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Editorials

Dr Royall T. Moore (1930-2014)

I regret to inform readers of the death of Dr Royall T. Moore, formerly of the School of Applied Biological and Chemical Sciences University of Ulster, on August 17th 2014. Roy had a keen interest in the use of ultrastructure in the taxonomy of ascomycete and basidiomycete fungi. His paper, Fine Structure of Mycota. 7. Observations on Septa of Ascomycetes and Basidiomycetes, co-autored with JH McAlear (Amer J Bot 49:86-94) was highly cited in the field. Roy's company at yeast symposia was always appreciated.

Dr James A. Barnett (1923-2015)

I regret to announce the recent death of Dr. James Barnett. The yeast researcher community will remember James's willingness to tell things the way they are. He is of course best known for his series of taxonomic keys of the yeasts, last updated in 2000. I am specially fond of his series of papers on the history of yeast research, which are now available as a collection from ASM Science. His wife Linda commented that "Having been a young 'civilian on active service' during the war years and working under a number of distinguished scientists analyse the flood of incoming intelligence data, James early learned to make his own assessments of people and situations. He was never afraid of those in power and would often make a direct approach to the highest authority when he considered them to be in error, or abusing their position. James was very sorry to give up his lab work in his mid-70s, but never thought of 'retiring' and continued working at his various articles to the last. It was simply his way of life." An obituary prepared by two colleagues appears in this issue.

Dr. Forbes Wardrop (1973-2015)

Jean Chagnon of Lallemand Inc., Montreal, informed me of the untimely death of Dr. Forbes Wardrop. Quoting Jean: "Dr. Forbes Wardrop unfortunately passed away this past Easter day a few days shy of his 43rd birthday. Forbes will be remembered for his readiness to give generously to everyone who would ask of his time and insights. He did this with his usual modesty, wit and brilliance. Forbes was passionate about scientific enquiry, yeasts, and the beers, spirits and wines that yeasts help produce. He had joined Lallemand's research team more than 15 years ago, having moved to Montreal from the University of Abertay Dundee after completing there his PhD under the guidance of Professor Graeme Walker. He quickly became one of the key members of our great team. He contributed to the development of many of our now well-established processes and of our successful new yeasts and yeast-derived products that are today used widely by breweries, distilleries and wineries throughout the world as well as bakery products, dietary supplements and, feed additives. His early departure from this world is a tragic loss not only to his immediate family and colleagues but also to his extended family of all persons passionate, like him, about yeasts."

MA Lachance, Editor

Essay - The Nagoya Protocol in our daily work

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The Nagoya Protocol[1] on ACCESS TO GENETIC RESOURCES AND THE FAIR AND EQUITABLE SHARING OF BENEFITS ARISING FROM THEIR UTILIZATION was adopted by the 10th Conference of the Parties (COP10) on 29 October 2010 in Nagoya, Japan, and entered into force on October 12th, 2014. It is the legally binding instrument for implementation at national level of the Convention of Biological Diversity (CBD), which had entered into force on December 29th, 1993. The three objectives of the CBD are (i) the conservation of biological diversity, (ii) the sustainable use of its components, and (iii) the fair and equitable sharing of benefits arising from its utilisation. The CBD recognizes the sovereign rights of countries over their own biological resources and the so-called genetic resources contained therein, and access to such resources in a country that is Party to the CBD requires Prior Informed Consent (PIC) from the competent authority in that country and settling on Mutually Agreed Terms (MAT) between provider and user. Parties are, however, free to determine if access to genetic resources naturally occurring within their own territories will be subject to such requirements or not. But every Party must assure that, within its territory, genetic resources originating from other Parties are utilised in accordance with the CBD and that benefits arising from the utilisation of these genetic resources or traditional knowledge associated with these genetic resources are shared fairly and equitably. The Nagoya Protocol thus provides the long awaited guidance for Parties to implement their national access and benefit sharing (ABS) legislation, and effectively achieve the third objective of the CBD.

Where to find information

The ABS Clearing House (ABSCH)[2] database has been set up recently where each Party should provide information on the national legislation and policy measures, and where each Party can also publish any issued internationally-recognized certificates of compliance (IRCC, i.e., permits or equivalents) and checkpoint communiqués sent or received. Most of the 59 Parties to the Nagoya Protocol (there are now in total 91 signatory countries) still have to put in place national ABS legislation, and are not yet able to deal with requests for PIC. Consequently, most countries have not uploaded such information into the ABSCH at this time. Documents which are accessible in the ABSCH are not always available in English. It may be hoped that the situation will improve soon. At this time, all Parties do provide contact information for National Focal Point staff, and these persons can be expected to answers specific questions. It should be noted that some countries have CBD-based legislation in place since before Nagoya, by which permits are required for collecting *in situ* biological resources, as for example has been the case in Brazil for some time. Also the export of genetic resources from the country of origin may be illegal without proper Material Transfer Agreement (MTA).

Implementation of ABS legislation: the EU Regulation as an example

To govern ABS in the European Union, Regulation (EU) 511/2014[3] was adopted by the European Parliament and the Council on April 16th, 2014. It entered into force on June 9th, 2014, and applies since the Nagoya Protocol entered into force for the Union (October 12th, 2014). Key-articles of this Regulation shall only apply a year later, from Oct 12th 2015. In article 4 of this Regulation obligations for users in the EU are laid down, such as to "exercise due diligence", i.e., to ascertain that genetic resources (and associated traditional knowledge) utilised have been accessed in accordance with applicable ABS legislation. Further obligations include to share benefits arising from the utilisation of the genetic resources in agreement with MAT and in accordance with legal requirements, and to transfer and utilise genetic resources (and associated traditional knowledge) only in accordance with MAT (if required), and to seek, keep and transfer to subsequent users the IRCC, information on the content of the MAT for subsequent users, etc. Furthermore, users are obliged to keep the information relevant to ABS for 20 years after the end of the period of utilisation. Article 7 of the Regulation deals with due diligence declaration requirements for users and how Member States should monitor user compliance, while article 9 describes how the Member States shall carry out checks on user compliance. Recipients of research funding utilising genetic resources and users of genetic resources bringing products to the EU market will have to make due diligence declarations. Detailed procedures will be settled in the Implementing Acts which are expected to enter into force in October 2015.

The importance of biological collections for research and development is recognized by Art. 5 of the Regulation. It offers biological resource centres within the EU the possibility to apply for admittance of their collections (or part thereof) to a Register of collections. Researchers obtaining a genetic resource from a registered collection will be considered to have exercised due diligence as regards the legality of access, and thus would likely benefit from less administrative burden and more legal certainty. If the Register will become a success is difficult to predict. For collections to qualify for the Register they will have to invest in order to meet administrative demands, and it is uncertain if they will see return on such investments. Registered collections could receive more requests for material, but at the same time researchers expect that the Nagoya Protocol is going to cause a reduction in their research activities on genetic resources, as collecting and sharing material may become more difficult and time-consuming and costly. This could also result in a reduction of numbers of strains deposited, or supplied by public collections.

Scope and how research could be affected

The scope of the Nagoya Protocol is still not fully clear. As regards the kind of biological material, it is certain that the Protocol does not affect human genetic resources, material collected in areas beyond national jurisdiction (e.g., Antarctica), or material governed by the International Treaty on Plant Genetic Resources for Food and Agriculture. As regards the temporal scope, at least for the European Union it has been made clear now that only material accessed in a Party to the Nagoya Protocol (i.e., collected *in situ*) after the entry into force of the Nagoya Protocol for the Union (Oct 12, 2014), is considered to be in scope of the Regulation. It is not unlikely that some Parties will adopt a wider temporal scope, which then may also include "new use" of materials collected earlier in the country of origin, and for example deposited in *ex situ* collections. As regards the utilisation of genetic resources, the definition provided in Art. 2 of the CBD, which reads "to conduct research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology", can be variously understood and calls for clarification by national authorities and review by the Conference of the Parties to the CBD. The European Commission has explained that for research activities in order to be in scope of the Regulation 511/2014, there has to be an element of development involved ("research and development are cumulative requirements") [4]. For research involving material that is (potentially) within scope of Nagoya Protocol, it is advisable to check whether any restrictions or requirements are applicable per PIC, MAT or MTA (if such is available) covering the material, or in general per national legislation of the country of origin of the material (at the time of collecting) or per legislation of the country where the work will be conducted (at the time it is to be conducted). The same is advisable before transferring materials to third party users, as this is may be subject to restrictions or not allowed at all!

Researchers who acquire biological material for research should always ascertain that it has been accessed lawfully. Parties to the Nagoya Protocol will take measures to assure that users of genetic resources who file for patents or bring products to the market will be checked for compliance to applicable ABS legislation. Even if from the onset of a project it is clear that use will be limited to non-commercial purposes, such as phylogenetic and taxonomic research or other descriptive work, this could be important. For example, strains of yeasts or other microbes may have to be deposited in a public collection as a requirement for valid publication of novel taxa, as reference material for published research, for conservation or other reasons. In order ascertain compliance, the collection curators will have to assess the status under Nagoya of any material offered for deposit, for which they will need at least the following information: the geographic origin of the strain (at least the country of origin, if applicable), the date of collecting the source samples *in situ*, and the person(s) who collected the source samples (including the legal entity on behalf of which the collecting was done). And, where such is required by applicable ABS legislation, the depositor will have to provide all available documents relevant to ABS at the time of deposit. This is crucial for providing the necessary legal certainty for both the collection preserving the strains and users who will acquire these strains from the collection. The collection will provide these strains to third parties under the same conditions as they were received by the collection.

Collections continue efforts to provide legal certainty and harmonize their practices

Since the entry into force of the CBD, microbial culture collections or "biological resource centres" have worked to reach compliance and harmonise practices. The project MOSAICC[5] developed a first voluntary code of conduct that provided a set of model clauses for PIC and MAT for providers and recipients

of microbial genetic resources, and MTAs for the deposit in public collections (also referred to as material accession agreements) and supply to users. The practice of sharing microbial strains and related information by scientists world-wide for research purposes, known as "microbial commons"[6,7,8] has been key to the development of mycology and microbiology over more than a century. Collections have been involved in several recent studies and meetings on the subject of microbial commons, which aimed at analysing current practices of sharing material and information by collections, researchers and their networks, and how this practice could be placed on a more solid scientific, and legally sound, institutional basis. The complicated issues of ownership was also addressed and a "bundle of rights"[9] attached to materials was proposed, which should be regulated by law and managed through agreements and contracts between stakeholders. The European Culture Collection's Organisation[10,11] (ECCO) developed the "ECCO-Core MTA"[12] in 2009, taking recommendations of MOSAICC into account. The Core MTA answered to the need of collections to have a harmonised MTA that settles terms for use of supplied microbial materials, and also effectively raises awareness with the users of microbial genetic resources about their obligations under the CBD, especially with regard to benefit sharing. Now, several of these initiatives are being worked up to assure compliance to the Nagoya Protocol, and the Microbial Resources Research Infrastructure (MIRRI)[13] is developing a best practice for ABS. By delivering transparency to users and providing countries (countries of origin of accessioned materials) public collections aim to contribute to the enhancement of trust among the various stakeholders in the ABS arena.

References

[1]https://www.cbd.int/abs/

[2]https://absch.cbd.int/

[3]Regulation (EU) No 511/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union; http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32014R0511

[4]European Commission (2014) Questions and answers on access and benefit sharing. EC MEMO-14-411 (http://europa.eu/rapid/press-release_MEMO-14-411_en.htm). Brussels, June 10th , 2014.

[5]Microorganism Sustainable use and Access regulation International Code of Conduct (http://bccm.belspo.be/projects/mosaicc/). A version that was updated in 2011 is provided at the BCCM website.

[6]Dijkshoorn L, de Vos P, Dedeurwaerdere T (2010). Understanding patterns of use and scientific opportunities in the emerging global microbial commons. Research in Microbiology 161: 407-413.

[7]Dedeurwaerdere, T (2010). Global microbial commons: institutional challenges for the global exchange and distribution of microorganisms in the life sciences. Research in Microbiology 161: 414-421.

[8]Dedeurwaerdere, T (2010). Self-governance and international regulation of the global microbial commons: introduction to the special issue on the microbial commons. International Journal of the Commons 4: 390-403. URN:NBN:NL:UI:10-1-100217.

[9]Dedeurwaerdere, T : Understanding ownership in the knowledge economy: the concept of the bundle of rights. BCCM News Edition 18 - Autumn 2005

[10]http://www.eccosite.org/

[11]Fritze D (2010) A common basis for facilitated legitimate exchange of biological materials, proposed by the European Culture Collections' Organisation (ECCO). International Journal of the Commons 4: 507-527. URN:NBN:NL:UI:10-1-100222.

[12]Janssens D, Tindal B, Green P, Garay E, Fritze D, Stalpers J, Smith D, Bimet F, Desmeth P (2009). The ECCO core Material Transfer Agreement for the supply of samples of biological material from the public collection. Article 7 of this standard MTA is cited here: "If the RECIPIENT desires to use the MATERIAL or MODIFICATIONS for COMMERCIAL PURPOSE(S), it is the responsibility of the RECIPIENT, in advance of such use, to negotiate in good faith the terms of any benefit sharing with the appropriate authority in the country of origin of the MATERIAL, as indicated by the COLLECTION's documentation." The full MTA text is downloadable from http://www.eccosite.org/.

[13]Microbial Resource Research Infrastructure (MIRRI) is an EU funded project (grant agreement no. 312251) that aims to build one pan-European infrastructure for microbial collections that will more effectively facilitate access to high-quality microorganisms, their derivatives and associated data and services, for research, development and applications.; www.mirri.org.

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

The following papers from the VKM were recently published.

1 Golubev WI. 2014. Mycocin sensitivity patterns of teleomorphs and *Cryptococcus* species in the Tremellales. Problems Med Mycol 16:19-21 (in Russian).

Anamorphic and teleomorphic representatives of the orderTremellales differ in their sensitivity to mycocins of tremellaceous yeasts, and in this respect the teleomorphs are intermingled with *Cryptococcus* species. As a rule, various isolates of a species are identical in mycocin sensitivity pattern that is a species-related property.

2 Golubev WI. 2015. Action spectra of *Schizosaccaromyces pombe* mycocins. Mycology Today in Russia 4:66 (In Russian).

Schizosaccaromyces pombe mycocins act against *Protomyces macrosporus, Taphrina bergeniae, T. carnea* and *T. tosquinetii* but do not against any representatives of the Saccharomycetes.

3 Golubev WI. 2015. Action spectrum of *Wickerhamomyces ciferrii* mycocin. Biotechnology (in press) (in Russian).

The mycocin secreted by *Wickerhamomyces ciferrii* has fungicidal action, and it is active against about 100 species of ascomycetous yeasts from 35 genera and 10 families of the order Saccharomycetales. Most of sensitive species belong to the family *Wickerhamomycetaceae* and taxa phylogenetically related. As a rule, the strains of the same species have identical response to the mycocin.

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

We are grateful to Dr. Fu-Li Lia, (Shandong Provincial Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China) and Dr. Ching-Fu Lee (National Hsinchu University, Taiwan) for visiting their labs in November-December 2014. Many thanks to the Organizing Committee for the invitation to participate at the International Symposium on Mycological Genomics and Diversity held at 5-6 December 2014 in Tonghai University, Taichung, Taiwan.

The following are papers for 2015 or submitted.

1 Naumov GI, Kondratieva VI, Naumova ES. 2015. Hybrid sterility of the yeast *Schizosaccharomyces pombe*: genetic genus and many species in statu nascendi? Microbiology (Moscow) 84(2):159–169. © Pleiades Publishing, Ltd.

2 Naumov GI, Naumova ES. 2015. Invertase overproduction may provide for inulin fermentation by selection strains of *Saccharomyces cerevisiae.* Microbiology (Moscow) 84(2):130–134. © Pleiades Publishing, Ltd.

3 Naumov GI, Shalamitskiy MYu, Martynenko NN, Naumova ES. 2015. Superfamily of pectinase genes *PGU* in the yeast genus *Saccharomyces*. Microbiology (Moscow). (submitted).

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 species-specific *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.

4 Naumov GI, Naumova ES. 2014. Complex composition and chromosomal polymorphism of the lactose loci in *Kluyveromyces* yeasts. International Symposium on Mycological Genomics and Diversity, 5–6 December 2014, Program Book, Taichung. Taiwan, pp. 3–5.

5 Mescheryakova EV, Kondratieva VI, Naumova ES, Naumov GI. 2015. *Komagataella kurtzmanii* Naumov et al. – a new biological species. Current Mycology in Russia. 4(1) New studies in fungal genetics, morphology and physiology pp. 75–77.

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

Letter to the Editor
Isomaltases of the yeast *Saccharomyces cerevisiae*

A recent publication by Xu Deng, et.al. (2014)* describes in detail the properties of four isomaltases encoded by the IMA genes in the yeast *Saccharomyces cerevisiae*. This work is a comprehensive study of isomaltases with respect to their biochemical and enzymological properties. All four isomaltases are functionally similar proteins with respect to the substrate specificities, they hydrolyze α-(1,6) disaccharides such as isomaltose but not the α-(1,4) bond of maltose. These results are consistent with previous publications (Khan and Eaton 1967, Yamamoto et. al 2004). Isomaltases, however, are structurally not identical proteins. For example, Ima2P and Ima3P only differ by three amino acids (Deng et. al. 2014), but the two proteins have different thermal stability. Certain specific amino acids such as proline may play an important role in determining the thermal stability of proteins. In this respect a paper was published by Khan and Haynes (1972), where two isomaltases were purified and certain of their physical properties were compared. Although the two proteins (isomaltases) were similar with respect to their molecular weight, substrate and serological specificities, they differed in the following ways: specific activity, Michaelis constant, and heat stability. On the basis of these results we suggested that the genes responsible for encoding isomaltases (α-methylglucosidase), as well the family of MAL genes may have arisen from a common ancestor by gene duplication.

References:

1 Deng X, Petitjean M, Teste MA, Kooli W, Tranier S, Francois JM, Parrou JL. 2014. Similarities and diffrences in the biochemical and enzymological properties of the four isomaltases from *Saccharomyces cerevisiae*. FEBS Open Bio 4:200-212.

2 Yamamoto K, Nakayama A, Yamamoto Y, Tabata S. 2004. Val216 decides the substrate specificity of α-glucosidase in *Saccharomyces cerevisiae*. Eur J Biochem 271:3414-3420.

3 Khan NA, Eaton NR. 1972. Purification and characterization of maltase and α-methylglucosidase from yeast. Biochim Biophys Acta 146:173-178.

4 Khan NA, Haynes RH. 1972. Genetic redundancy in yeast: non-identical products in a polymeric gene system. Molec Gen Genet 118:279-285.

* We thank Xu Deng et. al. for acknowledging and recommending our 1967 paper.

IV 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 Sylvester K, Wang QM, James B, Mendez R, Hulfachor AB, Hittinger CT. 2015. Temperature and host preferences drive the diversification of *Saccharomyces* and other yeasts: a survey and the discovery of eight new yeast species. FEMS Yeast Res 15:fov002.

Compared to its status as an experimental model system and importance to industry, the ecology and genetic diversity of the genus *Saccharomyces* has received less attention. To investigate systematically the biogeography, community members and habitat of these important yeasts, we isolated and identified nearly 600 yeast strains using sugar-rich enrichment protocols. Isolates were highly diverse and contained representatives of more than 80 species from over 30 genera, including eight novel species that we describe here: *Kwoniella betulae* f.a. (yHKS285^T = NRRL Y-63732^T = CBS 13896^T), *Kwoniella newhampshirensis* f.a. (yHKS256^T = NRRL Y-63731^T = CBS 13917^T), *Cryptococcus wisconsinensis* (yHKS301^T = NRRL Y-63733^T = CBS 13895^T), *Cryptococcus tahquamenonensis* (yHAB242^T = NRRL Y-63730^T = CBS 13897^T), *Kodamaea meredithiae* f.a. (yHAB239^T = NRRL Y-63729^T = CBS 13899^T), *Blastobotrys buckinghamii* (yHAB196^T = NRRL Y-63727^T = CBS 13900^T), *Candida sungouii* (yHBJ21^T = NRRL Y-63726^T = CBS 13907^T) and *Cyberlindnera culbertsonii* f.a. (yHAB218^T = NRRL Y-63728^T = CBS 13898^T), spp. nov. *Saccharomyces paradoxus* was one of the most frequently isolated species and was represented by three genetically distinct lineages in Wisconsin alone. We found a statistically significant association between *Quercus* (oak) samples and the isolation of *S. paradoxus*, as well as several novel associations. Variation in temperature preference was widespread across taxonomic ranks and evolutionary timescales. This survey highlights the genetic and taxonomic diversity of yeasts and suggests that host and temperature preferences are major ecological factors.

2 Coelho MA, Almeida JM, Hittinger CT, Gonçalves P. 2015. Draft genome sequence of *Sporidiobolus salmonicolor* CBS 6832, a red-pigmented basidiomycetous yeast. Genome Announc 3: e00444-15.

We report the genome sequencing and annotation of the basidiomycetous red-pigmented yeast *Sporidiobolus salmonicolor* strain CBS 6832. The current assembly contains 395 scaffolds, for a total size of about 20.5 Mb and a G+C content of ~61.3%. The genome annotation predicts 5,147 putative protein-coding genes.

V School of Environmental Science, Bovey Building, Room 3218, University of Guelph, Guelph, Ontario Canada N1G 2W1. Communicated by Hung Lee <hlee@uoguelph.ca>.

The following are the abstracts of papers published recently or are in press.

1 Pinel D, Colatriano D, Jiang H, Lee H & Martin VJJ. 2015. Deconstructing the genetic basis of spent sulfite liquor tolerance using deep sequencing of genome shuffled yeast. Biotechnol Biofuels. 8:53 - http://dx.doi.org/10.1186/s13068-015-0241-z

Background: Identifying the genetic basis of complex microbial phenotypes is currently a major barrier to our understanding of multigenic traits and our ability to rationally design biocatalysts with highly specific attributes for the biotechnology industry. Here, we demonstrate that strain evolution by meiotic recombination-based genome shuffling coupled with deep sequencing can be used to deconstruct complex phenotypes and explore the nature of multigenic traits, while providing concrete targets for strain development. Results: We determined genomic variations found within *Saccharomyces cerevisiae* previously evolved in our laboratory by genome shuffling for tolerance to spent sulphite liquor. The

representation of these variations was backtracked through parental mutant pools and cross-referenced with RNA-seq gene expression analysis to elucidate the importance of single mutations and key biological processes that play a role in our trait of interest. Our findings pinpoint novel genes and biological determinants of lignocellulosic hydrolysate inhibitor tolerance in yeast. These include the following: protein homeostasis constituents, including Ubp7p and Art5p, related to ubiquitin-mediated proteolysis; stress response transcriptional repressor, Nrg1p; and NADPH-dependent glutamate dehydrogenase, Gdh1p. Reverse engineering a prominent mutation in ubiquitin-specific protease gene UBP7 in a laboratory S. cerevisiae strain effectively increased spent sulphite liquor tolerance. Conclusions: This study advances understanding of yeast tolerance mechanisms to inhibitory substrates and biocatalyst design for a biomass-to-biofuel/biochemical industry, while providing insights into the process of mutation accumulation that occurs during genome shuffling.

2 Dashtban M, Wen X, Bajwa PK, Ho CY & Lee H. 2015. Deletion of *hxk1* gene results in derepression of xylose utilization in *Scheffersomyces stipitis*. J Indust Microbiol Biotechnol - http://dx.doi.org/10.1007/s10295-015-1614-9

A major problem in fermenting xylose in lignocellulosic substrates is the presence of glucose and mannose which inhibit xylose utilization. Previous studies showed that catabolite repression in some yeasts is associated with hexokinases and that deletion of one of these gene(s) could result in derepressed mutant strain(s). In this study, the *hxk1* encoding hexokinase 1 in *Scheffersomyces stipitis* was disrupted. The *Δhxk1* SS6 strain retained the ability to utilize the main hexoses and pentoses commonly found in lignocellulosic hydrolysates as efficiently as the wild-type (WT) strain. SS6 also fermented the dominant sugars to ethanol; however, on xylose, the *Δhxk1* strain produced more xylitol and less ethanol than the WT. On mixed sugars, as expected the WT utilized glucose ahead of xylose and xylose utilization did not commence until all the glucose was consumed. In contrast, the *Δhxk1* mutant showed derepression in that it started to utilize xylose even when considerable glucose (about 1.72 %, w/v) remained in the medium. Similarly, mannose did not repress xylose utilization by the Δhxk1 mutant and xylose and mannose were simultaneously utilized. The results are of interest in efforts to engineer yeast strains capable of efficiently utilizing glucose and xylose simultaneously for lignocellulosic biomass conversion.

VI Centre International de Ressources Microbiennes (CIRM-Levures), INRA/AgroParisTech, 78850 Thiverval-Grignon, France - http://www6.inra.fr/cirm/Levures. Communicated by Serge Casaregola <serge@grignon.inra.fr>.

The following papers were recently published or accepted for publication.

1 Jacques N, Zenouche A, Gunde-Cimerman N, Casaregola S. 2015. Increased diversity in the genus Debaryomyces from Arctic glacier samples. Antonie Van Leeuwenhoek 107:487-501.

Ice from Arctic glaciers contains large populations of yeasts. We studied 38 isolates from this environment, which were initially identified as *Debaryomyces* sp. related to *Debaryomyces hansenii* by sequence analysis of the D1/D2 domains of 26S rDNA. An analysis of the distribution of mitochondrial DNA insertions in the nuclear genome showed that 25 of these isolates were related to, but distinct from, *D. hansenii*. Sequence analysis of the *ACT1* gene of these 25 isolates revealed that they formed three different types of putative hybrids. In particular, 23 putative hybrids carried an *ACT1* sequence identical to that of three *Debaryomyces* strains, CBS 790, CLIB 660, CLIB 949, previously classified as associated with *D. hansenii* and an *ACT1* sequence of an undescribed taxon. The latter sequence displayed between 22 and 27 bp divergence (2.6-3.2 %) over 841 bp from sequences of closely related *Debaryomyces* sp., suggesting that this new taxon very likely represents a novel species for which no pure strain is available. Sequence comparisons of CBS 790, CLIB 660, and CLIB 949 with related *Debaryomyces* type strains also revealed an important sequence divergence. The putative hybrids described in this study could be differentiated from non-hybrid isolates and other *Debaryomyces* species on the basis of their use of a number of carbon sources.

2 Froissard M, Canonge M, Pouteaux M, Cintrat B, Mohand-Oumoussa S, Guillouet SE, Chardot T, Jacques N, Casaregola S. 2015. Lipids containing medium-chain fatty acids are specific to post-whole genome duplication Saccharomycotina yeasts. BMC Evol Biol. (in press).

Yeasts belonging to the subphylum Saccharomycotina have been used for centuries in food processing and, more recently, biotechnology. Over the past few decades, these yeasts have also been studied in the interest of their potential to produce oil to replace fossil resources. Developing yeasts for massive oil production requires increasing yield and modifying the profiles of the fatty acids contained in the oil to satisfy specific technical requirements. For example, derivatives of medium-chain fatty acids (MCFAs, containing 6-14 carbons) are used for the production of biodiesels, cleaning products, lubricants and cosmetics. Few studies are available in the literature on the production of MCFAs in yeasts. We analyzed the MCFA content in *Saccharomyces cerevisiae* grown in various conditions. The results revealed that MCFAs preferentially accumulated when cells were grown on synthetic media with a high C/N ratio at low temperature (23 °C). Upon screening deletion mutant strains for genes encoding lipid droplet-associated proteins, we found two genes, LOA1 and TGL3, involved in MCFA homeostasis. A phylogenetic analysis on 16 Saccharomycotina species showed that fatty acid profiles differed drastically among yeasts. Interestingly, MCFAs are only present in post-whole genome duplication yeast species. In this study, we produced original data on fatty acid diversity in yeasts. We demonstrated that yeasts are amenable to genetic and metabolic engineering to increase their MCFA production. Furthermore, we revealed that yeast lipid biodiversity has not been fully explored, but that yeasts likely harbor as-yet-undiscovered strains or enzymes that can contribute to the production of high-value fatty acids for green chemistry.

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

Recent publications.

1 Richard P, Viljanen K, Penttilä M. 2015. Overexpression of *PAD1* and *FDC1* results in significant cinnamic acid decarboxylase activity in *Saccharomyces cerevisiae*. AMB Express 5:12 - doi:10.1186/s13568-015-0103-x.

The *S. cerevisiae PAD1* gene had been suggested to code for a cinnamic acid decarboxylase, converting *trans*-cinnamic acid to styrene. This was suggested for the reason that the over-expression of *PAD1* resulted in increased tolerance toward cinnamic acid, up to 0.6 mM. We show that by over-expression of the *PAD1* together with the *FDC1* the cinnamic acid decarboxylase activity can be increased significantly. The strain over-expressing *PAD1* and *FDC1* tolerated cinnamic acid concentrations up to 10 mM. The cooperation of Pad1p and Fdc1p is surprising since the *PAD1* has a mitochondrial targeting sequence and the *FDC1* codes for a cytosolic protein. The cinnamic acid decarboxylase activity was also seen in the cell free extract. The activity was 0.019 μmol per minute and mg of extracted protein. The overexpression of *PAD1* and *FDC1* resulted also in increased activity with the hydroxycinnamic acids ferulic acid, p-coumaric acid and caffeinic acid. This activity was not seen when *FDC1* was overexpressed alone. An efficient cinnamic acid decarboxylase is valuable for the genetic engineering of yeast strains producing styrene. Styrene can be produced from endogenously produced L-phenylalanine which is converted by a phenylalanine ammonia lyase to cinnamic acid and then by a decarboxylase to styrene.

2 Djordjević R, Gibson B, Sandell M, de Billerbeck GM, Bugarski B, Leskošek-Čukalović I, Vunduk J, Nikićević N, Nedović V. 2015. Raspberry wine fermentation with suspended and immobilized yeast cells of two strains of *Saccharomyces cerevisiae*. Yeast 32:271–279 - DOI: 10.1002/yea.3060

The objectives of this study were to assess the differences in fermentative behaviour of two different strains of *Saccharomyces cerevisiae* (EC1118 and RC212) and to determine the differences in composition and sensory properties of raspberry wines fermented with immobilized and suspended yeast cells of both strains at 15 °C. Analyses of aroma compounds, glycerol, acetic acid and ethanol, as well

as the kinetics of fermentation and a sensory evaluation of the wines, were performed. All fermentations with immobilized yeast cells had a shorter lag phase and faster utilization of sugars and ethanol production than those fermented with suspended cells. Slower fermentation kinetics were observed in all the samples that were fermented with strain RC212 (suspended and immobilized) than in samples fermented with strain EC1118. Significantly higher amounts of acetic acid were detected in all samples fermented with strain RC212 than in those fermented with strain EC1118 (0.282 and 0.602 g/l, respectively). Slightly higher amounts of glycerol were observed in samples fermented with strain EC1118 than in those fermented with strain RC212.

3 Krogerus K, Magalhães F, Vidgren V, Gibson B. 2015. New lager yeast strains generated by interspecific hybridization. J Indust Microbiol Biotechnol 42:769-778.

The interspecific hybrid *Saccharomyces pastorianus* is the most commonly used yeast in brewery fermentations worldwide. Here, we generated de novo lager yeast hybrids by mating a domesticated and strongly flocculent *Saccharomyces cerevisiae* ale strain with the *Saccharomyces eubayanus* type strain. The hybrids were characterized with respect to the parent strains in a wort fermentation performed at temperatures typical for lager brewing (12 °C). The resulting beers were analysed for sugar and aroma compounds, while the yeasts were tested for their flocculation ability and α-glucoside transport capability. These hybrids inherited beneficial properties from both parent strains (cryotolerance, maltotriose utilization and strong flocculation) and showed apparent hybrid vigour, fermenting faster and producing beer with higher alcohol content (5.6 vs 4.5 % ABV) than the parents. Results suggest that interspecific hybridization is suitable for production of novel non-GM lager yeast strains with unique properties and will help in elucidating the evolutionary history of industrial lager yeast.

An improved model for prediction of fermentation and total diacetyl profile during brewery fermentation.

4 Krogerus K, Gibson BR, Hytönen E. 2015. An improved model for prediction of wort fermentation progress and total diacetyl profile. J Am Soc Brew Chem 73:90-99. DOI: 10.1094/ASBCJ-2015-0106-01

Diacetyl is normally considered to be an off-flavor in lager beer, and its removal prolongs the overall brewing process. Here, the effects of fermentation temperature (9, 12, and 15°C), initial wort pH (4.8, 5.1, and 5.3), and wort free amino nitrogen (FAN) content (222, 252, 287, and 366 ppm) on diacetyl formation and removal in lager beer fermentations were studied in order to develop an enhanced model for predicting fermentation progress (alcohol content, biomass, and pH) and diacetyl concentration. The relationships between model coefficients, temperature, pH, and FAN were calculated, and models predicted fermentation and diacetyl profiles with a good fit (overall relative mean square error less than 10.1%). The model was validated, and also applied to a larger-scale fermentation involving a different wort and yeast strain by adjusting model coefficients. The model can be used for predicting diacetyl concentrations and for brewing process parameter optimization in industrial fermentations.

VIII University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. Communicated by Bernard Prior <bap@sun.ac.za>.

Recent publications.

1 Van Zyl JH, Den Haan R, Van Zyl WH. 2014. Over-expression of native *Saccharomyces cerevisiae* exocytic SNARE genes increased heterologous cellulase secretion. Appl Microbiol Biotechnol 98:5567-78.

SNAREs (soluble NSF [N-ethylmaleimide-sensitive factor] attachment receptor proteins) are required at the majority of fusion events during intracellular membrane transport and play crucial roles in facilitating protein trafficking between the various membrane-enclosed organelles and the plasma membrane. We demonstrate increases in the secretion of the *Talaromyces emersonii* Cel7A (a cellobio-

hydrolase) and the *Saccharomycopsis fibuligera* Cel3A (a β-glucosidase), through the separate and simultaneous over-expression of different components of the exocytic SNARE complex in *Saccharomyces cerevisiae*. Over-expression of SNC1 yielded the biggest improvement in Te-Cel7A secretion (71 %), whilst SSO1 over-expression lead to the highest increases in Sf-Cel3A secretion (43.8 %). Simultaneous over-expression of differential combinations of these SNARE components yielded maximal increases of ~52 % and ~49 % for the secretion of Te-Cel7A and Sf-Cel3A, respectively. These increases generally did not cause deleterious growth effects, whilst differential improvement patterns were observed for the two reporter proteins (Sf-Cel3A and Te-Cel7A). Simultaneous over-expression of up to three of these components, in strains secreting the more efficiently expressed Sf-Cel3A, illustrated a slight decrease in osmotic tolerance at elevated NaCl concentrations, as well as a detectable decrease in ethanol tolerance at increased concentrations. This work illustrates the potential of engineering components of the anterograde secretory pathway, particularly its SNARE components, for the improvement of heterologous cellulase secretion.

2 Smith J, van Rensburg E, Görgens JF. 2014. Simultaneously improving xylose fermentation and tolerance to lignocellulosic inhibitors through evolutionary engineering of recombinant *Saccharomyces cerevisiae* harbouring xylose isomerase. BMC Biotechnol 14:41.

Yeasts tolerant to toxic inhibitors from steam-pretreated lignocellulose with xylose co-fermentation capability represent an appealing approach for 2nd generation ethanol production. Whereas rational engineering, mutagenesis and evolutionary engineering are established techniques for either improved xylose utilisation or enhancing yeast tolerance, this report focuses on the simultaneous enhancement of these attributes through mutagenesis and evolutionary engineering of *Saccharomyces cerevisiae* harbouring xylose isomerase in anoxic chemostat culture using non-detoxified pretreatment liquor from triticale straw. Following ethyl methanesulfonate (EMS) mutagenesis, *Saccharomyces cerevisiae* strain D5A (ATCC 200062 strain platform), harbouring the xylose isomerase (XI) gene for pentose co-fermentation was grown in anoxic chemostat culture for 100 generations at a dilution rate of 0.10 h^{-1} in a medium consisting of 60% (v/v) non-detoxified hydrolysate liquor from steam-pretreated triticale straw, supplemented with 20 g/L xylose as carbon source. In semi-aerobic batch cultures in the same medium, the isolated strain D5A(+H) exhibited a slightly lower maximum specific growth rate (μ_{max} = 0.12 ± 0.01 h^{-1}) than strain TMB3400, with no ethanol production observed by the latter strain. Strain D5A(+H) also exhibited a shorter lag phase (4 h vs. 30 h) and complete removal of HMF, furfural and acetic acid from the fermentation broth within 24 h, reaching an ethanol concentration of 1.54 g/L at a yield (Y(p/s)) of 0.06 g/g xylose and a specific productivity of 2.08 g/gh. Evolutionary engineering profoundly affected the yeast metabolism, given that parental strain D5A+ exhibited an oxidative metabolism on xylose prior to strain development. Physiological adaptations confirm improvements in the resistance to and conversion of inhibitors from pretreatment liquor with simultaneous enhancement of xylose to ethanol fermentation. These data support the sequential application of random mutagenesis followed by continuous culture under simultaneous selective pressure from inhibitors and xylose as primary carbon source.

3 Mehlomakulu NN, Setati ME, Divol B. 2014. Characterization of novel killer toxins secreted by wine-related non-*Saccharomyces* yeasts and their action on *Brettanomyces* spp. Int J Food Microbiol. 188:83-91.

Wine spoilage associated with *Brettanomyces bruxellensis* is a major concern for winemakers. An effective and reliable method to control the proliferation of this yeast is therefore of utmost importance. To achieve this purpose, sulphur dioxide (SO_2) is commonly employed but the efficiency of this chemical compound is subject to wine composition and it can elicit allergic reactions in some consumers. Biological alternatives are therefore actively sought. The current study focused on identifying and characterizing killer toxins which are antimicrobial compounds that show potential in inhibiting *B. bruxellensis* in wine. Two killer toxins, CpKT1 and CpKT2, from the wine isolated yeast *Candida pyralidae* were identified and partially characterized. The two proteins had a molecular mass above 50kDa and exhibited killer activity against several *B. bruxellensis* strains especially in grape juice. They

were active and stable at pH3.5-4.5, and temperatures between 15 and 25°C which are compatible with winemaking conditions. Furthermore, the activity of these killer toxins was not affected by the ethanol and sugar concentrations typically found in grape juice and wine. In addition, these killer toxins inhibited neither the *Saccharomyces cerevisiae* nor the lactic acid bacteria strains tested. These preliminary results indicated that the application of these toxins will have no effect on the main microbial agents that drive alcoholic and malolactic fermentations and further highlight the potential of using these toxins as agents to control the development of *B. bruxellensis* in grape juice or wine.

4 Fairbairn SC, Smit AY, Jacobson D, Prior BA, Bauer FF. 2014. Environmental stress and aroma production during wine fermentation. S Afric J Enol Vitic 35:168-177.

The sensory description of wine uses the widest range of descriptive terminology of all food products, reflecting the complex nature of a product whose character depends on the balance of hundreds of individual flavour-active compounds. There are many tools that can influence flavour profiles or wine styles, one of which is the choice of a specific yeast strain. Yeasts contribute to wine flavour by producing volatile metabolites with different flavour profiles. The impact of changing environmental conditions on the production of flavour compounds by yeast strains remains largely unexplored. This is the first study investigating the impact of two mild fermentation stresses, hyperosmotic and temperature stress, on aroma production in synthetic must by commercial *Saccharomyces cerevisiae* wine strains. Hyperosmotic stress was imposed by cultivation of the yeast for 21 days in the must containing either 0.3 or 0.5 M sorbitol. The transient temperature stresses were applied for 16 h at 8º or 37°C for either two or eight days after commencement of the fermentation. Greater glycerol and acetic acid levels were produced by most yeasts when only hyperosmotic stress was applied. Hyperosmotic and temperature stress conditions produced a limited number of significant changes to the profile of the esters, higher alcohols and volatile fatty acids. These changes differed significantly for each strain and stress treatment, suggesting that the fermentation conditions can significantly alter the aromatic profile of a wine, although these stress impacts cannot be predicted in general. The changes to the aromatic profile are specific to each individual wine yeast strain.

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Recent publications.

1 Selbman L, Zucconi L, Onofri S, Cecchini C, Isola D, Turchetti B, Buzzini P. 2014. Taxonomic and phenotypic characterization of yeasts isolated from worldwide cold rock-associated habitat. Fungal Biol 118:61-71.

Yeast strains isolated from rock samples collected from worldwide cold regions were identified by sequence analysis of the D1/D2 domains of the 26S rDNA gene and the ITS region followed by molecular phylogeny. Over 77% of yeasts isolates were Basidiomycota. *Cryptococcus* (orders Filobasidiales and Tremellales) and *Rhodotorula* (order Cystobasidiales) were the most frequent genera. About 40% of yeast isolates belonged to undescribed species. Almost all isolates were psychrotolerant. Urease and esterase were the most widely extracellular enzymatic activity at 4°C and 20°C. None of the strains exhibited extracellular protease, DNAse, cellulase, chitinase, and laccase activity. The taxonomic and ecological significance of yeasts associated to worldwide cold rocky habitats is discussed.

2 Selbmann L, Turchetti B, Yurkov A, Cecchini C, Zucconi L, Isola D, Buzzini P, Onofri S. 2014. Description of *Taphrina antarctica* f.a. sp. nov., a new anamorphic ascomycetous yeast species associated with Antarctic endolithic microbial communities and transfer of four *Lalaria* species in the genus *Taphrina*. Extremophiles 18: 707-721.

In the framework of a large-scale rock sampling in Continental Antarctica, a number of yeasts have been isolated. Two strains that are unable to grow above 20°C and that have low ITS sequence similarities with available data in the public domain were found. The D1/D2 LSU molecular phylogeny placed them in an isolated position in the genus *Taphrina*, supporting their affiliation to a not yet described species. Because the new species is able to grow in its anamorphic state only, the species *Taphrina antarctica* f.a. (forma asexualis) sp. nov. has been proposed to accommodate both strains (type strain DBVPG 5268^T, DSM 27485T and CBS 13532^T). *Lalaria* and *Taphrina* species are dimorphic ascomycetes, where the anamorphic yeast represents the saprotrophic state and the teleomorph is the parasitic counterpart on plants. This is the first record for this genus in Antarctica; since plants are absent on the continent, we hypothesize that the fungus may have focused on the saprotrophic part of its life cycle to overcome the absence of its natural host and adapt environmental constrains. Following the new International Code of Nomenclature for Algae, Fungi and Plants (Melbourne Code 2011) the reorganization of *Taphrina–Lalaria* species in the teleomorphic genus *Taphrina* is proposed. We emended the diagnosis of the genus *Taphrina* to accommodate asexual saprobic states of these fungi. *Taphrina antarctica* was registered in MycoBank under MB 808028.

3 Turchetti B, Selbmann L, Blanchette RA, Di Mauro S, Marchegiani E, Zucconi L, Arenz BE, Buzzini P. 2015. *Cryptococcus vaughanmartiniae* sp. nov. and *Cryptococcus onofrii* sp. nov.: two new species isolated from worldwide cold environments. Extremophiles 19:149-159.

Twenty yeast strains, representing a selection from a wider group of more than 60 isolates were isolated from cold environments worldwide (Antarctica, Iceland, Russia, USA, Italian and French Alps, Apennines). The strains were grouped based on their common morphological and physiological characteristics. A phylogeny based on D1/D2 ribosomal DNA sequences placed them in an intermediate position between *Cryptococcus saitoi* and *Cryptococcus friedmannii*; the ITS1 and ITS2 rDNA phylogeny demonstrated that these strains belong to two related but hitherto unknown species within the order Filobasidiales, albidus clade. These two novel species are described with the names *Cryptococcus vaughanmartiniae* (type strain DBVPG 4736^T) and *Cryptococcus onofrii* (type strain DBVPG 5303^T).

4 Pezzolla D, Marconi G, Turchetti B, Zadra C, Agnelli A, Veronesi F, Onofri A, Benucci GMN, Buzzini P, Albertini E, Gigliotti G. 2015. Influence of exogenous organic matter on prokaryotic and eukaryotic microbiota in an agricultural soil. A multidisciplinary approach. Soil Biol Biochem 82:9-20.

The effects on bacterial, yeast and fungal communities present within an agricultural soil treated with a pig slurry-derived digestate were studied using a multidisciplinary (biochemical and 454 pyro-sequencing platform) approach. Biochemical analyses showed a significant increase of CO2 emissions from soil 5 days after the amendment with digestate, whereas soil microbial biomass (C-biomass) increased significantly only after 12 and 30 days. PLFAs analysis revealed a significant increase in Gram-negative bacteria 90 days after the amendment. Results from 454 pyrosequencing revealed the presence of OTUs attributed to bacteria, yeasts and filamentous fungi. Proteobacteria, Bacteroidetes and Firmicutes exhibited a significant predominance in the first 5 days, whereas Ascomycota became predominant 90 days after the amendment. Overall, both bacterial and yeast + fungal richness exhibited a decreasing trend from 0 to 90 days after the amendment. Canonical analysis of principal coordinates showed that the cumulative effect of amendment and incubation time explained approximately 45% and 36% of the total variance observed in the bacterial and yeast + fungal communities, respectively. The correlation among some bacterial and fungal OTUs suggested the probable existence of specific biological interactions among different phyla. The results reported represent a picture of the changes of soil microbial diversity in relation with some agronomic practices, such as organic amendments.

5 Labbani FZK, Turchetti B, Bennamoun L, Dakhmouche S, Roberti R, Corazzi L, Meraihi Z, Buzzini P. 2015. A novel killer protein from *Pichia kluyveri* isolated from an Algerian soil: purification and characterization of its in vitro activity against food and beverage spoilage yeasts. Antonie van Leeuwenhoek 107:961-970.

A novel killer protein (Pkkp) secreted by a *Pichia kluyveri* strain isolated from an Algerian soil was active against food and beverage spoilage yeasts of the genera *Dekkera*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Torulaspora*, *Wickerhamomyces* and *Zygosaccharomyces*. After purification by gel filtration chromatography Pkkp revealed an apparent molecular mass of 54 kDa with SDS-PAGE. Minimum inhibitory concentrations (MICs) of purified Pkkp exhibited a high in vitro activity against *Dekkera bruxellensis* (MICs from 64,000- to 256,000-fold lower than that exhibited by potassium metabisulphite) and *Saccharomyces cerevisiae* (MICs from 32,000- to 64,000-fold lower than potassium sorbate). No in vitro synergistic interactions (calculated by FIC index - Σ FIC) were observed when Pkkp was used in combination with potassium metabisulphite, potassium sorbate, or ethanol. Pkkp exhibited a dose–response effect against *D. bruxellensis* and *S. cerevisiae* in a low-alcoholic drink and fruit juice, respectively. The results of the present study suggest that Pkkp could be proposed as a novel food-grade compound useful for the control of food and beverage spoilage yeasts.

6 Garofalo C, Osimani A, Milanović V, Aquilanti L, De Filippis F, Stellato G, Di Mauro S, Turchetti B, Buzzini P, Ercolini D, Clementi F. 2015. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. Food Microbiol 49:123-133.

Kefir grains are a unique symbiotic association of different microorganisms, mainly lactic acid bacteria, yeasts and occasionally acetic acid bacteria, cohabiting in a natural polysaccharide and a protein matrix. The microbial composition of kefir grains can be considered as extremely variable since it is strongly influenced by the geographical origin of the grains and by the sub-culturing method used. The aim of this study was to elucidate the bacteria and yeast species occurring in milk kefir grains collected in some Italian regions by combining the results of scanning electron microscopy analysis, viable counts on selective culture media, PCR-DGGE and pyrosequencing. The main bacterial species found was *Lactobacillus kefiranofaciens* while *Dekkera anomala* was the predominant yeast. The presence of sub-dominant species ascribed to *Streptococcus thermophilus*, *Lactococcus lactis* and *Acetobacter* genera was also highlighted. In addition, *Lc. lactis*, *Enterococcus* sp., *Bacillus* sp., *Acetobacter fabarum*, *Acetobacter lovaniensis* and *Acetobacter orientalis* were identified as part of the cultivable community. This work further confirms both the importance of combining culture-independent and culture-dependent approaches to study microbial diversity in food and how the combination of multiple 16S rRNA gene targets strengthens taxonomic identification using sequence-based identification approaches.

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These are recent publications of our laboratory.

1 Gancedo JM, Flores CL, Gancedo C. 2015. The repressor Rgt1 and the cAMP-dependent protein kinases control the expression of the *SUC2* gene in *Saccharomyces cerevisiae*. Biochim Biophys Acta 1850:1362–1367.

In *Saccharomyces cerevisiae* galactose, glycerol or ethanol hindered invertase induction by low glucose, but lactate did not. During growth in lactate, deletion of genes *RGT1* or *MTH1* caused a marked increase in invertase levels, and elimination of the Rgt1-binding site in the *SUC2* promoter caused also invertase induction. PKA activity decreased invertase levels in cells growing in lactate, and increased them during growth in lactate + 0.1% glucose. Conclusions: The low level of expression of SUC2 in the absence of glucose is mainly due to repression by the Rgt1-Mth1 complex. Repression is dependent on PKA activity, but not on any specific Tpk isoenzyme. The results show that previously overlooked regulatory elements, such as Rgt1 and Tpks, participate in the control of *SUC2* expression in *S. cerevisiae*.

2 Flores CL, Gancedo C. 2015. The gene *YALI0E20207g* from *Yarrowia lipolytica* encodes an N-Acetylglucosamine kinase implicated in the regulated expression of the genes from the N-Acetylglucosamine assimilatory pathway. PLOS ONE doiI:10.1371/journal.pone.0122135

The non-conventional yeast *Yarrowia lipolytica* possesses an ORF, *YALI0E20207g*, which encodes a protein with an amino acid sequence similar to hexokinases from different organisms. We have cloned that gene and determined several enzymatic properties of its encoded protein showing that it is an N-acetylglucosamine (NAGA) kinase. This conclusion was supported by the lack of growth in NAGA of a strain carrying a *YALI0E20207g* deletion. We named this gene *YlNAG5*. Expression of *YlNAG5* as well as that of the genes encoding the enzymes of the NAGA catabolic pathway, identified by a BLAST search, was induced by this sugar. Deletion of *YlNAG5* rendered that expression independent of the presence of NAGA in the medium and reintroduction of the gene restored the inducibility, indicating that *Yl*Nag5 participates in the transcriptional regulation of the NAGA assimilatory pathway genes. Expression of *YlNAG5* was increased during sporulation and homozygous *Ylnag5*/*Ylnag5* diploid strains sporulated very poorly as compared with a wild type isogenic control strain pointing to a participation of the protein in the process. Overexpression of *YlNAG5* allowed growth in glucose of an *Ylhxk1glk1* double mutant and produced, in a wild type background, aberrant morphologies in different media. Expression of *YlNAG5* in a *Saccharomyces cerevisiae hxk1 hxk2 glk1* triple mutant restored ability to grow in glucose.

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Recent publication.

1 Gombert AK, van Maris AJA. 2015. Improving conversion yield of fermentable sugars into fuel ethanol in 1st generation yeast-based production processes. Curr Opin Biotechnol 33:81–86.

Current fuel ethanol production using yeasts and starch or sucrose-based feedstocks is referred to as 1st generation (1G) ethanol production. These processes are characterized by the high contribution of sugar prices to the final production costs, by high production volumes, and by low profit margins. In this context, small improvements in the ethanol yield on sugars have a large impact on process economy. Three types of strategies used to achieve this goal are discussed: engineering free-energy conservation, engineering redox-metabolism, and decreasing sugar losses in the process. Whereas the two former strategies lead to decreased biomass and/or glycerol formation, the latter requires increased process and/or yeast robustness.

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

Recent publication.

1 Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, Falk R, Parnmen S, Lumbsch HT, Boekhout T. 2015. Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. Fungal Genet Biol - doi.org/10.1016/j.fgb.2015.02.009

Phylogenetic analysis of 11 genetic loci and results from many genotyping studies revealed significant genetic diversity with the pathogenic *Cryptococcus gattii/Cryptococcus neoformans* species complex. Genealogical concordance, coalescence-based, and species tree approaches supported the presence of distinct and concordant lineages within the complex. Consequently, we propose to recognize the current *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* as separate species, and five species within *C. gattii*. The type strain of *C. neoformans* CBS132 represents a serotype AD hybrid and is replaced. The newly delimited species differ in aspects of pathogenicity, prevalence for patient groups, as well as biochemical and physiological aspects, such as susceptibility to antifungals. MALDI-TOF mass spectrometry readily distinguishes the newly recognized species.

XIII Department of Biology, Faculty of Medicine Masaryk University, Kamenice 5, 62500 Brno, Czech Republic. Communicated by Professor Marie Kopecká <mkopecka@med.muni.cz> http://www.med.muni.cz/~mkopecka/ - http://www.researcherid.com/rid/H-4204-2014.

Papers published in 2014.

1 Kopecká M. 2014. Effects of microtubule and actin inhibitors on *Cryptococcus neoformans* examined by scanning and transmission electron microscopy. Chemotherapy 60:99-106. doi: 10.1159/000371413

Background: The aim of this basic research was to investigate by scanning and transmission electron microscopy the effects of cytoskeleton inhibitors on *Cryptococcus neoformans*. Methods: Cells treated by vincristine (VINC), methyl benzimidazol-2-ylcarbamate (BCM) and latrunculin A (LAT) in yeast extract peptone dextrose medium were examined by scanning (SEM) and transmission electron microscopy (TEM). Results: SEM and TEM showed VINC-inhibited cells, BCM-inhibited cells and LAT-inhibited cells that did not divide after 2 days, and had affected capsules. However, during 4 days resistant budding cells appeared in all samples. The most efficient inhibition occurred in combined VINC+LAT or BCM+LAT, where cells were dead without cytoplasm, and no resistant budding cells originated during 6 days. Conclusion: *C. neoformans* cells blocked by combined VINC + LAT or BCM + LAT for 6 days die without origin of resistant budding cells, but their empty cell-walls with affected capsules persist and imitate presence of yeast cells.

2 Kopecká M, Yamaguchi M, Kawamoto S. 2014. The Effects of the F-actin inhibitor latrunculin A on the pathogenic yeast *Cryptococcus neoformans*. Chemotherapy 60:185-190. http://www.karger.com/DOI/10.1159/000377619

Background: This basic research aimed to investigate the effects of actin inhibitor latrunculin A on human pathogen *Cryptococcus neoformans* by freeze-substitution and electron microscopy to identify how and why inhibited cells die. Methods: Cells treated by latrunculin A for 20 h in yeast extract peptone dextrose medium were investigated by phase contrast and fluorescent microscopy, freeze-substitution and transmission electron microscopy, counted in a Bürker chamber and absorbance was measured. Results: Control budding cells had actin patches, cables and rings and standard ultrastructure. Latrunculin A disrupted actin cytoskeleton and this inhibited cell growth and division; cells had ultrastructural disorder and contained small spherical mitochondria and thick aberrant cell walls. Budding cells lysed in buds and in septa. Conclusion: Latrunculin A has fungistatic, fungicidal and fungilytic effects on human pathogenic yeast *Cryptococcus neoformans*.

Papers published in 2015 (online).

3 Kopecká M, Yamaguchi M, Kawamoto S. 2015. Effects of the F-actin inhibitor latrunculin a on the budding yeast *Saccharomyces cerevisiae*. Microbiology 00:1-8; published online Apr 9 2015. doi: 10.1099/mic.0.000091.

XIV 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

The following are abstracts of recently published papers of the group.

1 Georgieva M, Staneva D, Uzunova K, Efremov T, Balashev K, Harata M, Miloshev G. 2015. The linker histone in *Saccharomyces cerevisiae* interacts with actin-related protein 4 and both regulate chromatin structure and cellular morphology. Int J Biochem Cell Biol 59:182–192. http://www.sciencedirect.com/science/article/pii/S1357272514003987

Chromatin structure promotes important epigenetic mechanisms that regulate cellular fate by organizing, preserving and controlling the way by which the genetic information works. Our understanding of chromatin and its functions is sparse and not yet well defined. The uncertainty comes from the complexity of chromatin and is induced by the existence of a large number of nuclear proteins that influence it. The intricate interaction among all these structural and functional nuclear proteins has been under extensive study in the recent years. Here, we show that Saccharomyces cerevisiae linker histone physically interacts with Arp4p (actin-related protein 4) which is a key subunit of three chromatin modifying complexes – INO80, SWR1 and NuA4. A single – point mutation in the actin – fold domain of Arp4p together with the knock-out of the gene for the linker histone in S. cerevisiae severely abrogates cellular and nuclear morphology and leads to complete disorganizing of the higher levels of chromatin organization.

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2 Georgieva M, Moyankova D, Djilianov D, Uzunova K, Miloshev G. 2015. Methanol extracts from the resurrection plant *Haberlea rhodopensis* ameliorate cellular vitality in chronologically ageing *Saccharomyces cerevisiae* cells. Biogerontology Mar 11 [Epub ahead of print] DOI 10.1007/s10522-015-9566-z. http://www.ncbi.nlm.nih.gov/pubmed/25758774

Bioactive substances that are found in many natural plant extracts are very important for the cosmetics, pharmaceutical industry and biotechnology. Especially interesting for these industries are the substances that possess cell revitalizing and anti-ageing properties. The endemic plant *Haberlea rhodopensis* is known for its ability to withstand drought and to revitalize when returned to optimal conditions after a long time in desiccation. It is a mere fact that this plant not only can completely resurrect from a dried state but is also able to bring back the natural biochemical compositions of its cells. As a result *H. rhodopensis* offers a wide field for investigation of the exact mechanisms of the revitalization process as well as broadens the search for unique bioactive chemical substances in its cells. Here, by using the yeast *Saccharomyces cerevisiae* as a model we have demonstrated that methanol extracts from the plant *H. rhodopensis* hold specific properties to revitalize and ameliorate cellular growth as well as to balance intracellular metabolic states. Our results add valuable knowledge on the effects of natural compounds on ageing and reinforce the idea of using yeast as a model organism in the development of rapid tests for studying the efficacy of different bioactive substances.

XV National Collection of Agricultural and Industrial Microorganisms, Faculty of Food Sciences, Corvinus University of Budapest, 1118, Budapest, Somlói út 14-16. Communicated by Gábor Péter <gabor.peter@uni-corvinus.hu>.

The following articles have been published since our last report.

1 Čadež N, Dlauchy D, Raspor P, Péter G. 2013. *Ogataea kolombanensis* sp. nov., *Ogataea histrianica* sp. nov. and *Ogataea deakii* sp. nov.,three novel yeast species from plant sources. Int J Syst Evol Microbiol 63:3115-3123.

Nine methanol-assimilating yeast strains isolated from olive oil sediments in Slovenia, extra virgin olive oil from Italy and rotten wood collected in Hungary were found to form three genetically separated groups, distinct from the currently recognized yeast species. Sequence analysis from genes of the small subunit (SSU) rRNA, internal transcribed spacer region/5.8S rRNA, large subunit (LSU) rRNA D1/D2 domains and translational elongation factor-1a (EF-1a) revealed that the three closely related groups represent three different undescribed yeast species. Sequence analysis of the LSU rRNA gene D1/D2 domains placed the novel species in the *Ogataea* clade. The three novel species are designated as *Ogataea kolombanensis* sp. nov. (type strain: ZIM 2322^T = CBS 12778^T= NRRL Y-63657^T), *Ogataea histrianica* sp. nov. (type strain: ZIM 2463^T = CBS 12779^T = NRRL Y-63658^T) and *Ogataea deakii* sp. nov. (type strain: NCAIM Y.01896^T = CBS 12735^T = NRRL Y-63656^T).

2 Nagy E, Niss M, Dlauchy D, Arneborg N, Nielsen DS, Péter G. 2013. *Yarrowia divulgata* f.a., sp. nov., a yeast species from animal-related and marine sources. Int J Syst Evol Microbiol 63:4818–4823.

Five yeast strains, phenotypically indistinguishable from *Yarrowia lipolytica* and *Yarrowia deformans*, were recovered from different animal-related samples. One strain was isolated from a bacon processing plant in Denmark, two strains from chicken liver in the USA, one strain from chicken breast in Hungary and one from minced beef in Hungary. Comparisons of the sequences of their large subunit rRNA gene D1/D2 domain and the internal transcribed spacer (ITS) regions revealed that, despite their phenotypic similarity, they represent a novel yeast species of the *Yarrowia* clade with *Y. deformans* being the genotypically closest relative (LSU rRNA gene D1/D2 and ITS region similarity of 97.0 and 93.7%, respectively). *Yarrowia divulgata* f.a., sp. nov. is proposed to accommodate these strains with F6-17^T (=CBS 11013^T=CCUG 56725^T) as the type strain. Some D1/D2 sequences of yeasts from marine habitats were found in the GenBank database that were identical to those of the strains of *Y. divulgata* f.a., sp. nov. Unfortunately, these strains were not available for our study.

3 Nagy E, Dlauchy D, Medeiros AO, Péter G, Rosa CA. 2014. *Yarrowia porcina* sp. nov. and *Yarrowia bubula* f.a. sp. nov., two yeast species from meat and river sediment. Antonie van Leeuwenhoek 105:697–707.

Eleven yeast strains representing two hitherto undescribed species were isolated from different kinds of meat samples in Hungary and one from the sediment of a tropical freshwater river in South-eastern Brazil. The analysis of the sequences of their large subunit rRNA gene D1/D2 domain and the internal transcribed spacer (ITS) regions placed the two new species in the *Yarrowia* clade. Some of the seven strains representing the first new species can mate and give rise to asci and form ascospores embedded in capsular material, which qualifies it as the third teleomorph species of the *Yarrowia* clade. The name *Yarrowia porcina* sp. nov. (type strain: NCAIM Y.02100^T = CBS 12935^T = NRRL Y-63669^T, allotype strain UFMG-RD131^A = CBS 12932^A) is proposed for this new yeast species, which, based on physiological characters, is indistinguishable from *Yarrowia lipolytica* and some other species of the genus. Considerable intraspecific variability was detected among the sequences of the large subunit rRNA gene D1/D2 domains of the seven strains. The variability among the D1/D2 sequences exceeded the divergence observed among the ITS sequences and in some cases more than 1 % substitution among the D1/ D2 sequences was detected. The conspecificity of these strains was supported by the low (0–3 substitutions) sequence divergence among their ITS sequences, the result of a parsimony network analysis utilizing the concatenated ITS and D1/D2 sequences and also by the fingerprint patterns generated by microsatellite primed PCR. No ascospore formation was observed in the group of the other five strains representing the second new species. These strains shared identical D1/D2 and ITS sequences. *Yarrowia bubula* f.a., sp. nov. (type strain: NCAIM Y.01998^T = CBS 12934^T = NRRL Y-63668^T) is proposed to accommodate these strains.

4 Čadež N, Fülöp L, Dlauchy D, Péter G. 2015. *Zygosaccharomyces favi* sp. nov., an obligate osmophilic yeast species from bee bread and honey. Antonie van Leeuwenhoek 107:645–654.

Five yeast strains representing a hitherto undescribed yeast species were isolated from bee bread and honey in Hungary. They are obligate osmophilic, i.e. they are unable to grow in/on high water activity culture media. Following isogamous conjugation, they form 1–4 spheroid or subspheroid ascospores in persistent asci. The analysis of the sequences of their large subunit rRNA gene D1/D2 domain placed the new species in the *Zygosaccharomyces* clade. In terms of pairwise sequence similarity, *Zygosaccharomyces gambellarensis* is the most closely related species. Comparisons of D1/D2, internal transcribed spacer and translation elongation factor-1a (EF-1a) gene sequences of the five strains with that of the type strain of *Z. gambellarensis* revealed that they represent a new yeast species. The name *Zygosaccharomyces favi* sp. nov. (type strain: NCAIM Y.01994^T = CBS 13653^T = NRRL Y-63719^T

= ZIM 2551^T) is proposed for this new yeast species, which based on phenotype can be distinguished from related *Zygosaccharomyces* species by its obligate osmophilic nature. Some intragenomic sequence variability, mainly indels, was detected among the ITS copies of the strains of the new species.

XVI Department of Genetics and Applied Microbiology, University of Debrecen, Egyetem-ter 1, H-4032 Debrecen, Hungary. Communicated by Matthias Sipiczki <gecela@post.sk>.

Recent publications.

1 Kaewwichian R, Jindamorakot S, Am-In S, Sipiczki M, Limtong S. 2015. *Hannaella siamensis* sp. nov. and *Hannaella phetchabunensis* sp. nov., two new anamorphic basidiomycetous yeast species isolated from plants. Int J Syst Evol Microbiol. doi: 10.1099/ijs.0.000101

Eight strains representing two novel anamorphic yeast species consisted of six strains isolated from the external surface of rice leaves (DMKU-RP72^T, DMKU-RP109, DMKU-RP119, YE-124 and YE-156) and a corn leaf (DMKU-CP430T) collected in Thailand, and two strains isolated from a composite flower (11-1114) and a fallen dead leaf (12-301) collected in Belize. On the basis of sequence analysis of the D1/D2 region of the large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS) region, they were assigned to be two novel *Hannaella* species. The seven strains (DMKU-RP72^T, DMKU-RP109, DMKU-RP119, YE-124, YE-156, 11-1114 and 12-301) differed from each other by 0-3 nucleotide substitutions in the D1/D2 region and 0-1 nucleotide substitution in the ITS region. In terms of pairwise sequences similarity of the D1/D2 region these seven strains were closest to *Hannaella zeae* but with 1.2-1.7% (7-9) nucleotide substitutions. The sequence of ITS region of these seven strains differed from *H. zeae* by 3.7-3.9% (16-17) nucleotide substitutions. Therefore, they were assigned as a single novel species and the name *Hannaella siamensis* sp. nov. is proposed. The type strain is DMKU-RP72^T (=BCC 69493^T = NBRC 110425^T = CBS 13533^T). Strain DMKU-CP430^T represented the second novel species was also closest to H. zeae but with 1.0% (6) nucleotide substitutions in the D1/D2 region and 3.2% (14) nucleotide substitutions in the ITS region. It was assigned to be *Hannaella phetchabunensis* sp. nov. (type strain DMKU-CP430^T = BCC 69492^T = NBRC 110424^T = CBS 13386^T).

2 Sipiczki M. 2015. *Starmerella syriaca* f.a., sp. nov., an osmotolerant yeast species isolated from flowers in Syria. Antonie Van Leeuwenhoek 107:847-856.

Four strains of a novel asexual ascomycetous yeast species were isolated from *Malva* sp. flowers in Syria. Sequencing of the regions spanning the small subunit, 5.8S, and the D1/D2 domains of the large subunit ribosomal RNA genes showed that the isolates were conspecific. Comparative analysis of these sequences and the corresponding sequences of the type strains of ascomycetous yeasts revealed that the novel species is phylogenetically related to members of the *Starmerella* clade. Its closest relative is *Candida vaccinii*. For the new species the name *Starmerella syriaca* is proposed. Its strains are osmotolerant and produce pseudohypha-like structures capable of penetrating agar media. The type strain is 2-1362^T (=CBS 13909^T = NCAIM Y.02138^T = CCY 090-003-001^T). The GenBank accession numbers for its nucleotide sequences are: JX515986 (D1/D2 LSU), JX515987 (ITS1-5.8S-ITS2) and JX515988 (SSU). Mycobank: MB 810090.

3 Papp L, Sipiczki M, Holb IJ, Miklos I. 2014. Optimal conditions for mycelial growth of *Schizosaccharomyces japonicus* cells in liquid medium: it enables the molecular investigation of dimorphism. Yeast 31:475-482.

The non-pathogenic dimorphic fission yeast, *Schizosaccharomyces japonicus*, could be a suitable model organism for investigation of the genetic background of mycelial growth, as it has a haploid chromosome set and its genome is sequenced. Since earlier results have suggested that its morphological transition required solid substrates, but molecular biological experiments would require hyphae production in a liquid medium, we wanted to find circumstances which would enable hyphae production in liquid media. Several external conditions were investigated, but the strongest inducer was fetal bovine

serum (FBS). Its positive effect could be hampered by heat and was dependent on pH, temperature and concentration of the serum. Other protein-containing compounds, such as peptone and bovine serum albumin or amino acids, proved to be ineffective or weak. Generally, the uninduced and induced mycelial growth of *Sz. japonicus* could be improved by lower external pH and higher temperature.

4 Nagy LG, Ohm RA, Kovacs GM, Floudas D, Riley R, Gacser A, Sipiczki M, Davis JM, Doty SL, de Hoog GS, Lang BF, Spatafora JW, Martin FM, Grigoriev IV, Hibbett DS. 2014. Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. Nat Commun 5:4471.

Convergent evolution is common throughout the tree of life, but the molecular mechanisms causing similar phenotypes to appear repeatedly are obscure. Yeasts have arisen in multiple fungal clades, but the genetic causes and consequences of their evolutionary origins are unknown. Here we show that the potential to develop yeast forms arose early in fungal evolution and became dominant independently in multiple clades, most likely via parallel diversification of Zn-cluster transcription factors, a fungal-specific family involved in regulating yeast-filamentous switches. Our results imply that convergent evolution can happen by the repeated deployment of a conserved genetic toolkit for the same function in distinct clades via regulatory evolution. We suggest that this mechanism might be a common source of evolutionary convergence even at large time scales.

5 Sipiczki M, Balazs A, Monus A, Papp L, Horvath A, Sveiczer A, Miklos I. 2014. Phylogenetic and comparative functional analysis of the cell-separation α-glucanase Agn1p in *Schizosaccharomyces*. Microbiology 160:1063-1074.

The post-cytokinetic separation of cells in cell-walled organisms involves enzymic processes that degrade a specific layer of the division septum and the region of the mother cell wall that edges the septum. In the fission yeast *Schizosaccharomyces pombe*, the 1,3-α-glucanase Agn1p, originally identified as a mutanase-like glycoside hydrolase family 71 (GH71) enzyme, dissolves the mother cell wall around the septum edge. Our search in the genomes of completely sequenced fungi identified GH71 hydrolases in Basidiomycota, Taphrinomycotina and Pezizomycotina, but not in Saccharomycotina. The most likely Agn1p orthologues in Pezizomycotina species are not mutanases having mutanase-binding domains, but experimentally non-characterized hypothetical proteins that have no carbohydrate-binding domains. The analysis of the GH71 domains corroborated the phylogenetic relationships of the *Schizosaccharomyces* species determined by previous studies, but suggested a closer relationship to the Basidiomycota proteins than to the Ascomycota proteins. In the *Schizosaccharomyces* genus, the Agn1p proteins are structurally conserved: their GH71 domains are flanked by N-terminal secretion signals and C-terminal sequences containing the conserved block YNFNA(Y)/HTG. The inactivation of the *agn1(Sj)* gene in *Schizosaccharomyces japonicus*, the only true dimorphic member of the genus, caused a severe cell-separation defect in its yeast phase, but had no effect on the hyphal growth and yeast-to-mycelium transition. It did not affect the mycelium-to-yeast transition either, only delaying the separation of the yeast cells arising from the fragmenting hyphae. The heterologous expression of *agn1(Sj)* partially rescued the separation defect of the *agn1Δ* cells of *Schizosaccharomyces pombe*. The results presented indicate that the fission yeast Agn1p 1,3-α-glucanases of *Schizosaccharomyces japonicus* and *Schizosaccharomyces pombe* share conserved functions in the yeast phase.

6 Sipiczki M. 2014. *Metschnikowia laotica* f.a., sp. nov., a dimorphic, pigment-producing yeast species isolated from fruit. Int J Syst Evol Microbiol 64:1847-1852.

Eight strains with identical sequences of the D1/D2 domains of the large subunit rRNA genes were isolated from fallen fruits in two distant localities in Laos. These strains represent a novel dimorphic budding yeast species producing invasive pseudo-hyphae and a brown pigment when growing on media containing quinic acid as the sole carbon source or tryptophan as the sole nitrogen source. Phylogenetic analysis of the sequences of the D1/D2 domains, the internal transcribed spacer (ITS) regions and the 18S

rRNA genes placed the novel species in the *Metschnikowia* clade close to *Candida torresii*, *Metschnikowia drosophilae* and *Candida danieliae*. The taxonomic name *Metschnikowia laotica* f.a., sp. nov., reflecting the geographical origin of the isolates, is proposed for the novel species. The type strain is 11-524^T (= CBS 12961^T = NCAIM Y.02124^T = CCY 64-4-1^T). The Mycobank number is MB 807383.

XVII International Centre for Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland, EH14 4AS and GG Stewart Associates, 13 Heol Nant Castan, Cardiff, Wales, CF14 6RP. Communicated by Graham G. Stewart <Profggstewart@aol.com> and <g.g.stewart@ac.uk> - www.ggstewartassociates.co.uk.

Recent papers:

1 Stewart, GG. 2014. Yeast mitochondria – Their influence on brewer's yeast fermentation and medical research. Master Brew Assoc Amer Tech Qu 51:3-11.

In *Saccharomyces cerevisiae* and related species (including brewer's yeast strains), the most frequent spontaneous mutation is the specific DNA contained in its mitochondria (mtDNA). This mutation is termed respiratory deficiency (RD) or petite because of the small size of the resulting colonies compared to the wild type respiratory sufficient (RS) colony. The phenotypic effects of the RD mutation in a brewer's yeast culture are profound and examples, particularly during wort fermentation, are discussed. In addition, fundamental research on yeast mitochondria has assisted our knowledge of human mitochondrial function and disease. There are a group of diseases caused by dysfunctional mitochondria, often as the result of mutation of the mtDNA. These diseases include: type two diabetes, deafness, hereditary optic neuropathy and epilepsy to name a few. Mitochondrial research with yeast has provided considerable information and has assisted medical research on many of the myopathies listed. Indeed, current research removing the mtDNA from the ovaries of a diseased patient and replacing it with unmutated mtDNA from a donor to produce healthy zygotes is a novel, but controversial, technique with significant potential. Importantly, in the context of this publication, it is important to emphasize, that without mitochondrial research on brewer's yeast and its close relatives, these medical developments would probably have been inhibited, impeded and protracted.

2 Stewart GG. 2014. The concept of nature–nurture applied to brewer's yeast and wort fermentations. Master Brew Assoc Amer Tech Qu 51:69-80.

The conflicting approaches of nature versus nurture have been extensively discussed during the past few years, but it is not a novel concept. Nature–nurture concepts were initially introduced by the anthropologist Francis Galton in his book *Hereditary Genius* published in 1869. Nevertheless, a consideration of nature-nurture concepts is relevant today including being applied to wort fermentation by brewer's yeast strains. In this context, nurture refers to the environment experienced by the yeast strain and nature is the yeast's genetic make-up. Nature-nurture together is the interaction of these two parameters. A yeast strain will perform differently when inoculated (pitched) into worts of different composition. Conversely, yeast strains will differentiate when fermenting wort of a standard composition. Nature-nurture concepts applying to brewer's wort fermentations are discussed when applied to the following parameters: ale/lager yeast differences, wort sugar uptake, ester formation, wort concentration (gravity), diacetyl metabolism, and yeast flocculation.

Recently published books:

3 Stewart GG. 2014. Brewing Intensification, Amer Soc Brew Chem, St. Paul, Mn, USA, ISBN: 978-0-12-242352-9.

The book discusses the concept of lean manufacturing as it is applied to brewing and distilling. It also covers the intensification of all aspects of both processes. Coverage includes brewing operations, wort composition, yeast, fermentation, maturation. Because intensification brings significant change to both processes, this book's final chapter outlines training guidelines to assist brewers and operators implement and keep up with intensification trends in both brewing and distilling processes.

4 Russell I and Stewart GG (eds.). 2014. Whisky: Technology, Production and Marketing, 2nd Edition, Academic Press, Boston MA, USA, ISBN: 978-0-12-401735-1.

The expansion of whisky production together with the growth of craft distilleries has significantly increased the need for an accessible and scientific guide to details of raw materials, processes, equipment, and the practical stages necessary to produce quality whisky. This second edition of Whisky: Technology, Production and Marketing involves an overview of the production techniques, the processes and the current technologies. It provides details of the relevant fermentation science and considers not only Scotch whisky but also Irish, North American, Japanese and Indian whiskies.

Biographical review.

5 Stewart GG. 2015. Seduced by yeast. J Am Soc Brew Chem 73:1-21.

This is a personal review linking progress through facets of yeast research and development in the United Kingdom and Canada during time spent in the brewing industry and the university environment. The paper is available through open access on the website of the American Society of Brewing Chemists: http://www.asbcnet.org/publications/journal/vol/2015/Documents/ASBCJ-2015-0202-01.pdf

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XVIII Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL, USA. Communicated by CP Kurtzman <cletus.kurtzman@ars.usda.gov>.

Recent publications.

1 Kurtzman CP & Robnett CJ. 2014. Description of *Kuraishia piskuri* f.a., sp. nov., a new methanol assimilating yeast and transfer of phylogenetically related *Candida* species to the genera *Kuraishia* and *Nakazawaea* as new combinations. FEMS Yeast Res 14:1028–1036.

The new anamorphic yeast *Kuraishia piskuri*, f.a., sp. nov. is described for three strains that were isolated from insect frass from trees growing in Florida, USA (type strain, NRRL YB-2544, CBS 13714). Species placement was based on phylogenetic analysis of nuclear gene sequences for the D1/D2 domains of large subunit rRNA, small subunit rRNA, translation elongation factor-1α and subunits B1 and B2 of RNA polymerase II B. From this analysis, the anamorphic species *Candida borneana*, *C. cidri*, *C. floccosa*, *C. hungarica* and *C. ogatae* were transferred to the genus *Kuraishia* as new combinations, and *Candida anatomiae*, *C. ernobii*, *C. ishiwadae*, *C. laoshanensis*, *C. molendini-olei*, *C. peltata*, *C. pomicola*, *C. populi*, *C. wickerhamii* and *C. wyomingensis* were transferred to the genus *Nakazawaea*.

2 Kurtzman CP & Robnett CJ. 2015. *Occultifur kilbournensis* f.a. sp. nov., a new member of the Cystobasidiales associated with maize (*Zea mays*) cultivation. Antonie van Leeuwenhoek 107:1323–1329.

During a study of microorganisms associated with maize cultivation, yeasts were isolated from overwintered stalks, cobs and surrounding soil, which were collected from an agricultural field in south-central Illinois, USA. Predominant among isolates were several species of *Cryptococcus* and a red yeast that D1/D2 LSU rRNA gene sequences revealed to be a new species of the basidiomycete yeast genus *Occultifur*. The species, which was not detected in the same field during the growing season, is described here as *Occultifur kilbournensis* (type strain NRRL Y-63695, CBS 13982; allotype strain NRRL Y-63699, CBS 13983; MycoBank accession number MB 811259). Mixture of the type and allotype strains resulted in formation of hyphae with clamp connections and a limited number of basidia following incubation on 5% malt extract agar at 15 C for two months. From analysis of D1/D2 and ITS nucleotide sequences, the new species is most closely related to *Occultifur externus*.

3 Kurtzman CP & Sugiyama J. 2015. Saccharomycotina and Taphrinomycotina: The Yeasts and Yeastlike Fungi of the Ascomycota. The Mycota VII Part B. Systematics and Evolution, 2nd edn. McLaughlin DJ and Spatafora J.W. (Eds.). Springer-Verlag, Berlin, pp. 3-33.

4 McCormick SP, Kato T, Maragos CM, Busman M, Lattanzio VM, Galaverna G, Dall-Asta C, Crich D, Price NP, Kurtzman CP. 2015. Anomericity of T-2 toxin-glucoside: masked mycotoxin in cereal crops. J Agric Food Chem. 63:731-738.

T-2 toxin is a trichothecene mycotoxin produced when *Fusarium* fungi infect grains, especially oats and wheat. Ingestion of T-2 toxin contaminated grain can cause diarrhea, hemorrhaging, and feed refusal in livestock. Cereal crops infected with mycotoxin-producing fungi form toxin glycosides, sometimes called masked mycotoxins, which are a potential food safety concern because they are not detectable by standard approaches and may be converted back to the parent toxin during digestion or food processing. The work reported here addresses four aspects of T-2 toxin-glucosides: phytotoxicity, stability after ingestion, antibody detection, and the anomericity of the naturally occurring T-2 toxin-glucoside found in cereal plants. T-2 toxin-β-glucoside was chemically synthesized and compared to T-2 toxin-α-glucoside prepared with *Blastobotrys* muscicola cultures and the T-2 toxin-glucoside found in naturally contaminated oats and wheat. The anomeric forms were separated chromatographically and differ in both NMR and mass spectrometry. Both anomers were significantly degraded to T-2 toxin and HT-2 toxin under conditions that mimic human digestion, but with different kinetics and metabolic end products. The naturally occurring T-2 toxin-glucoside from plants was found to be identical to T-2 toxin-α-glucoside prepared with *B. muscicola*. An antibody test for the detection of T-2 toxin was not effective for the detection of T-2 toxin-α-glucoside. This anomer was produced in sufficient quantity to assess its animal toxicity.

XIX Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California Davis, One Shields Ave, Davis, CA 95616 USA. Communicated by Kyria Boundy-Mills <klbmills@ucdavis.edu>.

The following are abstracts of papers published during the 2014 calendar year.

1 Boundy-Mills KL. 2014. Methods for the isolation and investigation of the diversity of cold-adapted yeasts and their ex situ preservation in worldwide collections. Cold-adapted yeasts, Springer Berlin Heidelberg pp 23-45.

Cold-adapted (psychrophilic and psychro-tolerant) yeasts have been isolated from a variety of substrates, using a variety of cultivation methods. Yeasts able to grow at as low as 0°C have been isolated from cold substrates such as glaciers, snow, and deep sea sediment, but also from temperate and tropical climates. A broad diversity of media and culture conditions have been used to isolate and cultivate these yeasts. Low temperature incubation is used to select for psychrophiles, thus – depending on the strains - relatively long incubation time (up to 14 weeks) may be required. Cold-adapted yeast strains belong to many species in many clades of Ascomycota and Basidiomycota. Numerous strains have been deposited in public culture collections. Online strain catalogs of some public yeast culture collections include searchable fields for growth temperatures, allowing selection of yeasts able to grow at desired temperatures. Culture-independent methods for profiling yeast diversity in mixed communities can be used to profile populations, allowing detection of yeasts whose DNA is present in a specimen but that were not cultivated.

2 Garay L, Boundy-Mills K, German J. 2014. Accumulation of high value lipids in single cell microorganisms: A mechanistic approach and future perspectives. J Agric Food Chem 62:2709-2727.

In recent years attention has been focused on the utilization of microorganisms as alternatives for industrial and nutritional applications. Considerable research has been devoted to techniques for growth, extraction, and purification of high value lipids for their use as biofuels and biosurfactants as well as high-value metabolites for nutrition and health. These successes argue that the elucidation of the mechanisms

underlying the microbial biosynthesis of such molecules, which are far from being completely understood, now will yield spectacular opportunities for industrial scale biomolecular production. There are important additional questions to be solved to optimize the processing strategies to take advantage of the assets of microbial lipids. The present review describes the current state of knowledge regarding lipid biosynthesis, accumulation, and transport mechanisms present in single-cell organisms, specifically yeasts, microalgae, bacteria, and archaea. Similarities and differences in biochemical pathways and strategies of different microorganisms provide a diverse toolset to the expansion of biotechnologies for lipid production. This paper is intended to inspire a generation of lipid scientists to insights that will drive the biotechnologies of microbial production as uniquely enabling players of lipid biotherapeutics, biofuels, biomaterials, and other opportunity areas into the 21st century.

3 McCluskey K, Wiest A, Boundy-Mills K. 2014. Chapter 4. Genome data drives change at culture collections. Fungal Genomics, Springer Berlin Heidelberg pp. 81-96.

Research with fungi has been collaborative for many years and has depended on the open sharing of resources either via public collections or via peer-to-peer exchanges. Formal culture collections, such as the Fungal Genetics Stock Center, the Centraalbureau voor Schimmelcultures, and the U.S. Department of Agriculture (USDA) Agriculture Research Service (ARS) Northern Regional Research Laboratory (NRRL) have facilitated this collaborative community and have ensured that research materials from one era are available as we transition to a modern postgenomics era. Culture collections have been identified as a key infrastructure element to promote the transition to the bioeconomy. Collections have leveraged the work of decades of researchers to provide ready-characterized material suitable for whole-genome analysis. Researchers at these collections are in the enviable position of having a veritable treasure trove of materials in their freezers.

4 Sitepu I, Garay L, Sestric R, Levin D, Block DE, German J, Boundy-Mills K. 2014. Oleaginous yeasts for biodiesel: Current and future trends in biology and production. J Biotechnol Adv 32:1336-1360.

Production of biodiesel from edible plant oils is quickly expanding worldwide to fill a need for renewable, environmentally-friendly liquid transportation fuels. Due to concerns over use of edible commodities for fuels, production of biodiesel from non-edible oils including microbial oils is being developed. Microalgae biodiesel is approaching commercial viability, but has some inherent limitations such as requirements for sunlight. While yeast oils have been studied for decades, recent years have seen significant developments including discovery of new oleaginous yeast species and strains, greater understanding of the metabolic pathways that determine oleaginicity, optimization of cultivation processes for conversion of various types of waste plant biomass to oil using oleaginous yeasts, and development of strainswith enhanced oil production. This review examines aspects of oleaginous yeasts not covered in depth in other recent reviews. Topics include the history of oleaginous yeast research, especially advances in the early 20th century; the phylogenetic diversity of oleaginous species, beyond the few species commonly studied; and physiological characteristics that should be considered when choosing yeast species and strains to be utilized for conversion of a given type of plant biomass to oleochemicals. Standardized terms are proposed for units that describe yeast cell mass and lipid production.

5 Sitepu I, Jin M, Fernandez J, Sousa L, Balan V, Boundy-Mills K. 2014. Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. Appl Microbiol Biotechnol 98:7645-7657.

Microbial oil is a potential alternative to food/plant derived biodiesel fuel. Our previous screening studies identified a wide range of oleaginous yeast species, using a defined laboratory medium known to stimulate lipid accumulation. In this study, the ability of these yeasts to grow and accumulate lipids was further investigated in synthetic hydrolysate (SynH) and authentic ammonia fiber expansion (AFEX™)-pretreated corn stover hydrolysate (ACSH). Most yeast strains tested were able to accumulate

lipids in SynH, but only a few were able to grow and accumulate lipids in ACSH medium. *Cryptococcus humicola* UCDFST 10-1004 was able to accumulate as high as 15.5 g/L lipids, out of a total of 36 g/L cellular biomass when grown in ACSH, with a cellular lipid content of 40 % of cell dry weight. This lipid production is among the highest reported values for oleaginous yeasts grown in authentic hydrolysate. Preculturing in SynH media with xylose as sole carbon source enabled yeasts to assimilate both glucose and xylose more efficiently in the subsequent hydrolysate medium. This study demonstrates that ACSH is a suitable medium for certain oleaginous yeasts to convert lignocellullosic sugars to triacylglycerols for production of biodiesel and other valuable oleochemicals.

6 Sitepu I, Selby T, Zhu S, Lin T, Boundy-Mills K. 2014. Carbon source utilization and inhibitor tolerance of 45 oleaginous yeast species. J Indust Microbiol Biotechnol 41:1061-1070.

Conversion of lignocellulosic hydrolysates to lipids using oleaginous (high lipid) yeasts requires alignment of the hydrolysate composition with the characteristics of the yeast strain, including ability to utilize certain nutrients, ability to grow independently of costly nutrients such as vitamins, and ability to tolerate inhibitors. Some combination of these characteristics may be present in wild strains. In this study, 48 oleaginous yeast strains belonging to 45 species were tested for ability to utilize carbon sources associated with lignocellulosic hydrolysates, tolerate inhibitors, and grow in medium without supplemented vitamins. Some well-studied oleaginous yeast species, as well as some that have not been frequently utilized in research or industrial production, emerged as promising candidates for industrial use due to ability to utilize many carbon sources, including *Cryptococcus aureus*, *Cryptococcus laurentii, Hannaella* aff. *zeae*, *Tremella encephala*, and *Trichosporon coremiiforme*. Other species excelled in inhibitor tolerance, including *Candida* aff. *tropicalis, Cyberlindnera jadinii*, *Metschnikowia pulcherrima*, *Schwanniomyces occidentalis* and *Wickerhamomyces ciferrii*. No yeast tested could utilize all carbon sources and tolerate all inhibitors tested. These results indicate that yeast strains should be selected based on characteristics compatible with the composition of the targeted hydrolysate. Other factors to consider include the production of valuable co-products such as carotenoids, availability of genetic tools, biosafety level, and flocculation of the yeast strain. The data generated in this study will aid in aligning yeasts with compatible hydrolysates for conversion of carbohydrates to lipids to be used for biofuels and other oleochemicals.

7 Sitepu I, Shi S, Simmons BA, Singer S, Boundy-Mills K, Simmons C. 2014. Yeast tolerance to the ionic liquid 1-ethyl-3-methylimidazolium acetate. FEMS Yeast Res 14:1286-1294.

Lignocellulosic plant biomass is the target feedstock for production of second generation biofuels. Ionic liquid (IL) pretreatment can enhance deconstruction of lignocellulosic biomass into sugars that can be fermented to ethanol. Although biomass is typically washed following IL pretreatment, small quantities of residual IL can inhibit fermentative microorganisms downstream, such as the widely used ethanologenic yeast, *Saccharomyces cerevisiae*. The aim of this study was to identify yeasts tolerant to the IL 1-ethyl-3-methylimidazolium acetate, one of the top performing ILs known for biomass pretreatment. One hundred and sixty eight strains spanning the Ascomycota and Basidiomycota phyla were selected for screening, with emphasis on yeasts within or closely related to the *Saccharomyces* genus and those tolerant to saline environments. Based on growth in media containing 1-ethyl-3-methylimidazolium acetate, tolerance to IL levels ranging 1–5% was observed for 80 strains. The effect of 1-ethyl-3-methylimidazolium acetate concentration on maximum cell density and growth rate was quantified to rank tolerance. The most tolerant yeasts included strains from the genera *Clavispora, Debaryomyces, Galactomyces, Hyphopichia, Kazachstania, Meyerozyma, Naumovozyma, Wickerhamomyces, Yarrowia,* and *Zygoascus*. These yeasts included species known to degrade plant cell wall polysaccharides and those capable of ethanol fermentation. These yeasts warrant further investigation for use in saccharification and fermentation of IL-pretreated lignocellulosic biomass to ethanol or other products.

XX Department of Microbial, Biochemical & Food Biotechnology, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa - http://www.ufs.ac.za/biotech. Communicated by James du Preez <dpreezjc@ufs.ac.za>.

Lodewyk Kock, a distinguished professor in this department and a former commissioner of the International Yeast Commission, retired at the end of 2014.

The following papers were recently published or accepted for publication.

1 Ells R, Kemp G, Albertyn J, Kock JLF & Pohl CH. 2013. Phenothiazine is a potent inhibitor of prostaglandin E_2 production by *Candida albicans* biofilms. FEMS Yeast Res 13:849-855.

2 Girhard M, Tieves F, Weber E, Smit MS & Urlacher VB. 2013. Cytochrome P450 reductase from *Candida apicola*: versatile redox partner for bacterial P450s. Appl Microbiol Biotechnol 97:1625-1635.

3 Motaung TE, Albertyn J, Kock JLF, Lee CF, Suh SO, Blackwell M & Pohl CH. 2013. *Trichosporon vanderwaltii* sp. nov., an asexual basidiomycetous yeast isolated from soil and beetles. Antonie van Leeuwenhoek 103:313-319.

4 Olivier AP, Swart CW, Pohl CH, Van Wyk PWJ, Swart HC, Coetsee E, Schoombie S, Smit J & Kock JLF. 2013. The "firing cannons" of *Dipodascopsis uninucleata* var. *uninucleata.* Can J Microbiol 59:413-416.

5 Swart CW, Dithebe K, Van Wyk PWJ, Pohl CH, Swart HC, Coetsee E, Lodolo E & Kock JLF. 2013. Intracellular gas bubbles deform organelles in fermenting brewing yeasts. J Inst Brew 119:15-16.

6 Ells R, Kilian W, Hugo A, Albertyn J, Kock JLF & Pohl CH. 2014. Virulence of South African *Candida albicans* strains isolated from different clinical samples. Med Mycol 52:246-253. DOI: 10.1093/mmy/myt013

Candida albicans is a dimorphic opportunistic pathogenic yeast that is commonly isolated from different anatomical sites and clinical samples. It possesses several virulence factors, including secretion of hydrolytic enzymes, the ability to adhere to abiotic surfaces and cells, and the ability to penetrate tissues. We determined the level of *in vitro* expression of virulence factors by South African clinical *C. albicans* strains and the correlation among them. Furthermore, we determined whether there is a correlation between the levels of virulence factors expressed by a strain and the anatomical site from which it was isolated. The overall virulence of strains expressing different levels of these virulence factors *in vitro* was examined using a chorioallantoic membrane (CAM) chicken embryo model of infection, with variations observed in the production of hydrolytic enzymes. Most strains were able to produce *in vitro* high levels of protease and phospholipase and medium levels of lipase. Using the quantitative agar invasion assay, most strains were found to be highly invasive. No relationships of virulence factors produced *in vitro* were observed, except for a weak negative correlation between protease activity and invasiveness, as well as protease activity and cell surface hydrophobicity. There was no indication that the *in vitro* differences in virulence factors were correlated with virulence in the CAM model. However, we found that the infection model is sensitive enough to distinguish different virulence levels of strains.

7 Kuloyo OO, du Preez JC, García-Aparicio MP, Kilian SG, Steyn L and Görgens J. 2014. *Opuntia ficus-indica* cladodes as feedstock for ethanol production by *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. World J Microbiol Biotechnol 30: 3173-3183. DOI: 10.1007/s11274-014-1745-6

The feasibility of ethanol production using an enzymatic hydrolysate of pretreated cladodes of *Opuntia ficus-indica* (prickly pear cactus) as carbohydrate feedstock was investigated, including a comprehensive chemical analysis of the cladode biomass and the effects of limited aeration on the fermentation profiles and sugar utilization. The low xylose and negligible mannose content of the cladode

biomass used in this study suggested that the hemicellulose structure of the *O. ficus-indica* cladode was atypical of hardwood or softwood hemicelluloses. Separate hydrolysis and fermentation (SHF) and simultaneous hydrolysis and fermentation (SSF) procedures using *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* at 40 °C and 35 °C, respectively, gave similar ethanol yields under non-aerated conditions. In oxygen-limited cultures *K. marxianus* exhibited almost double the ethanol productivity compared to non-aerated cultures, although after sugar depletion utilization of the produced ethanol was evident. Ethanol concentrations of up to 19.5 and 20.6 g l^{-1} were obtained with *K. marxianus* and *S. cerevisiae*, respectively, representing 66% and 70% of the theoretical yield on total sugars in the hydrolysate. Because of the low xylan content of the cladode biomass, a yeast capable of xylose fermentation might not be a prerequisite for ethanol production. *K. marxianus*, therefore, has potential as an alternative to *S. cerevisiae* for bioethanol production. However, the relatively low concentration of fermentable sugars in the *O. ficus-indica* cladode hydrolysate presents a technical constraint for commercial exploitation.

8 Theron CW, Labuschagné M, Gudiminchi RK Albertyn J & Smit MS. 2014. A broad-range yeast expression system reveals *Arxula adeninivorans* expressing a fungal self-sufficient cytochrome P450 monooxygenase as an excellent whole-cell biocatalyst. FEMS Yeast Res 14:556-566. DOI: 10.1111/1567-1364.12142

The feasibility of using a single vector to clone a cytochrome P450 monooxygenase (P450) in different yeasts and then compare whole cell hydroxylase activity was investigated. A broad-range yeast expression vector using the ylTEFp to drive expression of the cloned gene and the scTEFp to drive the hygromycin resistance marker gene, was used to clone the genes encoding two self-sufficient cytochrome P450 monooxygenases, CYP102A1 and CYP505A1. Both genes were cloned into *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* (two strains) and *Arxula adeninivorans*. 4-Hexylbenzoic acid (HBA), which is sub-terminally hydroxylated by both CYP102A1 and CYP505A1, was used to compare whole cell hydroxylase activity of transformants. *K. marxianus* and *A. adeninivorans* exhibited activity with both CYP102A1 and CYP505A1, while *S. cerevisiae* only displayed CYP102A1 activity and *Y. lipolytica* only CYP505A1 activity. The highest CYP102A1 activity (0.8 mM HBA converted in 24 h) was observed with concentrated resting cell suspensions of *S. cerevisiae*. The CYP505A1 activity observed with growing cultures of *A. adeninivorans* was however at least 12 times higher than the CYP102A1 activity of *S. cerevisiae* with up to 2 mM HBA converted within 6 h. The use of *K. marxianus* and *A. adeninivorans* for P450 expression has not previously been reported.

9 Akanni GB, du Preez JC, Steyn L and Kilian SG. 2015. Protein enrichment of an *Opuntia ficus-indica* cladode hydrolysate by cultivation of *Candida utilis* and *Kluyveromyces marxianus*. J Sci Food Agric DOI 10.1002/jsfa.6985

BACKGROUND: The cladodes of *Opuntia ficus-indica* (the prickly pear cactus) have a low protein content; for use as a balanced feed, supplementation with other protein sources is therefore desirable. We investigated protein enrichment by cultivation of the yeasts *Candida utilis* and *Kluyveromyces marxianus* in an enzymatic hydrolysate of the cladode biomass. RESULTS: Dilute acid pretreatment and enzymatic hydrolysis of sun-dried cladodes resulted in a hydrolysate containing (per litre) 45.5 g glucose, 6.3 g xylose, 9.1 g galactose, 10.8 g arabinose and 9.6 g fructose. Even though *K. marxianus* had a much higher growth rate and utilised L-arabinose and D-galactose more completely than *C. utilis*, it's biomass yield coefficient was lower due to ethanol and ethyl acetate production despite aerobic cultivation. Yeast cultivation more than doubled the protein content of the hydrolysate, with an essential amino acid profile superior to sorghum and millet grains. CONCLUSIONS: This *K. marxianus* strain was weakly Crabtree positive. Despite its low biomass yield, its performance compared well with *C. utilis*. This is the first report showing that the protein content and quality of *O. ficus-indica* cladode biomass could substantially be improved by yeast cultivation, including a comparative evaluation of *C. utilis* and *K. marxianus*.

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www.kuleuven.be/pme-bim. Communicated by S Crauwels <sam.crauwels@kuleuven.be>.

I recently defended by PhD thesis, conducted under the supervision of Profs. B. Lievens, G. De Samblanx, K. Marchal, K. Verstrepen, Catholic University of Leuven.

1 Sam Crauwels. Assessing genetic and phenotypic diversity of *Brettanomyces* yeasts.

Brettanomyces yeast species, with *Brettanomyces* (*Dekkera*) *bruxellensis* being the most important, are generally reported to be spoilage yeasts in the beer and wine industry due to the production of phenolic off-flavors. The aromas imparted, which can be described as 'medicinal and 'barnyard', are colloquially known as "Brett" character and are generally considered negative for beer and wine quality. However, the same compounds are regarded positively when produced during certain fermentation processes, such as the production of some styles of beer (e.g. lambic and gueuze). Despite its economic importance, surprisingly little is known about the biology, physiology and ecology of *Brettanomyces* yeasts. Herein, several aspects of *Brettanomyces* yeast biology and ecology were studied, with particular emphasis on *B. bruxellensis*, thus contributing to a better understanding of the biology and ecology of these important influencers of flavor profile. In the first chapter (Chapter I), we give a comprehensive literature overview of the state-of-the-art of *Brettanomyces* research, emphasizing areas that were particularly well explored at the start of this PhD study, including aroma-associated aspects and methods for detection and identification. We also focused on recent genetic and genomic studies providing novel insights into the biology and evolution of *B. bruxellensis*. In Chapter II, we assessed the genetic relationships between 50 *Brettanomyces* strains belonging to all species presently identified within the genus and isolated from different food products and beverages using established DNA fingerprinting methods. These methods included ribosomal RNA (rRNA) gene sequencing, random amplified polymorphic DNA (RAPD) PCR, arbitrarily primed (AP) PCR and repetitive element PCR fingerprinting (rep-PCR). Our results support earlier findings that *Brettanomyces* yeasts form a genetically diverse clade, even within a species, and are represented by several subgroupings. Further our results revealed an intriguing correlation between *B. bruxellensis* genotype groups and the respective source of isolation, suggesting niche adaptation. To further explore this relationship, we first sequenced a (beneficial) beer isolate of *B. bruxellensis* (VIB X9085; ST05.12/22) and compared its genome sequence with the genome sequences of two wine spoilage strains (AWRI 1499 and CBS 2499) (Chapter III). In addition to strain-specific single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels), structural genome variation was found between our strain and both wine strains, with some genomic regions specifically deleted in the beer strain. These included, but were not limited to, a region harboring the *B. bruxellensis* nitrate assimilation cluster and a region representing a cluster of genes mainly involved in carbon metabolism. Next, in Chapter IV, metabolic differences in carbon and nitrogen assimilation between different *B. bruxellensis* strains from different beverages (beer, wine and soft drink) were thoroughly assessed using Biolog Phenotype Microarrays. While some similarities of physiology were noted, many traits were variable among strains. Interestingly, some phenotypes were found that could be linked to strain origin, especially for the assimilation of particular α- and β-glycosides as well as α- and β-substituted monosaccharides. Based upon gene presence or absence, an α-glucosidase and β-glucosidase were found explaining the observed phenotypes. Further, using a PCR screen on a large number of *B. bruxellensis* isolates we have been able to specifically link a genomic deletion (e.g. harboring a β-glucosidase gene) to the beer strains, suggesting that this region may have a fitness cost for *B. bruxellensis* in certain fermentation systems such as brewing. Additionally, our work indicates that most beer strains are diploid, whereas the vast majority of wine strains are known to be triploid, suggesting that the additional set of chromosomes may confer a selective advantage in environments such as wineries. Finally, in Chapter V a series of fermentation tests were performed, in which different *B. bruxellensis* strains were inoculated in different media representative of different ecological niches of the

yeast (i.e. beer, wine and soft drink). Utilisation of different sugars was quantified and production of (off-) flavors was monitored after one month of fermentation. Our results revealed that not only the medium (which may contain different (off-) flavor precursors), but also the yeast strain mediates the formation of the typical *Brettanomyces* (off-) flavors. Moreover, a (moderate) correlation was found between the origin of the strains and their impact on the volatile composition of the media. Only strains originally isolated from wine produced typical *Brettanomyces* off-flavors when inoculated in wine, whereas strains originally isolated from beer or soft drink did not, for example. Vice versa, in strong golden pale ale (Duvel), beer strains behaved differently from the other strains. Altogether, these results suggest that the interaction between medium and strain affects the outcome of potential (off-) flavor production. Together, the components of this PhD study provide tools to discriminate *Brettanomyces* strains and reveal a first glimpse into the genetic diversity and genomic and phenotypic plasticity of *B. bruxellensis*. Our findings are relevant for the wine industry as well as for the beer industry. After all, deeper understanding of the ecology of *B. bruxellensis* not only provides novel insights into the evolution of this intriguing yeast species, it will also facilitate avoidance of wine spoilage and improvement of *B. bruxellensis* strains for brewing.

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

Recently accepted papers.

1 Santos ARO, Faria ES, Lachance MA, Rosa CA. 2015. *Ogataea mangiferae* sp. nov., a methylotrophic yeast isolated from mango leaves. Int J Syst Evol Microbiol - doi: 10.1099/ijs.0.000194.

2 Cadete RM, Cheab MAM, Santos RO, Safar SVB, Zilli JE, Vital MJS, Basso LC, Lee CF, Kurtzman CP, Lachance MA, Rosa CA 2015 *Cyberlindnera xylosilytica* sp. nov., a xylitol-producing yeast species isolated from lignocellulosic materials. Int J Syst Evol Microbiol - doi: 10.1099/ijs.0.000363.

Recent presentation.

3 Lachance MA, Hurtado E, Hsiang T. 2015. *Metschnikowia*: genus concepts in yeasts; getting the correct tree. The Second International Workshop on Ascomycete Systematics, Amsterdam, The Netherlands.

The sequencing revolution has caused an explosion in the number of new yeast species descriptions and an increase in the size of many yeast genera. Better-known ascomycete yeast genera such as *Saccharomyces* or *Pichia* have been reorganized into smaller units based on phylogenies inferred from sequence information. The genus *Metschnikowia* has forborn such reassignments. At current estimate, the clade containing all described species that fit a morphological *Metschnikowia* concept contains 45 ascosporic and 57 asexual species, as well as three ascosporic species assigned to the genus *Clavispora*. Cases could be made for subdividing the genus based on currently available phylogenies, carving out, for example, a subclade of large-spored, beetle-associated species and a small subclade that includes *M. orientalis* and *M. agaves* into separate genera. However, the subclades are poorly defined and hasty rearrangements would serve no purpose but to create names that would require further revision as better phylogenies become available. We have used Illumina HiSeq draft genome sequences to examine the phylogeny of six Hawaiian endemic *Metschnikowia* species. The analysis shows that phylogenies obtained in the past from rRNA genes or with a concatenation of actin, elongation factor 2, and RNA polymerase 2, are not congruent with the topology suggested by a majority of nearly a thousand genes selected for their information content, amenability to alignment, and strong phylogenetic signal to noise ratios. High-throughput sequencing now makes it possible to obtain better phylogenies, but until these are obtained, a conservative approach to nomenclature should be followed.

Obituary

Dr James Barnett (November 8 1923 - February 17 2015)

James Arthur Barnett devoted his long career to the study of yeasts, beginning in 1953 at the Low Temperature Station for Research in Biochemistry and Biophysics, Cambridge with work on taxonomy and nutrition and later at the Institute of Food Research, Norwich. Although an assiduous and meticulous experimentalist, his most lasting achievements are of a more scholarly nature. Together with Payne and Yarrow, he authored an immensely useful laboratory handbook, now in its third edition, *Yeasts: Identification and Characteristics* which describes how to identify each of 963 species of yeast, and what is known of their properties, many of which were independently checked by James. Then, aged 75, and an Honorary member of Faculty at the University of East Anglia, (where he had for many years enthusiastically run practical classes on yeasts), he embarked on another major project, in which he explored the history of research on yeasts. Written as a series of fourteen essays, initially published in the journal *Yeast*, these papers were then compiled into a single volume, *Yeast Research: a Historical Overview* (American Society of Microbiology Press, 2011). These articles are eloquent, immensely readable, and accurate, revealing as they do the foundations of the modern disciplines of microbiology and biochemistry, as well as the immense insights (and foibles) of the early scientists from Lavoisier (and predating Pasteur) in the late 18th century, to the near-present day. To avoid the errors of others, James consulted each of the original publications, many sourced from libraries across Europe. This model of scientific scholarship is another totem of James's intellectual legacy.

Forthcoming Meetings

32nd Specialized Symposium on Yeasts (ISSY 32)
Perugia, September 13-17 2015

On behalf of the Organizing Committee, I cordially invite you to the 32nd Specialized Symposium on Yeasts, which will be held in Perugia, Italy, in September 13-17, 2015.

General topic: Yeast Biodiversity and Biotechnology in the Twenty-First Century. The program will include opening and keynote lectures, selected oral presentations, and poster sessions covering all aspects of yeast ecology, physiology, taxonomy, food and non-food biotechnology, genetic and genomic. Special sessions will be dedicated to non-conventional yeasts and yeasts culture Collections. For further details on the program see: www.issy32.com

This Symposium back to Perugia after 27 years (the last was ISSY7, held in 1988). In this context, ISSY32 is dedicated to Prof. Alessandro Martini, Chair of ISSY7, yeast biologist and great Master of science and life.

ISSY32 is sponsored by the International Commission on Yeasts (ICY), by the Italian Society for Agricultural, Food and Environmental Microbiology (SIMTREA), by the Italian Society for General Microbiology and Microbial Biotechnology (SIMGBM).

Venue: ISSY32 will be held at Hotel Giò–Perugia Congress Centre, Perugia, Italy: http://www.hotelgio.it/en/#.VGIGh2cQNpB.
Hotel Giò – Perugia Congress Centre (inside the Hotel Giò Wine e Jazz Area with its 206 rooms) includes a number of meeting rooms and an Auditorium for up to 700 places. 2 Halls (Jazz Hall and Wine Hall) with areas expressly developed for informal meeting, living bar for coffee breaks and comfortable working and reading corners.

About the place: Perugia is a cosmopolitan city and home of two Universities. It hosts a worldwide famous jazz Festival during summertime and its University for Foreigners is a well-known place in which learn Italian. It is a walled city on a hilltop with amazing views over the valley and has several historical monuments and a lovely central square. Its history goes back to the ninth century BC. Besides, the beauty of the surrounding towns (Assisi, Todi) and nature (Trasimeno lake, Marmore falls) and the rich cultural heritage of the region will provide an excellent opportunity for informal encounters.

Perugia connections: the city Airport of Perugia (http://www.airport.umbria.it/en) represents the main access gateway from some European cities. Alternatively, the two airports of Rome, Fiumicino (http://www.adr.it/web/aeroporti-di-roma-en-/pax-fco-fiumicino
and Ciampino (http://www.adr.it/web/aeroporti-di-roma-en-/pax-cia-ciampino), which are about two hours from Perugia by public transport, are the closest possibilities.

I am looking forward to seeing you on middle September 2015 in Perugia, Italy.

Pietro Buzzini
Chair of the Organizing Committee of ISSY32.
Department of Agricultural, Food and Environmental Science,
Industrial Yeasts Collection DBVPG (www.dbvpg.unipg.it)
University of Perugia, Perugia (Italy)

14th International Congress on Yeasts (ICY14)
Awaji Yumebutai International Conference Center, Awaji Island, Japan, September 11-15, 2016

On behalf of the Organization Committee, I'm great honored to host the 14th International Congress on Yeasts (ICY14) at Awaji Yumebutai International Conference Center, Awaji Island, Japan, in September 11-15, 2016.

General topic of ICY14 is "Yeasts for Global Happiness". It means that yeast science & technology will contribute to the world in terms of food & beverage, health & medicine, energy & environment. In addition, this congress will be the first time held in Japan since ISSY2 in back to 1972, when I was a junior high school student. We'd like to send valuable message and information from Japan to the world. More importantly, I believe that this will be a great opportunity for young scientists as they can attend and present their research. In contrast, we senior must tell the fun and importance of yeast research to the next generation.

ICY14 is sponsored by the International Commission on Yeasts as part of the International Union of Microbiological Societies (IUMS). The ICY has been held once every four years since 1955. It provides an opportunity for presenting the latest research progress in yeast metabolism, physiology, genetics, genomics, regulation, ecology, systematics, phylogeny, food and beverage applications, biofuel production and clinical applications.

I am very much looking forward to seeing you on middle September in 2016 at Awaji Island, Japan!

Hiroshi Takagi, Head of the Organizing Committee of ICY14
http://icy2016.com/index.html

Brief News Item
Postdoctoral Position Available

Kyria Boundy-Mills, curator of the Phaff Yeast Culture Collection at the University of California Davis, invites US citizens to apply for postdoctoral funding from the US National Science Foundation to work in her laboratory. NSF has a funding program that targets work with biological collections. More information and application instructions are available online through the NSF website: http://www.nsf.gov/pubs/2015/nsf15501/nsf15501.htm

The Phaff Yeast Culture Collection is the fourth largest public collection of wild yeasts in the world, with over 7000 strains belonging to over 800 species in the public catalog: http://phaffcollection.ucdavis.edu.

Possible research areas for a postdoctoral project include new species descriptions, studies of stress tolerance, lipid production, glycolipid secretion, or other areas of interest to the postdoctoral candidate. The proposal for funding must be submitted to NSF by the postdoctoral candidate, but Dr. Boundy-Mills will assist. Proposals are due to NSF on November 3, 2015. Applicants must be US citizens. Funding is up to $200,000 total over two years.

Contact for more information: <klbmills@ucdavis.edu>, phone +1 (530) 754-5575.

50 Years Ago

Y E A S T

A News Letter for Persons Interested in Yeast

May 1965 Volume XIV, Number 1

Dr. W. Ch. Slooff of CBS, the Netherlands, reported that type strains of eleven new yeast species were deposited in the collection: *Candida cloaceae, C. fabianii, C. maltosa, C. parapsilosis, Endomycopsis parapsilosis, E. vini, Pichia delftensis, P. etchellsii, Saccharomyces inconspicuous, S vafer* and *Trichosporon penicillatum*.

Dr. M. C. Pignal, Université de Lyon, France, published several manuscripts including one on the genus Pichia *sensu lato*, proposing the new species *P. media* and several new combinations.

L. R. Hedrick and Marjorie Soyugenc, Illinois Institute of Technology, USA, presented a paper on yeasts from Lake Michigan bottom mud. Most known species of *Hansenula* were detected, except *H. mrakii*.

Dr. J. Santa María, Instituto Nacional de Investigaciones Agronomicas, Spain reported three papers published and two accepted, pertaining to saccharose and maltose utilization, new species *Saccharomyces onubensis,* sexually different states of *Hansenula californica,* and species of *Prototheca* associated with olive trees.

Dr. Paul A. Hartman, Iowa State University, USA, submitted the abstract of the Ph.D. Dissertation (1964) of his former student Dr. Harland Burmeister, who had moved on to the Northern Regional Research Laboratory, Peoria, IL. The thesis title was, "A study of the yeasts in ensiled high moisture corn." Total molds, coliform bacteria, aerobic bacteria and yeasts were enumerated, and nearly 1200 yeasts were isolated and identified from corn stored under several environmental conditions. A succession of yeasts predominated in various stages, from primarily *C. parapsilosis* and *C. intermedia* in early stages, to *H. anomala* and *C. krusei* after 12 days, correlating with production of lactic acid by bacteria.

Dr. S. Windisch, Institut für Gärungsgewerbe, West Berlin, reported publication of four articles on a new gene for maltose fermentation in *Saccharomyces carlsbergensis,* two new species of yeast-like fungi (*Protendomycopsis domschii* and *Endomyces laibachii*), and the yeast flora of marzipan, a high-sugar almond paste used in sweets. Only *Zygosaccharomyces rouxii* was found to cause deterioration. Dr. C. C. Emeis became a Privatdozent for microbiology, and published two papers, one involving counting bud scars to determine the age of yeasts grown under various conditions, and one on biological aspects of rapid fermentations.

Dr. H. J. Phaff, University of California Davis, USA, shared the abstract of an article published in the Ciferri Memorial Issue of Rivista di Patologia vegetale, concerning the yeast flora in a slime flux of *Ulmus carpinifolia*. *(Note: these yeasts are still available from the Phaff Yeast Culture Collection.)* He also reported publication of a

manuscript describing the characterization of bacteria that produce lytic enzymes capable of decomposing yeast cell walls. *(Note: the Wall Lytic "WL" series of bacteria mentioned in this publication are still available from the Phaff collection.)*

Professor J. M. Mitchison, University of Edinburgh, Scotland, reported publication in *Nature* of a method to prepare synchronous cultures of budding yeast, fission yeast, or *E. coli* using a linear sucrose gradient. The method was in use to study biochemical events in the cell cycle of *Schizosaccharomyces pombe* including RNA and DNA synthesis.

Dr. H. O. Halvorson, University of Wisconsin, USA, reported linkage mapping of mutations resulting in histidine requirement in *Saccharomyces lactis* and the Lindegren Breeding Stock.

Dr. C. C. Lindegren, Southern Illinois University, USA, announced publication of six articles since the previous issue of the Yeast News Letter. Subjects included pigmentation associated with mutants in lysine requirement of *Saccharomyces*, induction of alpha-glucosidase by glucose, alleles of the melezitose locus, and chromosomal rearrangements in *Saccharomyces* induced by the mutagen ethyl methanesulfonate.

Dr. A. Sols, Institute G. Marañon, Spain, described a publication in press that compared the relative activities of two pathways for utilization of non-sugar carbon sources, that involve fructosedipohsphatase or phosphofructokinase.

Dr. J. O. Lampen, Rutgers – The State University, New Jersey, USA, presented a review on "Secretion of Enzymes by Micro-Organisms" in April 1965 at the Symposium of the Society for General Microbiology.

Dr. C. Akin, Falstaff Brewing Corporation, St. Louis, Missouri, USA shared abstracts of two papers presented at the American Society of Brewing Chemists, May 9-14, 1965 and the American Society for Microbiology, April 25-29, 1965. The papers described kinetics of yeast sedimentation in aqueous suspensions, and removal of diacetyl in beer by *Saccharomyces cerevisiae*.

Dr. A. S. El-Nawawy and M. A. Fouda, Egyptian Ministry of Agriculture, Giza, U.A.R., described four papers presented at the first Conference for Applied Microbiology, held in Cairo. The presentations dealt with selection of yeast strains able to grow on pentose substrates, optimum conditions for production of fodder yeast, variation in *Saccharomyces cerevisiae* induced by gamma radiation, and physiological variation in *S. cerevisiae* attributed to dikaryosis.

Dr. Kenkichi Kodama, Kodama Brewing Co. Ltd., Japan, published three papers. One, titled "Studies on Wild Yeasts which Thrive in Sake-Moto", described the isolation of yeasts from 54 breweries in Akita and Chiba prefectures, including the proposed new species *Candida fabianii*, named in honor of Dr. Fabian of the U.S.A. Another paper described cultural properties of *Prototheca* species. In the discussion of new yeast species *Debaryomyces nilsonii,* the authors state that it must be placed in this genus due to spore morphology, but the fermentation of sugars and assimilation of carbon compounds was not similar to other species in this genus.

Brief News Items: The Editor announced the death of Dr. Harry Katznelson, Director of Canada Department of Agriculture's Microbiology Research Unit, at the age of 52. Dr. Esther Meyer retired from her position as associate professor of microbiology, University of Illinois, College of Medicine. Dr. L. J. Wickerham of the Northern Regional Research Laboratory, USDA, reported that Dr. Frank H. Stodola, Head of the Pioneering Laboratory fo the Northern Regional Research Laboratory received the Annual Pasteur Award from the Illinois Society for Microbiology, for his work on structural studies of the sphingolipids produced by *Hansenula ciferrii*. Also, Dr. Wickerham indicated that he was working on descriptions of two new species of *Hansenula*. Professor O. Verona, University of Pisa, Italy shared copies of two publications, one on blastomycetic flora of some marine animals, and one on cellulolytic activity of *Trichosporon* yeasts.

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