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# Yeast

## A Newsletter for Persons Interested in Yeast

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# Editorials

## Miroslav Gabriel

Dr. Marie Kopecká informs us that her friend, colleague, and excellent teacher, Miroslav Gabriel, MD, PhD, and Associate Professor in the Department of Biology of the Masaryk Medical Faculty of the University of Brno, died suddenly on June 7th, 2008. He will be dearly missed by all who knew him.

M.A. Lachance, Editor

**I Department of Applied Microbiology, Lund University, PO BOX 124, 22100 Lund Sweden. Communicated by João R.M. Almeida <joao.almeida@tmb.lth.se> - www.tmb.lth.se.**

Research on yeast at the Department of Applied Microbiology is divided into several areas. Under the topic "Metabolic engineering of *S. cerevisiae* for efficient bioethanol production from lignocellulosic raw materials", the following thesis has been completed in 2009.

1 Almeida JRM - Improving the Response of *Saccharomyces cerevisiae* to Lignocellulosic Hydrolysate Inhibitors in Ethanolic Fermentation. April 2009.

In this work, the enzymes ADH6, mutated-ADH1 and XR were identified as being responsible for NADPH-, NADH-, and NAD(P)H-dependent HMF and furfural reduction, respectively, in yeast. The tolerance and fermentation rates of *Saccharomyces cerevisiae* laboratory strains on defined medium and/or on lignocellulosic hydrolysate were improved by overexpression of the genes encoding for these enzymes. It was also shown that overexpression of furaldehyde reductases benefits product distribution in recombinant xylose-utilizing *S. cerevisiae* strains carrying the xylose reductase/xylitol dehydrogenase pathway during xylose fermentation. Finally, strains with higher furaldehyde conversion rates were shown to grow faster and ferment lignocellulosic hydrolysates faster. Evaluation of industrial strains of *S. cerevisiae* showed that the selection of a robust strain and its evaluation under representative conditions are essential for lignocellulosic hydrolysate fermentation, since strains perform differentially depending on the hydrolysate and the conditions employed. The use of fed-batch mode is advantageous not only because the inhibitors are kept at low concentrations and the capacity of the yeast to convert them is not surpassed, but also because it allows cells to adapt to the inhibitors in the lignocellulosic hydrolysate. Indeed, using microarray analysis, and *in vitro* and *in vivo* activity measurements it was demonstrated that short-term adaptation of the yeast to lignocellulosic hydrolysate increased detoxifying activities and induced the expression of genes related to the repair of cellular damage. The results presented in this work show that integration of process design and strain improvement strategies could be used to improve the yeast performance in ethanolic fermentation of lignocellulosic hydrolysates.

The thesis was based on the following papers.

2 Petersson A, Almeida JRM, Modig T, Karhumaa K, Hahn-Hägerdal B, Gorwa-Grauslund MF and Liden G 2006 A 5-hydroxymethyl furfural reducing enzyme encoded by the *Saccharomyces cerevisiae ADH6* gene conveys HMF tolerance. Yeast 23:455-64.

3 Laadan B, Almeida JRM, Radstrom P, Hahn-Hägerdal B and Gorwa-Grauslund M 2008 Identification of an NADH-dependent 5-hydroxymethylfurfural-reducing alcohol dehydrogenase in *Saccharomyces cerevisiae*. Yeast 25:191-198.

4 Almeida JRM, Modig T, Roder A, Liden G and Gorwa-Grauslund MF 2008 *Pichia stipitis* xylose reductase helps detoxifying lignocellulosic hydrolysate by reducing 5-hydroxymethyl-furfural (HMF). Biotechnol Biofuels 1:12.

5 Almeida JRM, Roder A, Modig T, Laadan B, Liden G and Gorwa-Grauslund MF 2008 NADH- vs NADPH-coupled reduction of 5-hydroxymethyl furfural (HMF) and its implications on product distribution in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 78:939-45.

6 Almeida JRM, Bertilsson M, Hahn-Hägerdal B, Liden G and Gorwa-Grauslund MF (Submitted) Carbon fluxes of xylose-consuming *Saccharomyces cerevisiae* strains are differentially affected by NADH- and NADPH usage in HMF reduction.

7 Almeida JR, Karhumaa K, Bengtsson O and Gorwa-Grauslund MF 2009 Screening of *Saccharomyces cerevisiae* strains with respect to anaerobic growth in non-detoxified lignocellulose hydrolysate. Bioresour Technol 100:3674-3677.

8 Modig T, Almeida JRM, Gorwa-Grauslund MF and Liden G 2008 Variability of the response of *Saccharomyces cerevisiae* strains to lignocellulose hydrolysate. Biotechnol Bioeng 100:423-429.

9 Almeida JRM, Modig T, Petersson A, Hahn-Hägerdal B, Lidén G and Gorwa-Grauslund MF 2007 Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 82:340-349.

10 Almeida JR, Bertilsson M, Gorwa-Grauslund MF, Gorsich S and Liden G 2009 Metabolic effects of furaldehydes and impacts on biotechnological processes. Appl Microbiol Biotechnol 82:625-38.

11 Almeida JRM and Hahn-Hägerdal B 2009 Developing *Saccharomyces cerevisiae* strains for second generation bioethanol: Improving xylose fermentation and inhibitor tolerance. Int Sugar J CXI:172-180.

## II Colección de Levaduras Quito Católica (CLQCA), Centro Neotropical para Investigación de la Biomasa, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Communicated by EJ Carvajal Barriga <EJCARVAJAL@puce.edu.ec>.

Recent publication.

1 SA James, EJ Carvajal Barriga, CJ Bond, K Cross, NC Núñez, PB Portero & IN Roberts 2009 *Candida carvajalis* sp. nov., an ascomycetous yeast species from the Ecuadorian Amazon jungle. FEMS Yeast Res (Published online).

In the course of a yeast biodiversity survey of different ecological habitats found in Ecuador, two yeast strains (CLQCA 20-011T and CLQCA20-014) were isolated from samples of rotten wood and fallen leaf debris collected at separate sites in the central region of the Ecuadorian Amazonia. These strains were found to represent a novel yeast species based on the sequences of their D1/D2 domain of the large subunit (LSU) rRNA gene and their physiological characteristics. Phylogenetic analysis based on LSU D1/D2 sequences revealed this novel species to be most closely related to *Candida asparagi*, *Candida fructus*, *Candida musae* and two as yet undescribed *Candida* species, with the six yeast taxa collectively forming a distinct species group within the *Clavispora* clade. The species name of *Candida carvajalis* sp. nov. is proposed to accommodate these strains, with CLQCA 20-011T (NCYC 3509T, CBS 11361T) designated as the type strain.

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

The following articles from our department have recently appeared or are in press.

1 WJ Müller WJ, Albertyn J and Smit MS 2007 Cycloheximide resistance in the *Lipomycetaceae*. Can J Microbiol 53: 509-513.

2 Labuschagne, M and Albertyn, J 2007 Cloning of an epoxide hydrolase encoding gene from *Rhodotorula mucilaginosa* and functional expression in *Yarrowia lipolytica*. Yeast 24: 69-78.

3 Van Heerden, A, Van Wyk, PWJ, Botes, PJ, Pohl, CH, Strauss, CJ, Nigam, S and Kock JLF 2007. The release of elongated, sheathed ascospores from bottle shaped asci in *Dipodascus geniculatus*. FEMS Yeast Research 7: 173-179.

4 Leeuw NJ, Swart CW, Ncango DM, Pohl CH, Sebolai OM, Strauss CJ, Botes PJ, Van Wyk PWJ, Nigam S and Kock JLF 2007 Acetylsalicylic acid as antifungal in *Eremothecium* and other yeasts. Antonie van Leeuwenhoek 91: 393-405.

5 Strauss CJ, Van Wyk PWJ, Lodolo EJ, Botes PJ, Pohl CH, Nigam S and Kock JLF 2007 Mitochondrial associated yeast flocculation – the effect of acetylsalicylic acid. J Inst Brew 113:42-47.

6 Kock JLF, Sebolai OM, Pohl CH, Van Wyk PWJ and Lodolo EJ 2007 Oxylipin studies expose aspirin as antifungal. FEMS Yeast Research 7:1207-1217.

7 Sebolai OM, Pohl CH, Botes PJ, Strauss CJ, Van Wyk PWJ, Botha A and Kock JLF 2007 3-Hydroxy fatty acids found in capsules of *Cryptococcus neoformans*. Canadian Journal of Microbiology 53: 809-812.

8 Sebolai OM, Pohl CH, Botes PJ, Van Wyk PWJ and Kock JLF 2008 The influence of acetylsalicylic acid on oxylipin migration in *Cryptococcus neoformans* var. *neoformans* UOFS Y-1378. Can J Microbiol 54: 91-96.

9 Sebolai OM, Pohl CH, Botes PJ, Van Wyk PWJ, Mizizi R, Swart CW and Kock JLF 2008 Distribution of 3-hydroxy oxylipins and acetyl salicylic acid sensitivity in *Cryptococcus* species. Can J Microbiol 54: 111-118.

10 Ncango DM, Swart CW, Goldblatt ME, Pohl CH, Van Wyk PWJ, Botes PJ and Kock JLF 2008 Oxylipin and mitochondrion probes to track yeast sexual cells. Can J Microbiol 54: 450-455.

11 Swart CW, Van Wyk PWJ, Pohl CH and Kock JLF 2008 Variation in yeast mitochondrial activity associated with asci. Can J Microbiol 54: 532-536.

12 Schäfer G, McEvoy CRE and Patterton H-G 2008 The *S. cerevisiae* linker histone Hho1p is essential for chromatin compaction in stationary phase, and is displaced by transcription. Proc Natl Acad Sci USA 105: 14838–14843.

The importance of core histones in the regulation of DNA function by chromatin is clear. However, little is known about the role of the linker histone. We investigated the role of H1 in *Saccharomyces cerevisiae* during extensive transcriptional reprogramming in stationary phase. Although the levels of linker histone Hho1p remained constant during growth to semi-quiescence, there was a genome-wide increase in binding to chromatin. Hho1p was essential for compaction of chromatin in stationary phase, but not for general transcriptional repression. A clear, genome-wide anti-correlation was seen between the level of bound Hho1p and gene expression. Surprisingly, the rank order of gene activity was maintained even in the absence of Hho1p. Based on these findings we suggest that linker histone Hho1p has a limited role in transcriptional regulation, and that the dynamically exchanging linker histone may be evicted from chromatin by transcriptional activity.

13 De Smidt O, du Preez JC & Albertyn J 2008 The alcohol dehydrogenases of *Saccharomyces cerevisiae*: a comprehensive review. FEMS Yeast Res 8: 967-978.

Alcohol dehydrogenases (ADHs) constitute a large family of enzymes responsible for the reversible oxidation of alcohols to aldehydes with the concomitant reduction of $NAD^+$ or $NADP^+$. These enzymes have been identified not only in yeasts, but also in several other eukaryotes and even prokaryotes. The ADHs of *Saccharomyces cerevisiae* have been studied intensively for over half a century. With the ever-evolving techniques available for scientific analysis and since the completion of the Yeast Genome Project, a vast amount of new information has been generated during the past 10 years. This review attempts to provide a brief summary of the wealth of knowledge gained from earlier studies as well as more recent work. Relevant aspects regarding the primary and secondary structure, kinetic characteristics, function and molecular regulation of the ADHs in *S. cerevisiae* are discussed in detail. A brief outlook also contemplates possible future research opportunities.

14 Ells R, Kock JLF, Van Wyk PWJ, Botes PJ and Pohl CH 2009 Arachidonic acid increases antifungal susceptibility of *Candida albicans* and *Candida dubliniensis*. J Antimicrob Chemother 63, 124-128.

Objectives: During *Candida albicans* infection, arachidonic acid (AA) is released from phospholipids of infected host cell membranes and used by *C. albicans* as the sole carbon source and for production of eicosanoids. AA can be incorporated into the phospholipids of yeasts, influencing the saturation level and fluidity of yeast cell membranes. It is suggested that the effectiveness of polyene (e.g. amphotericin B) and imidazole (e.g. clotrimazole) antifungals may depend upon the level of unsaturation and ergosterol in the membrane. Therefore, the aim of this study was to evaluate the effect of AA on the cell membrane and susceptibility of *C. albicans* and *Candida dubliniensis* biofilms towards amphotericin B and clotrimazole. Methods: Both yeasts were grown in the presence and absence of AA and the effect of amphotericin B and clotrimazole was examined by confocal laser scanning microscopy, determination of mitochondrial metabolism, unsaturation index of the phospholipid fractions and ergosterol content of the membranes. Results: AA had no effect on the viability of the cells in the biofilm; however, there was an increase in ergosterol levels as well as antifungal susceptibility of biofilms grown in the presence of AA. Conclusions: AA influences phospholipid unsaturation and ergosterol content of both yeasts *C. albicans* and *C. dublininensis*, increasing susceptibility towards the antifungals. Pretreatment of biofilms with polyunsaturated fatty acids may result in the reduction in antifungal dose needed to inhibit biofilms.

15 Albertyn J, Labuschagne M and Nel S 2009 Yeasts as eukaryotic expression systems. In: Diversity and Potential Biotechnological Applications of Yeasts. Eds: T Satyanarayana and G Kunze. ISBN: 978-1-4020-8291-7.

Yeast has been a favoured lower eukaryotic system for the expression and production of recombinant proteins for both basic research and practical applications, and the demand for foreign-gene expression systems is increasing rapidly. Despite the vast amount of information on the molecular biology and physiology of *Saccharomyces cerevisiae*, which has consequently been the first choice as host system for recombinant protein production in the past, several limitations have been identified in this expression system. These limitations have recently been relieved by the development of expression systems in other yeast species known as "non-conventional yeasts" or "non-*Saccharomyces*" yeasts. With the increasing interest in the biotechnological applications of these yeasts in applied and fundamental studies and processes, the term "non-conventional" yeast may well soon become redundant. As there is no universal expression system for heterologous protein production, it is necessary to recognize the merits and demerits of each system in order to make a right choice. This chapter will evaluate the competitive environment of non-conventional expression platforms represented by some of the best-known alternative yeasts systems including *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Hansenula polymorpha*, *Pichia pastoris* and more recently, *Arxula adeninivorans*.

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

Recent publications.

1 Golubev WI, Tomashevskaya MA 2009. Characterization of *Rhodotorula colostri* (Castelli) Lodder mycocin. Izvestiya RAN, ser. biol. 2 (in press).

A strain of *Rhodotocula colostri* that has fungicidal activity against related species of the genera *Rhodosporidium*, *Sporidiobolus*, and their anamorphs phylogenetically belonging to the order Sporidiobolales (Microbotryomycetes, Pucciniomycotina) was discovered. The agent secreted with molecular mass about 4ó5 kDa was active at pH values ranging from 3.5 to 5.5, thermolabile and protease-sensitive.

2 Kulakovskaya T, Shashkov A, Kulakovskaya E, Golubev W, Zinin A, Tsvetkov Y, Grachev A, Nifantiev N 2009 Extracellular cellobiose lipids from yeasts and their analogues: structures and fungicidal activities. J Oleo Sci 58:133-140.

Basidiomycetous yeasts *Cryptococcus humicola* and *Pseudozyma fusiformata* secrete cellobiose lipids into the culture broth. In the case of *Cr. humicola*, 16-(tetra-O-acetyl-β-cellobiosyloxy)-2-dihydrohexadecanoic acid was defined as major product and 16-(tetra-O-acetyl-β-cellobiosyloxy)-2, 15-dihydrohexadecanoic acid was defined as minor product, while *Ps. fusiformata* secreted mainly 16-[6-O-acetyl-2í-O-(3-hydroxyhexanoyl)-β-cellobiosyloxy)-2,15-dihydroxy-hexadecanoic acid. These compounds exhibit similar fungicidal activities against different yeasts including pathogenic *Cryptococcus* and *Candida* species. The cells of *Filobasidiella neoformans* causing systemic cryptococcosis completely died after 30-min incubation with 0.02 mg $mL^{-1}$ of cellobiose lipids. The same effect on ascomycetous yeasts, including pathogenic *Candida* species, is achieved at 0.1-0.3 mg $mL^{-1}$ of cellobiose lipids depending on the test culture used. Cellobiose lipid of *Ps. fusiformata* inhibits the growth of phytopathogenic fungi *Sclerotinia sclerotiorum* and *Phomopsis helianthi* more efficiently than cellobiose lipids from *Cr. humicola*. Fully O-deacylated analogue, namely 16-(β-cellobiosyloxy)-2-dihydroxyhexadecanoic acid, and totally synthetic compound, 16-(β-cellobiosyloxy)-hydroxyhexadecanoic acid, do not inhibit the growth of *F. neoformans* and *Saccharomyces cerevisiae*, while 16-(β-cellobiosyloxy)-2,15-dihydroxyhexadecanoic acid inhibits the growth of both test cultures but at higher concentrations than cellobiose lipids of *Cr. humicola* and *Ps. fusiformata*. The amide of 16-(β-cellobiosyloxy)-2,15-dihydroxyhexadecanoic acid possessed no fungicide activity. Thus, the structures of both the carbohydrate part and fatty acid aglycon moiety are important for the fungicidal activity of cellobiose lipids.

## V Department of Biology, Faculty of Medicine, Masaryk University, University Campus MU, Kamenice 5, A7, 62500 Brno, Czech Republic. Communicated by Marie Kopecká <mkopecka@med.muni.cz>.

The following are recent papers, a lecture, a research project supported by the Granting Agency of the Czech Republic and a new project for which support by Grant Agency of the Czech Republic is being sought.

Papers.

1 Kopecká, M, Gabriel M 2009 Microtubules and actin cytoskeleton of potentially pathogenic basidiomycetous yeast as targets for antifungals. *Chemotherapy* (S. Karger AG, Switzerland) - in press.

2 Kopecká M, Yamaguchi M 2009 Dissociation of the actin cytoskeleton-dependent pathway from the microtubule-dependent pathway in the cell cycle of yeast actin mutant: Electron and fluorescent microscopic study. Sent to FEMS Yeast Res.

3 Yamaguchi M, Kopecká M. Ultrastructural disorder of the secretory pathway in temperature-sensitive actin mutants of *Saccharomyces cerevisiae.* Sent to FEMS Microbiol Lett.

Lecture.

4 Kopecká M 2009 Yeast pathogens – the cytoskeleton and the cell wall as targets for antifungal agents. Lecture at the Scientific Board of the Faculty of Medicine, Masaryk University, Brno, Komenského nám. 2, Czech Republic, as part of the process for gaining the full professorship. June 18th, 2009.

Recently finished project supported by Granting Agency of the Czech Republic.

Human pathogenic fungi: the cytoskeleton as a potential target of inhibition of morphogenesis. Period 2006-2008, investigators: Marie Kopecká, Miroslav Gabriel, Augustin Svoboda.

Application for a new grant to the Granting Agency of the Czech Republic.

Cytoskeletal inhibitors as antifungal agents for human yeast pathogens. Investigators: Marie Kopecká and Augustin Svoboda (period 2010-2012).

## VI Institute of Fermentation Technology and Microbiology, Technical University of Lodz, 90-924 Lodz, Wolczanska 171/173, Poland. Communicated by Dorota Kregiel <dkregiel@p.lodz.pl>.

Recent publications.

1 Kregiel D, Berlowska J, Ambroziak W 2008 The succinate dehydrogenase activity assay *in situ* with blue tetrazolium salt in Crabtree-positive *Saccharomyces cerevisiae* strain. Food Technol. Biotechnol. 46(4):376–381.

The spectrophotometric method of SDH activity assay in azide-sensitive yeast *Saccharomyces cerevisiae* was developed. The permeabilization of yeast cells by 0.05% digitonin permited to study yeast enzymatic activity *in situ*. The reduction of blue tetrazolium salt (BT) to blue formazans (BTf) was conducted in the presence of phenazine methosulfate (PMS) as exogenous electron carrier - and sodium azide (SA) as inhibitor of cytochrome oxidase (Cyt) pathway. Various factors such as type of substrate, BT concentration, cell number, temperature and time of incubation and different Cyt pathway blockers were optimized. In earlier studies DMSO was selected as the best solvent for extraction of BTf from yeast cells. The linear correlation between permeabilized yeast cell density and amount of formed formazan was evidenced in the range from $9 \cdot 10^7$ to $5 \cdot 10^8$ cells per sample solution. Below the yeast cell concentration of $1 \cdot 10^7$ the absorbance values were to low to detect formazans with good precision. This standarized procedure allows the estimation of SDH activity in whole cells, depending on vitality level of yeast populations. Significant increases of succinate dehydrogenase activities were observed in sequential passages as the result of increase activity of strain and adaptation to cultivation conditions.

2 Berlowska J, Kregiel D, Ambroziak W 2009 The pyruvate decarboxylase activity assay in situ of different industrial yeast strains. Food Technol. Biotechnol. 47(1):96-100.

Cytoplasmic pyruvate decarboxylase EC 4.1.1.1 (PDC), is one of the key enzymes of yeast fermentative metabolism. PDC is the first enzyme which, under anaerobic conditions, leads to decarboxylation of pyruvate with acetaldehyde as the end product. The aim of this study was to develop a suitable method for PDC activity assay *in situ* for different industrial yeast strains. Yeast strains *Saccharomyces* sp. and *Debaryomyces* sp. grew under aerated conditions on an orbital shaker in nutrient medium

wort broth with 1 % or in fermentative medium with 12 % of maltose. Enzymatic assay was conducted in cell suspension treated with digitonin, as permabilisation agent, and with sodium pyruvate, as a substrate, at temperature of 30 °C. Metabolites of PDC pathway were detected using GC technique. Various parameters: type and concentration of substrate, minimal effective concentration of digitonin, cell density, reaction time and effect of pyrazole, as alcohol dehydrogenase inhibitor, were monitored to optimize PDC enzymatic assay *in situ*. In the concentration range of yeast cells from $1\times10^7$ to $1\times10^8$/mL the linear correlation between produced acetaldehyde and cell density was noticed. Only pyruvate was the specific substrate for pyruvate decarboxylase. In the presence of 0.05 M sodium pyruvate and 0.05 % digitonin, the enzymatic reaction was linear up to 20 minutes of assay. During incubation time there was no formation of ethanol and therefore pyrazole was not necessary for the assay.

## VII Laboratorio de Microbiología Aplicada y Biotecnología (Applied Microbiology and Biotechnology Lab.), Instituto de Investigaciones en Biodiversidad y Medio Ambiente (CONICET-UNComahue), Quintral 1250, (8400), Bariloche, Argentina. Communicated by Diego Libkind <libkind@crub.uncoma.edu.ar>.

Recent publications.


1 Libkind D, Arts M, van Broock M 2008 Fatty acid composition of cold-adapted carotenogenic basidiomycetous yeasts. Rev Argentina de Microbiología. 40:193-197.

2 Libkind D, Gadanho, M, van Broock M, Sampaio JP 2009 *Cystofilobasidium lacus-mascardii* sp. nov., a new basidiomycetous yeast species isolated from aquatic environments of the Patagonian Andes and *Cystofilobasidium macerans* sp. nov., the sexual stage of *Cryptococcus macerans*. Int J Syst Evol Microbiol 59:622-630.


Papers in press.


3 Moliné M, Libkind D, Diéguez MC, van Broock M - Photo-protective role of carotenoid pigments in yeasts: experimental study contrasting naturally occurring pigmented and albino strains. J Photochem Photobiol B: Biol.

4 Russo G, Libkind D, Ulloa RJ, de García V, Sampaio JP, van Broock MR - *Cryptococcus agrionensis* sp. nov., a basidiomycetous yeast of the acidic rock drainage ecoclade, isolated acidic aquatic environment of volcanic origin (River Agrio, Argentina). Int J Syst Evol Microbiol.

5 de Garcia V, Brizzio S, Russo G, Rosa CA, Boekhout T, Theelen B, Libkind D & van Broock M - *Cryptococcus spencermartinsiae* sp. nov., a basidiomycetous yeast isolated from glacial waters and apple fruits. Int J Syst Evol Microbiol.


## VIII Laboratório de Microbiologia, Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomia, 1349-017 Lisboa, Portugal. Communicated by M. Malfeito-Ferreira <mmalfeito@isa.utl.pt>.

Recent articles.


1 Barata A, Pagliara D, Piccininno T, Tarantino F, Ciardulli W, Malfeito-Ferreira M and Loureiro V 2008 The effect of sugar concentration and temperature on growth and volatile phenol production by *Dekkera bruxellensis* in wine. FEMS Yeast Research, 8, 1097-1102.


The wine spoilage yeast *Dekkera bruxellensis* was evaluated for the production of 4-ethylphenol under low concentrations (0.02 g/l to 20 g/l) of glucose and fructose in synthetic media. Measurable amounts of 4-ethylphenol were produced over 0.2 g/l of each sugar. The yeast growth rate and amount of biomass formed increased from 0.2 to 20 g/l of glucose or fructose, being accompanied by increasing production of 4-ethylphenol. In red wines, the production of 4-ethylphenol was only observed in the presence of growing populations of indigenous or inoculated strains of *D. bruxellensis*. The production rate of 4-ethylphenol varied between 22 and 93 $\mu$g/day either with inoculated strains or wild populations in bottled wines. The production rate of 4-ethylphenol as a function of the increase in the number of cells varied from 349 $\mu$g/l to 1882 $\mu$g/l per one log CFU/ml. The effect of temperature on cellular viability and 4-ethylphenol production was tested in red wines with indigenous or inoculated strains of *D. bruxellensis*. Incubation temperatures of 15, 20 and 25ºC permitted cellular growth and volatile phenol production. Increasing incubation temperatures to 36ºC induced full viability loss of 10 strains of *D. bruxellensis* within less than 12 hours.

2 Barata A, González S, Malfeito-Ferreira M, Querol A, and Loureiro V 2008 Sour rot-damaged grapes are sources of wine spoilage yeasts. FEMS Yeast Research, 8, 1008-1017.

The yeast species of sound and sour rot damaged grapes were analysed during fermentation and along grape ripening in the vineyard, using selective culture media. During 2003 and 2004 vintages, microvinifications were carried out using sound grapes to which were added different amounts of sour rotten grapes. The wine spoilage species *Zygosaccharomyces bailii* was only recovered during sour rotten fermentations, reaching 5.00 log CFU $ml^{-1}$ (2003) and 2.48 log CFU $ml^{-1}$ (2004) at the end of fermentation. The study of yeast populations along sour rot ripening process (2005 vintage) showed, since the veraison, that damaged grapes exhibited always higher total yeast counts and a much higher diversity of species. From a total of 22 ascomycetous species, 17 were only present in damaged grapes. The most frequent species were *Issatchenkia occidentalis* and *Zygoascus hellenicus*. The spoilage species *Z. bailii* and *Z. bisporus* were consistently isolated only from damaged grapes. This work demonstrates that one of the most dangerous wine spoilage species, *Z. bailii*, is strongly associated with sour rot grapes and survives during fermentation with *S. cerevisiae.* The use of selective media provided a more accurate characterisation of the grape contamination species.

## IX 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 J. Schnürer (Uppsala) for the invitation to visit his laboratory in February 2009.

The following are papers for 2009 or in press.

1 Naumov GI, Naumova ES 2009 Chromosomal differentiation of the sibling species *Pichia membranifaciens* and *Pichia manshurica.* Microbiology (Moscow), 78:214-217.

2 Naumov GI, Ivannikova YuV, Chernov IYu, Naumova ES 2009 Natural polymorphysm of the plasmid double-stranded RNA of the *Saccharomyces* yeasts. Microbiology (Moscow), 78 (2): 208–213.

3 Naumov GI 2009 New wine yeasts. On publication of the book Modern Preparative Forms of Yeasts for Wine-Making by N.N. Martynenko, Moscow: Rossel'khozizdat, 2006. Microbiology (Moscow) (in press).

4 Naumov GI, Naumova ES 2009 Polygenic control for fermentation of beta-fructosides in the yeast *Saccharomyces cerevisiae*: new genes *SUC9* and *SUC10*. Microbiology (Moscow) (submitted).

Using molecular karyotyping and genetic hybridization analysis we identified two new polymeric β-fructosidase genes *SUC9* and *SUC10* in the yeast *Saccharomyces cerevisiae*, which are located on chromosome XIV and on chromosome XVI/XIII doublet, respectively. The genes are responsible for fermentation of sucrose and raffinose. The genotypes on *SUC* genes of strains VKM Y-1831 and DBVPG 1340 are *SUC2 SUC9* and $suc2^0$ *SUC10*, respectively. The $suc2^0$ is a silent sequence. Scientific and applied significance of *SUC* genes is discussed.

5 Naumova ES, Serpova EV, Naumov GI. 2009. Genome variability of the yeast *Yarrowia lipolytica*. Microbiology (Moscow) (submitted).

Using sequence analysis of internal transcribed spacers ITS1 and ITS2, RAPD-PCR and pulsed-field gel electrophoresis of intact chromosomal DNAs, we investigated molecular and genetic peculiarities of genomes of 40 *Yarrowia lipolytica* strains. All strains showed nearly identical ITS-sequences. On the other hand, karyotypic analysis revealed significant differences in chromosomal patterns of *Y. lipolytica*. Number, sizes and order of individual chromosomes vary from strain to strain. Chromosome-length polymorphism of the *Y. lipolytica* strains was pronounced and independent of their geographic origin and source of isolation. Intra-species polymorphism of *Y. lipolytica* chromosomes is discussed.

6 Naumov GI, Kondratieva VI, Naumova ES. 2009. Taxonomic genetics of *Zygowilliopsis* yeasts. Russian Journal of Genetics (in press).

We have conducted genetic hybridization analysis of 16 natural *Zygowilliopsis* strains isolated in different geographical regions and maintained in different collections under species names of *Z. californica, Hansenula dimennae* and *Pichia populi*. Genetic relatedness was determined on the basis of mating, viability of hybrid progeny and meiotic recombination of markers. Four new biological species are recognized in the former monotypic genus *Zygowilliopsis*. Species *Z. californica* and *Zygowilliopsis* sp. 3 are probably include divergent geographical populations. It is necessary to reconsider the species composition of the genus *Zygowilliopsis* and genus belonging of the yeast *P. populi.* Genetic and molecular identifications of the *Zygowilliopsis* species are in perfect agreement.

7 Naumov GI, Naumova ES 2009 Comparative genetics of yeasts. A new β-fructosidase gene *SUC8* in *Saccharomyces cerevisiae.* Russian J Genetics (in press).

Molecular and genetic analyses show that distillers race XII, which is an ancestor for Pitergof and Gatchina genetic lines of *Saccharomyces cerevisiae,* has three polymeric â-fructosidase genes: *SUC2*, *SUC5* and *SUC8*. The latter gene is located on chromosome X and identified by us for the first time. In connection with literature data we discussed the presence of single *SUC2* gene in the yeast used for the international project on complete genome sequencing of *S. cerevisiae.*

**X Collection de Levures d'Intérêt Biotechnologique (CLIB), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H-V <nguyenhv@grignon.inra.fr>.**

## "Blind men and an elephant" cases in research on Saccharomyces paradoxus/bayanus/uvarum/*pastorianus*.

Readers can find the story that Asian young school children learn as a reading exercise at the following website: **http://en.wikipedia.org/wiki/Blind_Men_and_an_Elephant**. In the current era of the internet, when consulting the literature is a matter of a few mouse clicks, some facts apparently remain unknown, intentionally or not. Here are some examples in the taxonomy of *Saccharomyces*.

(a) *S. cariocanus* differs from a population of *S. paradoxus* by four translocations. Thus, *S. cariocanus* is not a distinct species but a strain of *S. paradoxus*:

Liti G, Barton DB, Louis EJ 2006 Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. Genetics 174:839-850.

(b) *S. uvarum* is not a synonym of *S. bayanus*. It was reinstated as real species (or pure line), and thus the name *S. uvarum* is valid (not a "so-called"). Furthermore *S. bayanus* CBS 380T has been shown to be a hybrid (or mixed line):

Nguyen H-V, Lépingle A, Gaillardin C 2000 Molecular typing demonstrates homogeneity of *S. uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae*, including *S. bayanus* type strain CBS380. System Appl Microbiol 23:71-85.

Pulvirenti A, Nguyen H-V, Caggia C, Giudici P, Rainieri S, Zambonelli C 2000 *S. uvarum*, a proper species within *Saccharomyces sensu stricto*. FEMS Microbiol Lett 192:191-196.

Nguyen H-V, Gaillardin C 2004 Evolutionary relationships between the former species *Saccharomyces uvarum* and the hybrids *Saccharomyces bayanus* and *Saccharomyces pastorianus*; reinstatement of *Saccharomyces uvarum* (Beijerinck) as a distinct species. FEMS Yeast Research 5:471-483.

In the above article we also demonstrated that the genome of *S. pastorianus* (lager yeast) contains sequences derived from *S. uvarum*: *HO*, *MET2*, *BAP2*, *GDH*, etc. Many recent papers dealing with the genomes of lager yeasts refer to the genome of *S. bayanus* but this genome was sequenced from strain MCYC623 (=CBS 7001) which we have shown belongs to *S. uvarum*.

Recent publications.

1 Nguyen HV 2008 Use of intergenic ipacer (IGS) rDNA amplification and fingerprinting for rapid identification and assignment of yeast strains. Abstract in 12th ICY, Kiev, Ukraine August 11-15, 2008 (partial)

Intergenic Spacer (rDNA) amplification and *Alu*I fingerprinting (IGSAF) revealed: (1) Mis-classification in the taxa: *Candida ernobi*/*C. populi*, *C. oleophila*/*C. shehatae* and *P. guilliermondi*/*C. blankii*. IGSAF was applied to check homogeneity of strains assigned in several taxa. The results showed misclassification in many cases: *C. populi* were found in *C. ernobi*, *C. shehatae*, *P. segobiensis* in *C. oleophila*, *C. blankii* in *P. guilliermondii*. (2) Presence of subgroups in *Arxula adeninivorans*. Generally in each taxon all the strains share with the type strain the same typical IGS pattern, but subgroups could be also distinguished as in *Arxula adeninivorans*. (3) Two distinct species: *C. zeylanoides* and *C. iberica*/*C. krissii*. *Candida zeylanoides* (Castellani) Langeron&Guerra, 1938 and *Candida iberica* (Ramirez&Gonzalez), 1972 were synonymized by S.A. Meyer, D.G. Ahearn and D.Yarrow in 1984 based on growth tests. While *C. krissii* (Goto, Yamasato & Iizuka) 1974 was considered as synonym with *C. zeylanoides* based on the D1D2 sequence comparison, IGSAF showed that *C. zeylanoides* CBS 619T and *C. iberica* CBS 6391$^T$ exhibited two totally different *Alu*I fingerprints while *C. krissii* CBS 6519$^T$ exhibited the same fingernprint as *C. iberica* CBS 6391$^T$. IGS sequence comparisons confirmed that *C. zeylanoides* is genetically distant from *C. iberica* and *C. krissii*.

2 Nguyen HV, Gaillardin C, Neuvéglise C 2009 Differentiation of *Debaryomyces hansenii* and *Candida famata* by rRNA gene intergenic spacer fingerprinting and reassessment of phylogenetic relationships among *D. hansenii*, *C. famata*, *D. fabryi*, *C. flareri* (= *D. subglobosus*) and *D. prosopidis*: description of *D. vietnamensis* sp. nov. closely related to *D. nepalensis*. FEMS Yeast Res 9:641-662.

The intergenic spacer rDNA amplification and *Alu*I fingerprinting (IGSAF) method detected four distinct groups among 170 *Debaryomyces hansenii* strains: *D. hansenii* var. *hansenii*; *Candida famata* var. *famata*; *D. hansenii* var. *fabryi* and *C. famata* var. *flareri*. IGS sequence comparison of representative strains showed that *D. hansenii* var. *hansenii* and *C. famata* var. *famata* belonged to one species, whereas *D. hansenii* var. *fabryi* and *C. famata* var. *flareri* belonged to two different ones. This confirmed the following three species recently reinstated: *D. hansenii* (=*C. famata*), *Debaryomyces fabryi* and *Debaryomyces subglobosus* (=*Candida flareri*). Accordingly, growth at 37°C may no longer be used to differentiate *D. hansenii* from *D. fabryi*. Riboflavin production is more specific for *D. fabryi* and *D. subglobosus* strains. IGSAF identified all the other 17 species of the genus Debaryomyces, six of them sharing with *D. hansenii* an rRNA gene unit harbouring two 5S rRNA genes. The phylogenetic tree established with IGS sequences was congruent with the one based on ACT1, GPD1 and COX2 sequences depicting a distinct *D. hansenii* clade close to the *D. subglobosus*, *Debaryomyces prosopidis* and *D. fabryi* clade. Description of *Debaryomyces vietnamensis* sp. nov. (type strain CBS 10535$^T$, MUCL 51648$^T$), closely related to *Debaryomyces nepalensis* is given.

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

The following articles have been published since our last report:

1 Péter, G, Tornai-Lehoczki J and Dlauchy D 2008 *Ogataea nitratoaversa* sp. nov., a methylotrophic yeast species from temperate forest habitats. Antonie van Leeuwenhoek. 94:217–222.

Three methanol-assimilating, nitrate-negative yeast strains representing a hitherto undescribed species, were isolated from leaf and rotten wood samples collected in temperate forests in Hungary. Analysis of the D1/D2 large subunit ribosomal RNA gene sequences placed the strains in the *Ogataea* clade. The three strains share identical D1/D2 and ITS sequences and significantly differ from the genetically most closely related species, *Pichia pilisensis*. Five substitutions in D1/D2 and more than 10% difference in the ITS regions were detected. A novel yeast species, *Ogataea nitratoaversa*, is proposed to accommodate these isolates. The type culture is NCAIM Y.01837$^T$ (CBS 10796, NRRL Y-48449). As the current description of the genus does not allow the inclusion of nitrate negative species, the emendation of the diagnosis of the genus *Ogataea* Yamada, Maeda and Mikata is proposed.

2 Péter G, Tornai-Lehoczki J and Dlauchy D 2009 *Candida ogatae* sp. nov., an anamorphic member of the Kuraishia clade. FEMS Yeast Res 9:328-333.

Three methanol-assimilating yeast strains representing a hitherto undescribed species were isolated from rotten wood and freshwater samples collected in Hungary. Analysis of the D1/D2 large subunit rRNA gene sequences placed the strains in the *Kuraishia* clade; however, no ascospore formation was observed. These strains differ from *Candida hungarica*, the genetically most closely related recognized species, by four and five substitutions in D1/D2 and by >1% and 4% differences in the internal transcribed spacer and in the mitochondrial small subunit rRNA gene regions, respectively. Some phenotypic differences were also observed. *Candida ogatae*, a novel yeast species, is proposed to accommodate these isolates. The type culture is NCAIM Y.01845$^T$ (CBS 10924, NRRL Y-48474).

3 Péter G, Tornai-Lehoczki J and Dlauchy D 2009 *Trichomonascus apis* sp. nov., a heterothallic yeast species from honeycomb. International Journal of Systematic and Evolutionary Microbiology. 59: 1371–1375.

Four strains of a novel heterothallic yeast species were isolated from pollen-storing cells of a honeycomb of honeybee (*Apis mellifera*) in Hungary. Analysis of the D1/D2 domain of the large-subunit (26S) rRNA gene sequences placed the strains in the *Trichomonascus* clade. The four strains share identical D1/D2 sequences and differ by 24 substitutions and nine indels from the genetically most closely related species, *Blastobotrys attinorum*. The name *Trichomonascus apis* sp. nov. is proposed for the novel species. The carbon-source assimilation spectrum of *T. apis* sp. nov. is rather broad. Unlike *B. attinorum*, it assimilates sucrose, trehalose, D-glucuronate and succinate and does not grow at 37 °C, thus enabling the two taxa to be distinguished. The type and isotype strains of *Trichomonascus apis* are NCAIM Y.01848$^T$ (=CBS 10922$^T$ =NRRL Y-48475$^T$) and NCAIM Y.01849$^{IT}$ (=CBS 10923$^{IT}$ =NRRL Y-48476$^{IT}$), respectively.

## XII Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Vienna. Communicated by Hansjörg Prillinger <hansjoerg.prillinger@boku.ac.at>.

The following is a recent publication.

1 Prillinger H, Wuczkowski M, Lopandic K, Bauer R, Molnár O, Sterflinger K 2009 *Schizonella caricis-atratae* (Ustilaginomycetes) a new cryptic species on *Carex atrata* from Austria. Mycological Progress 8:157-164.

Based on DNA-fingerprinting, ITS-DNA sequencing and physiological characteristics an new species of *Schizonella*, *Sch. caricis-atratae* is described. *Schizonella caricis-atratae* is a so called cryptic species, *i.e.* morphologically it is identical with *Sch. melanogramma*, of which differs in physiological and molecular phylogenetic characters. *Schizonella caricis-atratae* can be distinguished by the following tests: growth on myo-inositol, D,L-lactate, salicine, maltose, amethyl-D-glucoside, butane-2,3-diol, D-turanose, at 30°C; no growth on galactose. *Sch. caricis-atratae* can be isolated from *Carex atrata*. The type strain is HB 3, CBS 123477.

## XIII Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, 31270-901, Brazil. Communicated by C.A. Rosa <carlrosa@icb.ufmg.br>.

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

1 Rosa CA, Jindamorakot S, Limtong S, Nakase T, Pagnocca FC, Lachance MA 2009 *Candida golubevii* sp. nov, an asexual yeast related to *Metschnikowia lunata*. Int J Syst Evol Microbiol (in press).

Two strains of a new yeast species were isolated from insect frass and a flower in Thailand and Brazil, respectively. The strain from Thailand was isolated from insect frass collected in Than-Tong waterfall, Nong Khai Province, whereas the strain from Brazil was recovered from a flower of *Ipomoea* sp. collected on the banks of the Paraguai River in state of Mato Grosso do Sul. The sequences of the D1/D2 domains of the large subunit of the rRNA of both strains were identical. This new species belongs to the *Metschnikowia* clade and is related to *M. lunata*. No signs of sporulation were observed for the two strains on various culture media. The novel species, *Candida golubevii*, is proposed to accommodate these isolates. The type strain of *C. golubevii* sp. nov. is BCC $8332^T$ (=CBS$11362^T$ =NBRC $105679^T$).

2 Barbosa AC, Cadete RM, Gomes FCO, Lachance MA, Rosa CA 2009 *Candida materiae* sp. nov., a yeast species isolated from rotting wood in the Atlantic Rain Forest. Int J Syst Evol Microbiol (in press).

Three strains of new yeast species *Candida materiae* were isolated from rotting wood in an Atlantic Rain Forest site in Brazil. The analysis of the sequences of the D1/D2 domains of the large-subunit rDNA showed that this species belongs to the *Spathaspora* clade and is related to *C. jeffriessi* and *S. passalidarum*. Unlike *C. jeffriessi* and *S. passalidarum*, *C. materiae* does not ferment xylose. The type strain of *C. materiae* is UFMG-07-C15.$1B^T$ (=CBS $10975^T$ = CBMAI $956^T$).

3 Silva CLC, Vianna CR, Cadete RM, Santos RO, Gomes FCO, Oliveira ES, Rosa CA 2009 Selection, growth, and chemo-sensory evaluation of flocculent starter culture strains of *Saccharomyces cerevisiae* in the large-scale production of traditional Brazilian cachaça. Int J Food Microbiol 131:203-210.

The physiological and kinetic capabilities of 233 *Saccharomyces cerevisiae* isolates, originating from traditional Brazilian cachaça fermentation, were evaluated under laboratory conditions to select flocculent and non-$H_2S$ producing strains to be employed in beverage production. Three flocculent *S. cerevisiae* strains were selected, two non-$H_2S$ producing and one $H_2S$ producing, and their kinetic performances were analysed during two large-scale fermentation experiments in a traditional cachaça distillery. One non-flocculent $H_2S$-producing *S. cerevisiae* strain was also used for comparison with the flocculent strains. The results of mitochondrial DNA restriction analysis showed that the three flocculent starter *S. cerevisiae* strains, as well as the non-flocculent strain, remained in the process during the whole fermentation period, with cells numbering around $10^7$ cfu/ml. All selected strains produced ethanol yields that were typically higher in the distillery than in the laboratory conditions, except for strain UFMGA-1240. The greatest diversity of non-*Saccharomyces* yeasts was observed prior to day 21 of cachaça fermentation; *Pichia membranifaciens* and *Hanseniaspora guilliermondii* were the most frequently isolated species. These yeasts were present in lower densities throughout the whole process. The cachaça produced by the selected strains contained concentrations of chemical compounds in accordance with current Brazilian legislation, and all cachaças scored well in sensory effective tests. In addition to the advantage of being flocculent, the strain UFMGA-1031 is non-$H_2S$ producing and also produces cachaça with good sensory acceptance. Therefore, this flocculent and non-$H_2S$ producing *S. cerevisiae* strain is highly suitable as a starter for production of high quality traditional cachaça.

4 Rosa CA, Morais PB, Lachance MA, Santos RO, Melo WG, Viana RH, Bragança MA, Pimenta RS 2009 *Wickerhamomyces queroliae* sp. nov. and *Candida jalapaonensis* sp. nov., two yeast species isolated from Cerrado ecosystem in North Brazil. Int J Syst Evol Microbiol 59:1232-1236.

5 Tiago FCP, Martins FS, Rosa CA, Nardi RM, Machado DCC, Nicoli JR 2009 Physiological characterization of non-*Saccharomyces* yeasts from agro-industrial and environmental origins with possible probiotic function. World J Microbiol Biotechnol 25:657-666.

6 Rosa CA, Jindamorakot S, Limtong S, Nakase T, Lachance MA, Fidalgo-Jiménez A, Daniel HM, Pagnocca FC, Inacio J, Morais PB 2009 Synonymy of the yeast genera *Moniliella* and *Trichosporonoides* and proposal of *Moniliella fonsecae* sp. nov. and five new species combinations. Int J Syst Evol Microbiol 59:425-429.

7 Marini MM, Gomes FCO, Silva CLC, Cadete RM, Badotti F, Oliveira ES, Cardoso CR, Rosa CA 2009 The use of selected starter *Saccharomyces cerevisiae* strains to produce traditional and industrial cachaça: a comparative study. World J Microbiol Biotechnol 25:235-242.

8 Pimenta RS, Silva FL, Silva JFM, Morais PB, Braga DT, Rosa CA, Correa Jr A 2008 Biological control of *Penicillium italicum, P. digitatum* and *P. expansum* by the predacious yeast *Saccharomycopsis schoenii* on oranges. Braz J Microbiol 39:85-90.

9 Medeiros AO, Kohler LM, Hamdan JS, Missagia BS, Barbosa FAR, Rosa CA 2008 Diversity and antifungal susceptibility of yeasts from tropical freshwater environments in Southeastern Brazil. Water Res 42:3921-3929.

10 Martins FS, Miranda IC, Nicoli JR, Rosa CA, Neves MJ 2008 Effect of trehalose levels on the screening of yeast as probiotic by in vivo and in vitro assays. Braz J Microbiol 39:50-55.

11 Gabler IG, Barbosa AC, Vilela R, Lyon S, Rosa CA 2008 Incidence and anatomic localization of oral candidiasis in patients with AIDS hospitalized in a public hospital in Belo Horizonte, Minas Gerais. J Appl Oral Sci 16:247-250.

## XIV CREM – Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Communicated by Á. Fonseca <amrf@fct.unl.pt> and <J.P. Sampaio jss@fct.unl.pt>.

The following papers are in press or were recently published:

1 K Findley, M Rodriguez-Carres, B Metin, J Kroiss, Á Fonseca, R Vilgalys and J Heitman. 2009. Phylogeny and phenotypic characterization of pathogenic *Cryptococcus* species and closely related saprobic taxa in the Tremellales. Eukaryotic Cell 8: 353-361.

The basidiomycetous yeasts *Cryptococcus neoformans* and *Cryptococcus gattii* are closely related sibling species that cause respiratory and neurological disease in humans and animals. Within these two recognized species, phylogenetic analysis reveals at least six cryptic species defined as molecular types (VNI/II/B, VNIV, VGI, VGII, VGIII, and VGIV) that comprise the pathogenic *Cryptococcus* species complex. These pathogenic species are clustered in the *Filobasidiella* clade within the order Tremellales. Previous studies have shown that the *Filobasidiella* clade also includes several saprobic fungi isolated from insect frass, but information evaluating the relatedness of the saprobes and pathogens within this cluster is limited. Here, the phylogeny encompassing a subset of species in the Tremellales lineage that clusters closely with the pathogenic *Cryptococcus* species complex was resolved by employing a multilocus sequencing approach for phylogenetic analysis. Six highly conserved genomic loci from 15 related basidiomycete species were sequenced, and the alignments from the concatenated gene sequences were evaluated with different tree-building criteria. Furthermore, these 15 species were subjected to virulence and phenotype assays to evaluate their pathogenic potential. These studies revealed that *Cryptococcus amylolentus* and *Tsuchiyaea wingfieldii*, two nonpathogenic sibling species, are the taxa most closely related to the pathogens *C. neoformans* and *C. gattii* and together with *Filobasidiella depauperata* form a *Cryptococcus* sensu stricto group. Five other saprobic yeast species form the *Kwoniella* clade, which appears to be a part of a more distantly related sensu lato group. This study establishes

a foundation for future comparative genomic approaches that will provide insight into the structure, function, and evolution of the mating type locus, the transitions in modes of sexual reproduction, and the emergence of human pathogenic species from related or ancestral saprobic species.

2 MF Landell, J Inácio, Á Fonseca, MH Vainstein and P Valente. 2009. *Cryptococcus bromeliarum* sp. nov., an orange-coloured basidiomycetous yeast isolated from bromeliads in Brazil. Int J Syst Evol Microbiol 59: 910-913.

During a survey of yeasts associated with the phylloplane of several bromeliad species in Itapuã Park in southern Brazil, we isolated four orange-coloured strains which were found to represent a novel anamorphic tremellaceous (Tremellales, Agaricomycotina, Basidiomycota) yeast species, *Cryptococcus bromeliarum* sp. nov. (type strain BI20$^T$ = CBS 10424$^T$ = NRRL Y-48112$^T$). PCR-fingerprinting profiles of the four strains with primers M13 and $(GTG)_5$ were almost identical, which suggested conspecificity among the isolates. On the basis of D1/D2 26S rDNA sequence analysis, *C. bromeliarum* is phylogenetically closely related to other orange-coloured *Cryptococcus* species, namely *Cryptococcus armeniacus*, *C. amylolyticus*, *C. tibetensis* and *C. cistialbidi*, but differed from these species by at least six nucleotide substitutions and was thus considered a separate species. Physiological differences from *C. armeniacus*, *C. amylolyticus* and *C. cistialbidi* included the inability of *C. bromeliarum* to assimilate citrate and to form starch-like compounds. Differentiation from *C. tibetensis* can be achieved by the ability of the latter to assimilate ethylamine.

3 Á Fonseca, T Boekhout and JW Fell. 2008. Validation of the basidiomycetous yeast species *Cryptococcus flavus* and *C. liquefaciens*. Mycotaxon 106: 503–504.

Novel combinations are proposed to validate the binomials of two currently accepted species belonging to the basidiomycetous yeast genus *Cryptococcus*.

4 Libkind D, Gadanho M, van Broock M and Sampaio JP. 2009. *Cystofilobasidium lacus-mascardii* sp. nov., a new basidiomycetous yeast species isolated from aquatic environments of the Patagonian Andes and *Cystofilobasidium macerans* sp. nov., the sexual stage of *Cryptococcus macerans.* Int J Syst Evol Microbiol 59:622-630.

5 Gadanho M and Sampaio JP 2009. *Cryptococcus ibericus* sp. nov., *Cryptococcus aciditolerans* sp. nov. and *Cryptococcus metallitolerans* sp. nov., a new ecoclade of anamorphic basidiomycetous yeast species from an extreme environment associated with acid rock drainage in São Domingos pyrite mine, Portugal. Int J Syst Evol Microbiol (in press).

6 Leandro MJ, Fonseca C and Gonçalves P. 2009. Hexose and pentose transport in ascomycetous yeasts: an overview. FEMS Yeast Research 9:511-525.

The biochemical characterization of sugar uptake in yeasts started five decades ago and led to the early production of abundant kinetic and mechanistic data. However, the first accurate overview of the underlying sugar transporter genes was obtained relatively late, due mainly to the genetic complexity of hexose uptake in the model yeast *Saccharomyces cerevisiae*. The genomic era generated in turn a massive amount of information, allowing the identification of a multitude of putative sugar transporter and sensor-encoding genes in yeast genomes, many of which are phylogenetically related. This review aims to briefly summarize our current knowledge on the biochemical and molecular features of the transporters of hexoses and pentoses in yeasts, when possible establishing links between previous kinetic studies and genomic data currently available. Emphasis is given to recent developments concerning the identification of D-xylose and L-arabinose transporter genes, which are thought to be key players in the optimization of *S. cerevisiae* strains for bioethanol production from lignocellulose hydrolysates.

## XV Canadian Institute of Fisheries Technology, Dalhousie University, P.O. Box 1000; Halifax, Nova Scotia, Canada B3J 2X4. Communicated by Alex Speers <aspeers@dal.ca>.

I am pleased to announce the creation of a website detailing opportunities in brewing and distilling science training, sponsored by the Institute of Brewing and Distilling (UK). See **http://myweb.dal.ca/aspeers/**.

Abstract submitted to the the MBAA Convention in La Quinta Oct 1-4, 2009.

1 Speers A, Reid A-J, Kruger L 2009 Use of a miniaturized fermentation assay to assess the fermentation characteristics of six yeast strains.

Yeast strain selection is a time intensive and resource expensive process. Strain selection depends on various factors including flavour production, flocculation behaviour, attenuation limit and fermentation speed. However it is unpractical to test hundreds of potential yeast strains for these characteristics at production scale. The aim of this research is to test if the physical differences in fermentation characteristics affected by yeast strain can be identified and compared using a miniaturized fermentation assay. Industrial wort was fermented with six different yeast strains (A, SMA, and Labatt Culture Collection (LCC) 125, 1208, 1209, and 1240) in small-scale test tube fermentation vessels. The yeast were either ale (LCC 125, 1209, 1240) or lager (A, SMA) and varied in their genotypes and flocculation phenotypes (either NewFlo or Flo1). Each fermentation was conducted at 21 oC with wort supplemented with 4% (w/v) glucose and completed in 72 hours (Lake et al., 2008. J. ASBC). Turbidity and density measurements were collected throughout the fermentations and non-linear modelling was used to compare each yeast strainís fermentation parameters. This method showed differences between strains ín attenuation of the wort, as well as some differences in maximum fermentation rates. The results provide evidence that small-scale fermentations can be used to compare the fermentability of different yeast strains, as well as their maximum rate of fermentation and the time at which this occurs. This method could be useful in preliminary screening of new yeast strains in large-scale production.

## XVI Laboratorio di Micologia Medica. Dipartimento di Sanità Pubblica-Microbiologia-Virologia. Università degli Studi di Milano, via Pascal 36, 20133 Milano, Italy. Communicated by AM Tortorano <annamaria.tortorano@unimi.it>.

The Laboratory of Medical Mycology in the Section Public Health of the Department of Public Health-Microbiology-Virology of the University of Milan has a large collection of yeasts, mainly from documented human cases of deep-seated *Candida* and *Cryptococcus* infections. The Lab is participating in the current Europe-wide candidemia survey of the European Confederation of Medical Mycology (ECMM) and in another ongoing Italian candidemia survey. Two main topics of research in the field of yeasts - *Cryptococcus* and cryptococcosis, and *Candida* and candidosis - are ongoing.

*Cryptococcus* and cryptococcosis.

Recent publications.

1 Viviani MA, Cogliati M, Esposto MC, Lemmer K, Tintelnot K, Valiente MFC, et al. 2006 Molecular analysis of 311 *Cryptococcus neoformans* isolates from a 30-month ECMM survey of cryptococcosis in Europe. FEMS Yeast Res 6(4):614-9.

During a European Confederation of Medical Mycology (ECMM) prospective survey of cryptococcosis in Europe (from July 1997 to December 1999) 655 cases were reported from 17 countries; 565 of the completed questionnaires were evaluable. Cryptococcosis was associated with HIV infection in 77% of cases (range 57.5-94%). Assessment of the laboratory data highlighted the lack of defined standard procedures for the diagnosis of cryptococcosis: the antigen test was not usually used for screening, the disease was mainly recognised when meningitis occurred (65% of patients) and, with the exception of a few cases, the extent of the infection was not investigated. *Cryptococcus neoformans* was the etiological agent in all of the cases except for six caused by C. gattii and four by other *Cryptococcus* species. A total of 311 C. *neoformans* strains were serotyped by Crypto Check latex agglutination, genotyped by PCR-fingerprinting using the $(GACA)_4$ oligonucleotide as a single primer, and their mating type was determined by PCR of the STE20 alleles. Serotype A was the most represented (51% of the isolates), followed by serotype D (30%) and serotype AD (19%). PCR-fingerprinting analysis significantly increased the percentage of hybrid strains to 30%, as 6% of the serotype A and 28% of the serotype D isolates were of the VN3 or VN4 hybrid genotype. In addition, the mating type determinations revealed the MATa serotype A allele in one haploid strain and 28 hybrids, and hybrid isolates with a single mating type (four Aá and two Dá) were also identified. This is the first prospective survey to be carried out in Europe which has attempted to investigate the epidemiology of cryptococcosis and the population structure of C. *neoformans*, and the results obtained thus far show the widespread involvement of AD hybrid strains in C. *neoformans* infections.

2 Cogliati M, Esposto MC, Tortorano AM, Viviani MA 2006 *Cryptococcus neoformans* population includes hybrid strains homozygous at mating-type locus. FEMS Yeast Res 6(4):608-13.

Recent attempts to characterise the hybrid strains of *Cryptococcus neoformans* have led to the identification of a cryptic population of hybrid strains ('H strains') with double DNA content but only a single mating-type allele. To verify a set of hypotheses concerning their origin, we investigated 14 previously isolated H strains and ten F1-progeny strains arising from H99 and JEC20 mating. The double DNA content was tested by flow cytometry; the presence of only one mating type was tested by amplifying 12 mating-type-specific genes and one gene unlinked with the mating-type locus (URA5). Analysis of the F1 progeny identified two H strains, and electrophoretic karyotyping confirmed the occurrence of genetic recombination. The simultaneous presence of the homozygous and heterozygous loci, and the fact that all of the F1-progeny strains presented a recombinant karyotype, suggest that the H strains originated from the post-meiotic random fusion of two of the four recombinant nuclei. Further studies are required to elucidate the role of the homozygous mating-type loci in the virulence of C. *neoformans*.

3 Cogliati M, Esposto MC, Liberi G, Tortorano AM, Viviani MA 2007 *Cryptococcus neoformans* typing by PCR fingerprinting using $(GACA)_4$ primers based on *C. neoformans* genome project data. J Clin Microbiol 45:3427-30.

Four $(GACA)_4$ PCR fingerprinting sequences, used as markers to identify serotypes A and D and AD hybrids, were retrieved in four *Cryptococcus neoformans* genome databases. Their locations, both in serotype A and D genomes, were confirmed by chromosomal hybridization with specific probes. Two sequences were recognized to code for hypothetical functional proteins.

4 Esposto MC, Cogliati M, Tortorano AM, Viviani MA 2009 Electrophoretic karyotyping of *Cryptococcus neoformans* AD-hybrid strains Mycoses 52 (1): 16-23.

This study investigated the differences in genome structure between haploid serotype A and D isolates and AD-hybrid strains of *Cryptococcus neoformans*, and the correlation between the karyotype of A and D strains with their mating ability. The electrophoretic karyotyping of 16 AD-hybrid, eight haploid serotype-A MATá, and eight haploid serotype-D MATáC. *Cryptococcus* isolates was performed. These 32 isolates presented, two by two, the same genotype and flow cytometry profile. Five clusters were identified, each including VNI (serotype A), VNIV (serotype D) haploid strains and VNIII AD hybrids. Similarly, mating types were also randomly distributed in the five clusters. In addition, AD-hybrid isolates, with double content of DNA, showed only a slight increase in both the number of chromosomal bands and the calculated genome size compared with haploid isolates. Data support the hypothesis that hybrid isolates are aneuploids (2n+x) rather than eudiploids (2n). In addition, a set of six mating type a strains were karyotyped and then used for mating experiments carried out crossing the haploid isolates with similar or different karyotype profile strains. Isolates with completely different karyotype were able to mate confirming that meiosis occurred even in the presence of chromosomes of different lengths.

5 Viviani MA, Tortorano AM 2009 *Cryptococcus*. In: Anaissie EJ, McGinnis MR, Pfaller MA (eds) Clinical Mycology. Churchill Livingston 2nd edition 2009: 231-250.

Publications submitted or in press.

6 Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz M, Esposto MC, Fisher M, Gilgado F, Hagen F, Kaocharoen S, Litvintseva AP, Mitchell TG, Simwami SP, Trilles L, Viviani MA, and Kwon-Chung KJ 2009 Multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii.* Med Mycol In press.

7 Kantarcioglu S, Boekhout T, Viviani MA, Del Poeta M, Theelen B, Cogliati M, Yücel A, and Altas K 2009 A *Cryptococcus neoformans* strain isolated from a case of microscopy and latex antigen negative cryptococcal meningitis. Submitted.

8 Cogliati M, Chandrashekar N, Esposto MC. Chandramuki A, Petrini B, and Viviani MA 2009 A survey on cryptococcosis reveals the presence of a clonal *Cryptococcus gattii* serotype-C population in Bangalore, India. Submitted.

Recent conference presentations.

9 Cogliati M, Chandrashekar N, Prigitano A, Esposto MC, Petrini B, Chandramuki A, Viviani MA 2008 Clinical isolates from an indian hospital: an unexpected detection of a serotype-C *Cryptococcus gattii* population. 7th International Conference on *Cryptococcus* and Cryptococcosis, 11-14 September, Nagasaki, Japan. Pg. 52, SY-05-04

Genotyping of 152 strains of *Cryptococcus neoformans* species complex from Bangalore showed that 146 isolates were VNI-Aa, one VNIV-Da, one VNIII-Da, and four *C. gattii* VGIV-Ca. Serotype-C isolates were all identical by multilocus sequence typing and, by comparison with a larger number strains, with one serotype-C isolate from Botswana.

*Candida* and candidosis.

Recent publications.

10 Viviani MA, Cogliati M, Esposto MC, Prigitano A, Tortorano AM 2006 Four-year persistence of a single *Candida albicans* genotype causing bloodstream infections in a surgical ward proven by multilocus sequence typing. J Clin Microbiol 44(1):218-21.

The present study represents the first application of multilocus sequence typing to retrospectively investigate a suspected outbreak of *Candida albicans* bloodstream infection cases that occurred in the same hospital ward between July 1987 and October 1991. Results demonstrated that eight bloodstream infections were caused by the same strain, endemic in the ward, over a 4-year period.

11 Tortorano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L, Grillot R 2006 Candidaemia in Europe: epidemiology and resistance. Int J Antimicrob Agents 27(5):359-66.

Despite the widespread use of antifungals for prophylaxis, Candida bloodstream infection (BSI) remains the most frequent life-threatening fungal disease. From an analysis of multi-institutional surveys of Candida BSIs performed in Europe, including the large prospective survey by the European Confederation of Medical Mycology (2089 episodes from seven countries), a limited role of species with decreased susceptibility to azoles in causing BSIs and a low proportion of antifungal resistance was evident. Large prospective epidemiological surveys using common databases are needed to monitor trends in incidence and changes in species distribution, to identify new at-risk patients and to evaluate the impact of the introduction into the market of new antifungal agents.

12 Van Asbeck E, Clemons K, Markham A, Stevens D and the Candida parapsilosis global epidemiology group 2008 Molecular epidemiology of the global and temporal diversity of *Candida parapsilosis*. Scand J Infect Dis 40 (10): 827-834.

We examined the global epidemiology of *C. parapsilosis* and assessed the discriminatory capabilities of restriction fragment length polymorphism (RFLP) and RAPD typing methods. We used EcoRI digestion of cellular DNA to generate RFLP; RAPD analysis on genomic DNA. Band profiles were used to distinguish and group isolates. From 7 diverse geographic areas, 536 isolates obtained over 35 y were placed into 23 RFLP subgroups. Subtype VII-1 was dominant worldwide (82.4% of isolates). Dividing the isolates into VII-1 versus non-VII-1 showed temporal variation for the USA pre-1995 versus post-1995 ($p < 0.0001$) and versus Europe pre-1995 ($p < 0.0001$). Genotype distribution differed among localities ($p < 0.0001$); Mexico was unique ($p < 0.05$) due to the high proportion of non-VII-1. The prevalence of *C. parapsilosis* RFLP type VII-1 apparently has risen in the USA and current isolates show some variation in distribution of types in some non-USA localities. There were no differences in distribution of types comparing babies versus adults, or bloodstream isolates versus colonizing or environmental isolates. RAPD typing showed 3 major profiles, but was less discriminatory.

13 Veraldi S, Tortorano AM, Lunardon L, Persico MC, Francia C 2008 Mycological evaluations in chronic leg ulcers. Wounds 20 (9): 250-253.

Recent conference presentations.

14 Tortorano AM 2008 *Candida* infections in the ICU. XII IUMS, Istanbul, 6-8 August 2008, MIP-49.

An overview on yeast infections in ICU patients was presented The gastrointestinal insults that may arise as a consequence of ICU management procedures are responsible for the vulnerability of these patients to haematogenous dissemination of *Candida* species, such as *C. albicans, C. glabrata, C. tropicalis*, that form part of their commensal flora of the gastrointestinal tract. The alteration of the skin barrier, as in the presence of IV lines, favours the acquisition of yeasts, such as *C. parapsilosis*, colonizing the patient's skin or the hands of the healthcare workers. In addition, the vascular catheters, as well as other implantable devices, may be hematogenous seeded by *Candida,* such as *C. albicans, C. glabrata* etc, coming from distant local infection. Formation of biofilm on implanted biomaterials increases resistance to antifungal agents, protects *Candida* from host defences, and causes failure of devices.

15 Tortorano AM 2008 Epidemiology of *Candida* infections: European focus. 18th ECCMID, Barcelona 19-22 April 2008. Clin Microbiol Infect 14 (S7): S66-S67.

Invasive candidiasis and candidaemia are the most common systemic fungal infections observed in hospitals. The increased prevalence of risk factors for these infections over the past two decades have dramatically increased their incidence during that period. These infections are frequently severe, with a crude mortality rate of 38%.The nature of systemic *Candida* infections appears to be changing. Until recently, the majority of infections were caused by *Candida albicans*, but this species is becoming less common as non-*albicans Candida* species begin to proliferate, particularly in certain patient types. In a recent survey of the epidemiological and mycological profile of *Candida* species in Europe, while *Candida albicans* was still the most common cause of infection overall, 43.6% of infections were caused by non-*albicans Candida* species. Furthermore, in surgical patients and those with solid tumours, and in patients aged >=70 years, the prevalence of *C. glabrata* approached 20% The significance of these changes in *Candida* epidemiology may be profound, since non-*albicans Candida* species seem to be associated with an increased risk of mortality compared with *C. albicans (C. krusei*: 55.3%; *C. glabrata*: 45.0%; *C. tropicalis*: 41.4%; *C. albicans*: 38.5%). However, these differences may be explained by the increased prevalence of these species in patients with more severe underlying illness. While isolates of *C. albicans* are generally susceptible to most antifungal agents, non-*albicans Candida* species, particularly *C. glabrata* and *C. krusei*, are often non-susceptible to older agents, such as fluconazole. This has important implications for the selection of appropriate antifungal therapy in patients with systemic *Candida* infections.

Recent PhD thesis.

16 Dho G 2009 ECMM-FIMUA Working Group on deep-seated fungal infections in Intensive Care Units.: results of a two year survey. Post-Doctoral thesis, University of Milano.

## XVII Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by M.A. Lachance <lachance@uwo.ca>.

See also Dr Rosa's communication. The following paper was published rescently.

1 Wardlaw AM, Berkers TE, Man KC and Lachance MA 2009 Population structure of two beetle-associated yeasts: comparison of a New World asexual and an endemic Nearctic sexual species in the *Metschnikowia* clade. Antonie van Leeuwenhoek (published online).

The genetic structure of two related yeast species, one sexual and one asexual, was compared using polymorphic DNA markers. Although both yeasts propagate by asexual budding of haploid cells, *Metschnikowia borealis* reproduces sexually when compatible strains come in contact. To what extent this has occurred in nature was not known. As *Candida ipomoeae* is a closely related, asexual species, the two yeasts provide an excellent model system to assess the role of sexual reproduction in a biogeographic context. Natural isolates of the two species were characterized using several polymorphic DNA markers. As predicted for an organism whose reproduction is strictly clonal, *C. ipomoeae* exhibited low haplotype diversity, high linkage disequilibrium, and high population differentiation. In contrast, *M. borealis* had unique haplotypes in most isolates, lower population differentiation, and little linkage disequilibrium, demonstrating that sexual recombination is prevalent. Geographic gradients were identified in both species, indicating that historical and climatic factors both play a role in shaping the populations. The spatial structure is also thought to be influenced by the ecology of the small floricolous beetles (family Nitidulidae) that vector the yeasts. For example, Hawaiian strains of *C. ipomoeae* show evidence of having undergone a genetic bottleneck, most likely when the vector was introduced to the islands. The two haplotypes found in Hawaii were nearly identical and were also found in North and Central America. *M. borealis* had a more continuous distribution where the genetic markers follow latitudinal and longitudinal gradients.

Conference presentations.

2 Lachance MA 2009 Of Yeasts and Beetles: the *Metschnikowia* saga. Great Lakes-St.Lawrence Mycology Workshop, May 2009, University of Toronto.

3 Wijayanayaka D 2009 The species boundaries of Candida species in the *Starmerella* clade. Great Lakes-St.Lawrence Mycology Workshop, May 2009, University of Toronto.

4 Berkers T 2009 The yeast-beetle-nematode triangle. Canadian Botanical Society, Halifax NS.

Yeasts are known to enter symbiotic associations with beetles or other insects, but most of these interactions are not well understood. My research explores the ecology of the yeast *Metschnikowia borealis*, specifically its relationship to *Conotelus obscurus*, a flower-inhabiting nitidulid beetle that always carries this yeast species. The life cycle and biogeography of *M.borealis* have been characterized. However, the species cannot be fully understood without also considering its relationship with the beetle host. I have investigated the beetle's life history and anatomy with the purpose of determining whether the yeast is a deterrent to nematodes that are often observed in the beetles. Because the large, barbed ascospores of *M. borealis* may be important in the infection process, I tested the hypothesis that presence of ascospores in individual beetles is negatively correlated with nematode load. It isn't. I have searched for possible mechanisms by which the beetles carry and propagate the yeast. Preliminary results of scanning electron microscopy suggest that the yeast is vectored internally in a specialized structure. The next step will be to evaluate critically the effect of yeasts and nematodes on the reproductive output of the beetles.

# Forthcoming Meetings

## ISSY28, Bangkok, Thailand

On behalf of the Organizing Committee, I would like to invite yeast researchers from around the world to attend ISSY28 in Bangkok during September 2010. This will be the first time a yeast symposium is held in Asia and Bangkok is situated right in the middle of the region. It is hoped that many new participants from Asia and Oceania will join this meeting.

Although there had been political unrests earlier this year, the situation has returned to normal and the Thai people will no longer support any more aggressions that have harmed the country's economy and reputation. The current government is now stable and we can be sure that these incidents will not happen again.

The details of the yeast symposium will be announced at ISSY27 in Paris this August. We are planning this meeting to make it satisfying both scientifically and culturally. Let the world renowned Thai hospitality welcome you to Thailand, the Land of Smile.

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**For up-to-date announcements, please see the YNL website**

**http://publish.uwo.ca/~lachance/Future%20meetings.html**

# Fifty Years Ago in the Yeast Newsletter
# (Volume VIII Number 1, May 1959)

"Dr. **Wm. Bridge Cooke**, U. S Public Health Service, Cincinnati, Ohio, pointed out the following article to the Editor, as he thought it to be of interest to readers of the Yeast News Letter. [...]

**J. Zsolt**, Institute for Plant Physiology, University , Szeged, Hungary. The Evolution of Domesticated yeasts, and some related problems. Acta Botanica: Academiae Scientiarum Hungaricae. 5(1-2): 233-257. 1959 [...]

The timely tasks of yeast taxonomy and phylogeny are the following viz.:

(i) Detection of new yeasts.

(ii) Formation of homogenous groups of known forms.

(iii) Tracing the origin of individual properties.

(iv) Construction of the family tree within small groups."

Dr. **Samuel P. Meyers** reports:

"Two graduate students, Mr. **Jack Fell** and Mr. **Donald Ahearn**, have conducted various aspects of this research, leading to their degree, Master of Science in Marine Biology.**"**