.82 μM for compound 1 and 0.48-1.95 μM for compound 2. In the checkerboard microtiter test, both compounds exhibited synergistic activity with amphotericin B against *C. gattii*. Ultrastructural analysis revealed several signs of damage in *C. gattii* and *C. neoformans* cells treated with compounds 1 and 2, including deformities in cell shape, depressions on the surface, and withered cells. The cells of *C. gattii* treated with compounds 1 and 2 showed less loss of cellular material in comparison to those treated with amphotericin B. The difference in cellular material loss increased in a test compound concentration-dependent manner. Consistent with this observation, compounds 1 and 2 were able to internalize propidium iodide (PI) in *C. gattii* cells. In addition, compound 2 induced the formation of several pseudohyphae, suggesting that it could reduce virulence in *C. gattii* cells. The study results show that these natural products led to membrane damage; however, this may not be the main target of action. These compounds have potential antifungal activity and could be useful in further studies for developing more effective combination therapies with amphotericin B and reducing side effects in patients.

4 Gomes FC, Safar SV, Santos AR, Lachance MA, Rosa CA. 2016. *Kockovaella libkindii* sp. nov., a yeast species isolated from water tanks of bromeliad. Int J Syst Evol Microbiol - doi: 10.1099/ijsem.0.001471.

During a study of yeast community associated with water tanks (phytotelmata) of the bromeliad *Vriesea minarum*, two strains of a new stalk-forming yeast species were found. The sequences of the region spanning the ITS and D1/D2 domains of the large subunit rRNA gene showed that this species belongs to the genus *Kockovaella*. The new species differs by 14 or more nucleotide substitutions in the D1/D2 domains and by 26 or more substitutions in the ITS-5.8S region from all other *Kockovaella* species. We describe this species as *Kockovaella libkindii* sp. nov. The type strain of *Kockovaella libkindii* sp. nov. is UFMG-CM-Y6053T (= CBS 12685T). The MycoBank number is MB 817710.

5 Morais CG, Lara CA, Borelli BM, Cadete RM, Moreira JD, Lachance MA, Rosa CA 2016 *Satunispora bothae* sp. nov., a new species isolated from rotting wood. Int J Syst Evol Microbiol, 66: 3810-3813.

Two strains representing a new *Saturnispora* species were isolated from rotting wood samples collected in an Atlantic Rain Forest site in Brazil. Analyses of the sequences of the D1/D2 domains of the rRNA gene showed that this new species belongs to a subclade in the *Saturnispora* clade formed by *S. sanitii, S. sekii, S. silvae* and *S. suwanaritii*. The new species differed in D1/D2 sequences by 60 or more nucleotide substitutions from these species. The strains produced asci with one to four hemispherical ascospores. The new species *Saturnispora bothae* sp. nov. is proposed to accommodate these isolates. The type strain of *Saturnispora bothae* sp. nov. is UFMG-CM-Y292T (=CBS 13484T). The MycoBank number is MB 817127.

6 Cadete RM, de Las Heras AM, Sandstrom AG, Ferreira C, Gírio F, Gorwa-Grauslund MF, Rosa CA, Fonseca C. 2016. Exploring xylose metabolism in *Spathaspora* species: XYL1.2 from *Spathaspora passalidarum* as the key for eficiente anaerobic xylose fermentation in metabolic engineered *Saccharomyces cerevisiae.* Biotechnol Biofuels 9:167.

BACKGROUND: The production of ethanol and other fuels and chemicals from lignocellulosic materials is dependent of efficient xylose conversion. Xylose fermentation capacity in yeasts is usually linked to xylose reductase (XR) accepting NADH as cofactor. The XR from *Scheffersomyces stipitis*, which is able to use NADH as cofactor but still prefers NADPH, has been used to generate recombinant xylose-fermenting

Saccharomyces cerevisiae. Novel xylose-fermenting yeasts species, as those from the *Spathaspora* clade, have been described and are potential sources of novel genes to improve xylose fermentation in *S. cerevisiae*.
RESULTS: Xylose fermentation by six strains from different *Spathaspora* species isolated in Brazil, plus the *Sp. passalidarum* type strain (CBS 10155(T)), was characterized under two oxygen-limited conditions. The best xylose-fermenting strains belong to the *Sp. passalidarum* species, and their highest ethanol titers, yields, and productivities were correlated to higher XR activity with NADH than with NADPH. Among the different *Spathaspora* species, *Sp. passalidarum* appears to be the sole harboring two XYL1 genes: *XYL1*.1, similar to the XYL1 found in other *Spathaspora* and yeast species and XYL1.2, with relatively higher expression level. XYL1.1p and *XYL1*.2p from *Sp. passalidarum* were expressed in *S. cerevisiae* TMB 3044 and *XYL1*.1p was confirmed to be strictly NADPH-dependent, while *XYL1*.2p to use both NADPH and NADH, with higher activity with the later. Recombinant *S. cerevisiae* strains expressing *XYL1*.1p did not show anaerobic growth in xylose medium. Under anaerobic xylose fermentation, *S. cerevisiae* TMB 3504, which expresses *XYL1*.2p from *Sp. passalidarum*, revealed significant higher ethanol yield and productivity than *S. cerevisiae* TMB 3422, which harbors *XYL1p* N272D from *Sc. stipitis* in the same isogenic background (0.40 *vs* 0.34g.gCDW^{-1} and 0.33 vs 0.18g.g.CDW $^{(-1)}$h$^{(-1)}$, respectively).
CONCLUSION: This work explored a new clade of xylose-fermenting yeasts (*Spathaspora* species) towards the engineering of *S. cerevisiae* for improved xylose fermentation. The new *S. cerevisiae* TMB 3504 displays higher XR activity with NADH than with NADPH, with consequent improved ethanol yield and productivity and low xylitol production. This meaningful advance in anaerobic xylose fermentation by recombinant *S. cerevisiae* (using the XR/XDH pathway) paves the way for the development of novel industrial pentose-fermenting strains.

7 Santiago IF, Rosa CA, Rosa LH. 2016. Endophytic symbiont yeasts associated with the Antarctic angiosperms *Deschampsia antarctica* and *Colobanthus quitensis.* Polar Biol - doi: 10.1007/s00300-016-1940-z.

Fungal diversity in Antarctic seems to be greater than what is known and remains largely unexplored. In this study, we identified the endophytic symbiont yeasts associated with leaves of the angiosperms *Deschampsia antarctica* and *Colobanthus quitensis* living on King George Island, Antarctica using a culture-based approach. One hundred and twelve yeast isolates were obtained from the tissue of the different plants sampled. These yeasts were identified using sequencing of the D1/D2 domains of the LSU region of the rRNA gene as *Cryptococcus victoriae*, *Cystobasidium laryngis*, *Rhodotorula mucilaginosa*, *Sporidiobolus ruineniae* and *Leucosporidium* aff. *golubevii*. The psychrophilic yeast *C. victoriae* was the most abundant species associated with the two angiosperms. *Cystobasidium laryngis* occurs only in the leaves of *D. antarctica*. In contrast, *R. mucilaginosa*, *S. ruineniae* and *L.* aff. *golubevii* occurred only in *C. quitensis*. Phylogenetic analysis indicates the Antarctic endophytic yeast strains are closely related to taxa obtained from substrates located in different habitats of the world. However, the endophytic yeast C. victoria was closely related to psychrophilic taxa isolated from Antarctica, but also from the Arctic, Alpine and Himalayan environments. The abundance of endophytic yeasts associated with Antarctic angiosperms suggests a possible symbiotic relationship with their plant hosts, which may provide shelter and growing conditions suitable for the yeasts' survival, dispersal and colonization other Antarctic environments. In contrast, the endophytic yeasts might directly or indirectly promote the fitness of their host plants by producing metabolites beneficial to plant survival in the extreme environments of Antarctica.

8 Lopes MR, Morais CG, Kominek J, Cadete RM, Soares MA, Uetanabaro APT, Fonseca C, Lachance MA, Hittinger CT, Rosa CA. 2016. Genomic analysis and D-xylose fermentation of three novel *Spathaspora* species: *Spathaspora girioi* sp. nov., *Spathaspora hagerdaliae* fa, sp. nov. and *Spathaspora gorwiae* fa, sp. nov. FEMS Yeast Res 16: fow044.

XVI International Centre for Brewing and Distilling, Heriot Watt University, Edinburgh, Scotland EH14 4AS. and GGStewart Associates, 13 Heol Nant Castan, Rhiwbina, Cardiff, Wales, CF14 6RP. Communicated by Graham G. Stewart

<Profggstewart@aol.com> and <g.g.stewart@hw.ac.uk> www.ggstewartassociates.co.uk.

Recent publications:

1 Stewart GG. 2015. Yeast quality assessment, management and culture maintenance. In: Brewing Microbiology: Managing Microbes, Ensuring Quality and Valorising Waste. Annie E Hill (ed.). Elsevier Woodhead, Oxford, UK. Chapter 2, pp. 11-29.

2 Sammartino M and Stewart GG. 2015. Graham G. Stewart: A lifelong relationship with yeast. Master Brewers Association of the Americas. Technical Quarterly 52:146-151.

3 Stewart GG. 2016. BEERS/Raw materials and wort production. In: Encyclopedia of Health and Food. Caballero B, Finglas P. and Toldra F (eds.). Elsevier, Oxford, UK. Chapter 58, pp. 355-363.

4 Stewart GG. 2016. Adjuncts. In: Brewing Materials and Processes: A Practical Approach to Beer Excellence. Bamforth CW (ed.), Elsevier, Oxford, UK. Chapter 2, pp. 27-44.

5 Stewart GG, Maskell DL and Speers A, 2016. Brewing fundamentals – fermentation. Master Brewers Association of the Americas. Technical Quarterly 53:2-22.

6 Stewart GG. 2016. Beer shelf life and stability. In: The Stability and Shelf Life of Food and Beverages. Subramanian, SM (ed.). Elsevier, Oxford, UK, Chapter 10, pp. 293-309.

7 Stewart GG. 2016. Industrial uses of yeast – brewing and distilling. New Food, 19:20-24.

8 Stewart GG. 2016. *Saccharomyces cerevisiae* in the production of beer. Beverages 2016. MDPI, Basel, Switzerland – in press.

9 Stewart GG. 2016. Brewing intensification through the lens of the craft brewer. Master Brewers Association of the Americas. Technical Quarterly 53(4) – in press.

XVII Yeast Genetics & Molecular Biology Lab, Department of Studies in Biochemistry, University of Mysore, Mysore, India. Communicated by Akshay Vishwanatha <agnibhat@gmail.com>.

Recent publication.

1 Vishwanatha A, Rallis C, Subramanyaswamy S B, Michael D'Souza C J, Bähler J, Schweingruber M E. 2016. Identification of nuclear genes affecting 2-deoxyglucose resistance in *Schizosaccharomyces pombe*. FEMS Yeast Res - doi:10.1093/femsyr/fow061

Fission yeast *Schizosaccharomyces pombe* was screened for genes involved in regulation of sensitivity to 2-Deoxyglucose (2-DG). 2-DG is a toxic glucose analog used as an anticancer drug and an antimetabolite. In a first approach we selected for genes causing 2-DG resistance when overexpressed by screening a *Sau3A* genomic library ligated into shuttle vector pURL18. Plasmid borne genes were cloned into expression vector pREP4X and verified for their involvement in control of 2-DG resistance. We identified a gene providing strong resistance to 2-DG which we termed *odr1* (SPBC215.10), and gene *ysp2* (SPAC13A11.05) which showed only weak resistance. Invertase assay indicate that overexpression of *odr1* leads to hyperderepression of invertase. We speculate that the product of the newly identified *odr1* gene might have a similar function as the previously identified DOG1 and DOG2 proteins in *S. cerevisiae* which exhibit 2-DG-6-phosphate specific phosphatase activity. In a second approach, a haploid deletion library was screened to identify 2-DG resistant mutants. From initial screening we identified 14 strains and in subsequent growth analyses we confirmed four genes *snf5*, *ypa1*, *pas1* and *pho7* to be 2-DG resistant. In liquid medium, deletion of these genes conferred 2-DG resistance preferentially under glucose-repressed conditions. Results of invertase assays carried out at various conditions indicate that

these deletion strains are defective in glucose repression. Compared with the control strains which exhibit a glucose repressible invertase phenotype, all the 2-DG resistant deletion strains display a rather constitutive invertase expression. Strain *pas1* exhibits a remarkable high activity both at repressed as well as derepressed conditions. Although the identified genes are involved in regulation of a variety of processes such as chromatin remodeling (*snf5)*, transcription regulation (*pho7)* and cell cycle (*ypa1* and *pas1*), their roles in providing resistance to 2-DG is still unexplored. These genes are not known to be involved in controlling 2-DG resistance in *S. cerevisiae* indicating that 2-DG resistance in budding and fission yeast is achieved, at least partially, by different mechanisms. In view of reports from recent clinical trial that cancer patients treated with 2-DG exhibit 2-DG resistance (Raez *et al.* 2013), identification of genes modulating 2-DG sensitivity in eukaryotic model system *S. pombe* might prove useful.

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

The following have been accepted or published since the last issue of the Yeast Newsletter..

1 Lachance MA, Collens JD, Peng XF, Wardlaw AM, Bishop L, Hou LY, Starmer WT 2016 Spatial scale, genetic structure, and speciation of Hawaiian endemic yeasts. Pacific Sci 70:389-408.

Two Hawaiian endemic yeast species, *Metschnikowia hawaiiensis* and *Metschnikowia hamakuensis,* were examined by means of multilocus characterization. In spite of their narrow range of distribution, restricted to the island of Hawai'i, both species were found to be polymorphic at several loci. Alleles of different loci were distributed independently within local populations, confirming that sexual reproduction prevails among these facultatively asexual organisms. No alleles were shared across species, confirming their reproductive isolation. Although the sample size for the northern species, *M. hamakuensis,* is much less (N = 7) than that for its southern relative, *M. hawaiiensis* (*N* = 161), their genetic diversity is comparable. Genetic differentiation was detected in *M. hawaiiensis* populations at both regional (ca. 30 – 40 km) and local (ca. 1 km) scales. A single isolate recovered in a separate locality exhibited considerable allelic divergence from others, indicating that genetic isolation can occur over relatively short distances and suggesting a first step towards cessation of gene flow, which is required for speciation.

2 Morais CG, Lara CA, Borelli BM, Cadete RM, Moreira JD, Lachance MA, Rosa CA 2016 *Saturnispora bothae* sp. nov., a new species isolated from rotting wood. Int J Syst Evol Microbiol 66: 3810-3813.

See abstract under Dr. Rosa's communication.

3 Gomes FCO, Safar SVB, Santos ARO, Lachance MA, Rosa CA 2016 *Kockovaella libkindii* sp. nov., a yeast species isolated from water tanks of bromeliad. Int J Syst Evol Microbiol - doi: 10.1099/ijsem.0.001471.

See abstract under Dr. Rosa's communication.

4 Lachance MA 2016 *Metschnikowia*: half tetrads, a regicide and the fountain of youth. Yeast 33:563–574.

The purpose of this review is to introduce *Metschnikowia* to the yeast researcher community and to convince readers that the genus is a worthwhile object of study in developmental biology, genetics, ecology and biotechnology. *Metschnikowia* sits at the foundation of modern immunology, having been instrumental in the discovery of animal phagocytosis. Some 81 species form a monophyletic group within the Metschnikowiaceae, which also include the smaller genus *Clavispora* and a few clades of *Candida* species. The family stands out by the habit of forming, by meiosis, only two ascospores, which in *Metschnikowia* are needle shaped. In some cases, the spores can reach enormous proportions, exceeding 200 µm in length; in others, ascus formation is preceded by the development of chlamydospores. The adaptive value of such features remains to be elucidated. Extensive genetic studies are lacking, but attempts to apply

methods developed for model species have been successful. Some species are found at the plant–insect interface whereas others are pathogens of aquatic animals and have served as model organisms in the exploration of host-parasite theory. Some species are globally distributed and others exhibit extreme endemism. Many species are remarkably easy to recover by sampling their known habitats. *M. pulcherrima* and close relatives may play an important role in wine quality and produce pulcherrimin, an iron-dipeptide complex that can interfere with the growth of other microorganisms. Some symbiotic species incapable of growth in culture media have been assigned to the genus, but their kinship with the group remains to be demonstrated.

5 Lachance MA 2016 Paraphyly and (yeast) classification. Int J Syst Evol Microbiol - DOI: 10.1099/ijsem.0.001474

Yeast systematics has wholeheartedly embraced the phylogenetic approach. Central to this has been the unspoken convention that taxa at all ranks be strictly monophyletic. This can result in a proliferation of small genera and instances of nomenclatural instability, counter to the expected benefit of phylogenetic systematics. But the literature abounds with examples, at all taxonomic levels, where paraphyly is a reality that can no longer be ignored. The very concepts of Bacteria or Archaea, under the constraint of monophyly, are in peril. It is therefore desirable to effect a shift in practices that will recognize the existence of paraphyletic taxa.

Recent meetings

43rd Annual Conference on Yeasts, Czechoslovak Comission on Yeast Smolenica Castle, Slovakia, May 10-13 2016

The 43rd Annual Conference on Yeasts was held 10-13 May 2016 at Smolenice Castle, Slovakia. The abstract book is available at on http://yeastconference.sk/archive.

Emília Breierová <Emilia.Breierova@savba.sk>

Training course of Microbial Resources Information Management and Utilization for Developing Countries, September 6-23, 2016, Beijing, China

A training course on Microbial Resources Information Management and Utilization for Developing Countries was held September 6-23, 2016. The course was supported by the Bureau of International cooperation of the Chinese Academy of Sciences (CAS).

The course was hosted by the WFCC-MIRCEN world data center for microorganisms (WDC) and organized by the Word Federation for Culture Collection (WFCC), the United Nations Educational, Scientific and cultural organization (UNESCO), the Institute of Microbiology, Chinese Academy of Science (IMCAS), the CODATA Task Group on Advancing informatics for Microbiology (TG-AIM). Fifteen researchers from developing countries including Argentina, Brazil, Bulgaria, China, Fiji, Greece, India, Iran, Russia, Romania, and Thailand participated in this training course to acquire knowledge on microbial culture collections around the world, data management systems, and data standards. The participants received up-to-date information regarding the main databases, including WFCC and CCINFO, the GCM on-line catalogue, taxonomic rules and regulations, required ISO certificates, and Bioinformatics in general. The relatively long duration of the course provided participants with the opportunity to communicate between scientific groups in different countries and to identify their weaknesses and their strengths for promoting partnerships, joint projects, and networking between microbial resource centers.

S. Nasr <shaghayegh2963@yahoo.com>

Forthcoming Meetings

44rd Annual Conference on Yeasts, Czechoslovak Comission on Yeast May 2-5 2017, Smolenice Castle, Slovakia

The 44th Annual Conference on Yeasts is being planned for 2-5 May 2017 at Smolenice Castle, Slovakia. On-line registration will open at http://yeastconference.sk/ in December.

Emília Breierová <Emilia.Breierova@savba.sk>

9th International Fission Yeast Meeting May 14-19 2017, Banff, Alberta, Canada

The 9th International Fission Yeast Meeting is coming to Canada and will be held May 14-19, 2017, in Banff, Alberta, at the Banff Centre Conference Facility. This is particularly exciting because the first Fission Yeast Workshop was held in conjunction with the Thirteenth International Congress on Yeast Genetics and Molecular Biology at the Banff Centre in 1986. In 2017 we are expecting 350-450 guests representing Pombe labs from around the globe. The organizing committee consists of Paul Young, Queen's, Gordon Chua, Calgary, and Dallan Young, Calgary. For further information, contact:

Paul G. Young, Biology, 2443 Biosciences, Queen's University
Kingston, ON K7L 3N6 <youngpg@queensu.ca>

33rd International Specialised Symposium on Yeast - ISSY33 University College Cork, Cork, Ireland, 26-29 June 2017

The 33rd International Specialised Symposium on Yeast (ISSY33), organised under the auspices of the International Commission on Yeasts, with support from the Microbiology Society, will take place at University College Cork, Cork, Ireland. The title of the meeting is 'Exploring and Engineering Yeasts for Industrial Application'.

Yeasts are unicellular fungi used in many sectors of biotechnology to make products such as beverages, foods, pharmaceuticals and chemicals. This meeting will examine the basic physiology and metabolism of industrial yeast strains. The potential to further exploit the natural biodiversity of yeasts to create select or create new strains for applications will also be considered. New genetic tools and approaches have opened up new possibilities for reprogramming pathways to produce novel products in yeast and there will be a particular focus on yeast cell factories.

Key topics: Exploration of yeast biodiversity for industrially relevant traits - Hybrid genomes of industrial yeasts: analysis and engineering - Engineering novel (to yeast) product pathways - Cell factory product pitches - New tools for yeast genome engineering - New synthetic pathways in yeast - Analysing and engineering regulatory networks in yeast - Metabolomics and proteomics of industrial yeasts - Evolutionary approaches for yeast strain improvement.

Registration will open soon:

http://www.microbiologysociety.org/events/event-listing/index.cfm/33rd-international-specialized-symposium-on-yeast

28th International Conference on Yeast Genetics and Molecular Biology
Prague Congress Centre, Prague, Czech Republic, August 27 to September 1, 2017

Dear yeast researchers and friends: It is our great pleasure to invite you to the 28th International Conference on Yeast Genetics and Molecular Biology (www.yeast2017.cz) to be held at Prague Congress Centre in Prague, Czech Republic, from August 27th to September 1st, 2017. The conference is intended to attract all yeast researchers. The symposia and workshops cover highly significant research fields and are open for all yeast species. To make sure that the conference gives an overview of the latest developments in yeast research we will focus on keynote speakers, invited speakers and selected contributions of participants.

The Prague Congress Centre is one of the modern architectural landmarks of Prague. From the Centre, visitors have a unique view of the city, including the Prague Castle, former residence of kings of Bohemia and present seat of the president. The Centre is ideally accessible by the underground and the north-south highway, especially when the free public transportation will be provided by the Major of the city Prague to all participants. We look forward to welcoming you in Prague.

Jiri Hasek <hasek@biomed.cas.cz> (on Behalf of the Local Organizing Committee)

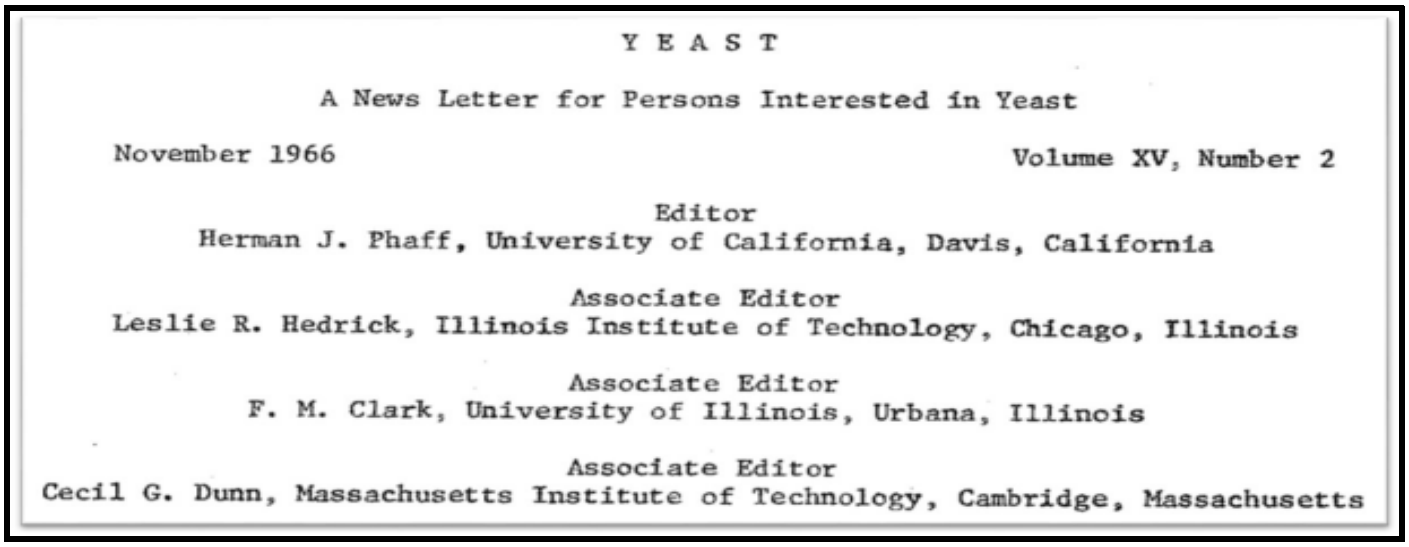
Brief News Item

Postdoctoral/Researcher Position Sought

I have acquired comprehensive laboratory skills throughout intense training in the area of yeast molecular biology, biochemistry, and yeast genetics, leading to my PhD thesis in Molecular Biology. At the moment I am working as a Postdoctoral Fellow in the Department of Biochemistry at the University of Saskatchewan in Canada and looking for a position in line to my profile and interest. My major experience is related to metabolic engineering and heterologous expression in budding yeast. Alongside with the conventional yeast *S. cerevisiae*, I have also worked with methylotrophic yeasts such as *P. pastoris*, the non-conventional *K. lactis*, as well as bacterial expression systems. Besides, I was engaged in a project on lipid metabolic engineering in *S. cerevisiae*. Working with *P. pastoris*, I studied methanol utilization pathway (MUT) genes and have conceived of a project targeting methanol free fed-batch and continuous cultures with *AOX1* promoter-driven expression. The aim is to maximize the yield of target proteins through genetic modification of the host strain and substrate feeding strategy optimization. I would be happy to find collaborators and a host institution in order to pursue this project.

Oleg Tyurin <oleg.tyurin77@gmail.com>

50 Years Ago



Y E A S T

A News Letter for Persons Interested in Yeast

November 1966 Volume XV, Number 2

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Dr. W. Ch. Slooff of CBS, the Netherlands, reported that type strains of eleven new yeast species were deposited in the collection: *Candida benhamii, C. beverwijkii, Rhodotorula gluinis var. rufusa, R. slooffii, Sporobolomyces japonicus, Torulopsis kestoni, T. mogii,* and *T. westerdijkii*.

Dr. Roger D. Goos, ATCC, Rockville, Maryland, USA listed 20 *Hansenula* strains received from Illinois Institute of Technology, USA and 22 strains of *Aureobasidium* and *Prototheca* received from Sanitary Engineering Center, Cincinnati, Ohio, USA.

Dr. Samuel P. Meyers of the University of Miami, USA reported visits with Drs. Tubaki and Hasegawa at the institute for Fermentation in Osaka, with Miss Slooff at CBS and Dr. Kreger van Rij in Groningen, and Dr. Gunkel in Helgoland, Germany. He and Dr. Gunkel collected yeasts from the Elbe River and the North Sea. Dr. Fell continued his Antarctic yeast collections.

Prof. O. Verona of Universita di Pisa, Italy, shared summaries of two studies on cellulosolytic activity of fungi and yeasts from pulpwood, and fungicidal activity of yeasts from pulpwood.

Dr. S. Sugama of the Institute of Brewing, Tokyo, Japan communicated abstracts of recent publications. (1) The degree of contamination of starter cultures by wild yeasts was determined to range from 0.5% to 38%. (2) The yeast florae of ceilings and floors in a sake production facility were determined by isolating and identifying 165 yeast strains. During production, over 95% of yeasts were *Hansenula;* off season, the yeast microflora resembled that of garden soil. (3) Effective selective conditions for isolation of *Saccharomyces cerevisiae* from natural samples include 2.5% ethyl acetate, limited oxygen, pH 4, and sealing the Petri plate with vinyl tape.

Dr. James Lewis, Louisiana State University, listed five recent publications on the ultrastructure of *Geotrichum candidum*.

Prof. Thomas D. Brock of Indiana University described the partial purification and characterization of sex-specific agglutination factors from *Hansenula wingei.*

The abstract of the PhD thesis of **Dr. U. Leupold** of the University of Bern, Switzerland described analysis of allele-specific suppressor mutations in *Schizosaccharomyces pombe.*

Research in yeast genetics at the University of Strathclyde, Royal College, Glasgow, Scotland was described by **Dr. John Johnston**, and included genetic stability of diploid, triploid and tetraploid cultures, and genetics of drug (nystatin) resistance.

Work in progress on invertase biosynthesis and yeast cell membrane were described by **Dr. J. O. Lampen** of Rutgers, The State University, New Jersey, USA.

Dr. Heikki Suomalainen of the State Alcohol Monopoly, Helsini, Finand listed eleven publications from 1965 and 1966 dealing with yeast protoplasts, production of vinegar, nicotinic acid and nicotinamide adenine dinucleotide, vitamins, nucleotides, and biotin.

Prof. L. Leistner of the Institut für Bacteriologie und Histologie, West Germany studied the correlation of yeasts to water activity, redox potential and flavor in fermented sausage.

Dr. J. G. Kleyn of the University of Puget Sound, Tacoma, Washington, USA announced two publications on dwarf cell formation in some *Saccharomyces* and effects of soybeans on yeast growth and beer flavor.

Dr. R. B. Gilliland of A. Guinness Son & Co. Ltd., Dublin, Ireland shared abstracts of two recent publications, one regarding an *Acetobacter* that is lethal to yeasts in bottled beer, and another describing *Saccharomyces diastaticus,* separated based on starch fermentation.

Dr. John R. Wilmot of The Stroh Brewery, Detroit, Michigan, USA reported that Curtis C. Scheifinger recently joined the laboratory of the Stroh Brewery Company to develop a yeast research program on yeast metabolism related to beer production.

Dr. J. Delente of the Falstaff Brewing Corporation, St. Louis, Missouri, USA presented a paper at the Society for Industrial Microbiology meeting in College Park, Maryland in August 1966 on fermentationn of mixtures of maltose and glucose by *Saccharomyces carlsbergensis*.

Dr. N. J. W. Kreger-van Rij reported publications including the description of *Kluyveromyces osmophilus*, the taxonomy of *Pichia*, and yeast ascospores observed under the electron microscope.

Drs. Herman J. Phaff, Martin W. Miller and Emil M. Mrak published a book titled "The Life of Yeasts – Their Nature, Activity, Ecology, and Relation to Mankind". [Ed. note - This book was translated into other languages, and used for decades in microbiology and mycology classes at UC Davis and worldwide.]

Biological Abstracts offered a free three-issue trial of their new "Abstracts in Mycology."

Dr. Anna Kockova-Kratochvilova chaired the highly successful Second International Symposium on Yeasts in Bratislava, July 1966, which had 150 participants from 22 countries. Excursions included a tour on the Danube River and visits to a large brewery and wine producing area in the interior of Czechoslovakia. At this meeting, a resolution was passed to establish a Council of prominent yeast workers, chaired by Dr. Kocková-Kratocvílová, secretary Minárik (CSSR), members Beran (CSSR), Eddy (Great Britain), Elinov (USSR), Klaushofer (Austria), Kudrjavcev (USSR), Leopold (Switzerland), Müller (German Democratic Republic), Nagai (Japan), Necas (CSSR). Phaff (USA), Robinow (Canada), Suomalainen (Finland), Wickerham (USA), Wikén (Holland), Windisch (German Federal Republic). The "Yeast News Letter", edited by Herman Phaff, would serve as the means of communication for actions and decisions of the Council. The Council will coordinate the international cooperation including setting up a Nomenclature Committee tasked with standardizing methods of yeast taxonomy, and coordinating the organization of Yeast Collections. A list of permanent culture collections will be prepared and presented at the next Symposium in 1969. Authors of descriptions of new species were encouraged to deposit type strains in as many culture collections as possible. [Ed. Note: This resolution resulted in establishment of the International Commission on Yeasts.]

A questionnaire was sent to all recipients of the **Yeast News Letter**, requesting contact information of all institutes and scientists engaged in the study of yeasts, and of regional collections of yeasts. The questionnaire also asked if recipients wanted to cooperate on standardization of methods, and for nomination of people to serve on the Nomenclatural Committee.

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