

# Yeast

**A Newsletter for Persons Interested in Yeast**

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Marc-André Lachance, Editor  
University of Western Ontario, London, Ontario, Canada N6A 5B7  
<lachance@uwo.ca>

<http://publish.uwo.ca/~lachance/YeastNewsletter.html>

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## Associate Editors

Peter Biely  
Institute of Chemistry  
Slovak Academy of  
Sciences  
Dúbravská cesta 9, 842 3  
8 Bratislava, Slovakia

Patrizia Romano  
Dipartimento di Biologia, Difesa  
e Biotecnologie Agro-Forestali  
Università della Basilicata,  
Via Nazario Sauro, 85, 85100 Potenza,  
Italy

Kyria Boundy-Mills  
Herman J. Phaff Culture  
Collection  
Department of Food Science and  
Technology  
University of California Davis  
Davis California 95616-5224

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## **Editorials**

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### **Frank Spencer 1922-2010**

I regret to announce the recent death of Dr. J.F.T. Spencer, after some years of ill health. Many of us knew Frank, not only for his accomplishments in yeast genetics, systematics, physiology, and biochemistry, but also for his passion for politics and his love of art and nature. I had the privilege to spend time with Dorothy and Frank exploring yeast biodiversity in the northern regions of Argentina. This is where I learned to enjoy Frank's mischievous sense of humour. An obituary was kindly provided by members of his family.

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### **ISSY 28 Bangkok, Thailand**

Congratulations to Charoen Charoenchai and his organizing team for an excellent specialized symposium. The meeting was attended by a good cross-section of researchers from across the international community. The Montien Riverside Hotel was a wonderful venue and the scientific and social programs were of the highest quality, introducing us in particular to the vibrant activity in Thai research in the area of yeast biotechnology and biodiversity. Our tour of the Royal Palace, our river cruise, and the gentle and welcoming way of the Thai people will remain in our memory for years to come.

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### **Publications of N. van Uden**

Dr. Álvaro Fonseca informed us that a new website has been created by Maria Loureiro Dias where the late Professor van Uden's publications, as well as some biographic notes, can be found:

<http://www.spmicrobiologia.pt/vanuden/>

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

MA Lachance, Editor

**A new yeast research resource is now available from ATCC**

A set of new yeast strains called Yeast Diversity Library (YDL) is now available for breeding, population studies and other R&D. Dr. Timberlake and his colleagues generated nearly three hundred strains from a select group of sixteen *Saccharomyces cerevisiae* laboratory and industrial strains whose genome sequence are largely known. These researchers performed systematic, pair-wise mating of sixteen MAT $\alpha$  haploids with congenic MAT $\alpha$  haploids in all possible combinations in producing a total of 256 F1 hybrid strains. These strains, together with the 32 F0 parent strains, are arrayed on three 96-well microtiter plates. For detailed information on strain generation and characterization,

see the reference (Timberlake WE, Frizzell MA, Richards KD, & Gardner RC. A new yeast genetic resource for analysis and breeding. Yeast DOI: 10.1002/yea.1821 Epub ahead of print). The Yeast Diversity Library (YDL) product is available from ATCC (catalog number is GSA-9PS). It includes three 96-well microtiter plates, named as GSA-9P1, GSA-9P2 and GSA-9P3. Each well on the plate contains a unique strain as 150 ml of cell suspension in YPAD with 10% glycerol. A small version of the yeast diversity library is offered as Compact Diversity Library (CDL) with ATCC catalog number GSA-10. Contact: Jim Zhou ([jjzhou@atcc.org](mailto:jjzhou@atcc.org)), ATCC Mycology Collection.

Publications from the ATCC Mycology Collection.

- 1 JL Houseknecht, EL Hart, SO Suh, and JJ Zhou 2010 Yeasts in the *Sugiyamaella* clade associated with wood-ingesting beetles and the proposal of *Candida bullrunensis* sp. nov. Int J Syst Evol Microbiol doi: 10.1099/ijs.0.026427-0

During a survey of yeasts associated with wood-ingesting insects, six strains of the *Sugiyamaella* clade were isolated from the gut of passalid and tenebrionid beetles and their inhabited decayed wood. Phylogeny based on ribosomal RNA gene sequences placed these yeasts into *Sugiyamaella smithiae*, *Su. americana*, *Candida lignohabitans*, and a novel species closely related to *Su. americana*. The only strain of the novel

species, EH008, was unquestionably distinguished from its relatives by the DNA sequences and other taxonomic characteristics. Ascospore production was not observed under the laboratory conditions tested. Therefore, we propose this new species as *Candida bullrunensis* (EH008<sup>T</sup> = ATCC MYA-4660<sup>T</sup> = CBS 11840<sup>T</sup>).

- 2 P Gujjari, SO Suh, CFu Lee, and JJim Zhou 2010 *Trichosporon xylopinii* sp. nov., a hemicellulose-degrading yeast isolated from wood-inhabiting beetle *Xylopinus saperdioides*. Int J Syst Evol Microbiol doi: 10.1099/ijs.0.028860-0

Four arthroconidium-producing yeasts were isolated from the gut of wood-inhabiting tenebrionid and passalid beetles. The ribosomal RNA genes of these yeast strains were sequenced, compared and analyzed. The sequence results and other taxonomic characterizations placed two of the strains into *Trichosporon porosum*, and the remaining strains, EH024 and EH026 which were isolated from *Xylopinus saperdioides* (Coleoptera: Tenebrionidae), to a novel *Trichosporon* species in the Porosum clade. Strain EN6S23 was independently isolated from forest soil in Taiwan and was identified as the same novel

species based on its identical sequences in the ITS and the D1/D2 region of the LSU rRNA gene and similar physiological characteristics to those of strains EH024 and EH026. The three strains can assimilate cellulose and xylan as sole carbon source, and are clearly distinguished from their closest taxon *T. porosum* by 14 nucleotide differences in the ITS and the D1/D2 region. These strains did not reproduce sexually under the laboratory conditions tested. The novel species is proposed as *Trichosporon xylopinii* (type strain EH024<sup>T</sup> = ATCC MYA-4670<sup>T</sup> = CBS 11841<sup>T</sup>).

Current publications.

- 1 Barnett JA 2010 A history of research on yeasts 14: medical yeasts part 2, *Cryptococcus neoformans*. Yeast 27: 693–707.
- 2 Barnett JA & Barnett L (in the press) *Yeast Research: a Historical Overview*. ASM Press, approx 430 pp. ISBN 978-1-55581-516-5

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**III CBS Fungal Biodiversity Centre, Department of Yeast and Basidiomycete Research, Uppsalaalan 8, NL-3584CT Utrecht, The Netherlands. Communicated by Ferry Hagen <[f.hagen@cbs.knaw.nl](mailto:f.hagen@cbs.knaw.nl)>.**

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Recent publications.

- 1 Cafarchia C, Latrofa MS, Figueredo LA, da Silva Machado ML, Ferreiro L, Guillot J, Boekhout T, Otranto D 2010 Physiological and molecular characterization of atypical lipid-dependent *Malassezia* yeasts from a dog with skin lesions: adaptation to a new host? *Med Mycol* - in press.
- 2 Illnait-Zaragozí MT, Martínez-Machín GF, Fernández-Andreu CM, Hagen F, Boekhout T, Klaassen CH, Meis JF 2010 Microsatellite typing and susceptibilities of serial *Cryptococcus neoformans* isolates from Cuban patients with recurrent cryptococcal meningitis. *BMC Infect Dis* 10:289.
- 3 Hagen F, Illnait-Zaragozi MT, Bartlett KH, Swinne D, Geertsen E, Klaassen CH, Boekhout T, Meis JF 2010 *In vitro* antifungal susceptibilities and Amplified Fragment Length Polymorphism genotyping of a worldwide collection of 350 clinical, veterinary, and environmental *Cryptococcus gattii* isolates. *Antimicrob Agents Chemother.* 54:5139-5145.
- 4 Hagen F, Boekhout T 2010 The search for the natural habitat of *Cryptococcus gattii*. *Mycopathologia* 170:209-211.
- 5 Guinea J, Hagen F, Peláez T, Boekhout T, Tahoune H, Torres-Narbona M, Bouza E 2010 Antifungal susceptibility, serotyping, and genotyping of clinical *Cryptococcus neoformans* isolates collected during 18 years in a single institution in Madrid, Spain. *Med Mycol* 48:942-948.
- 6 Wuczkowski M, Passoth V, Turchetti B, Andersson AC, Olstorpe M, Laitila A, Theelen B, van Broock M, Buzzini P, Prillinger H, Sterflinger K, Schnürer J, Boekhout T, Libkind D 2010 Description of *Holtermanniella takashimae* sp. nov., *Holtermanniella* gen. nov. and proposal of the order Holtermanniales to accommodate Tremellomycetous yeasts of the *Holtermannia* clade. *Int J Syst Evol Microbiol* - in press.

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**IV Culture Collection of Yeast (CCY), Institute of Chemistry, Slovak Academy of Sciences Dúbravská cesta 9, 842 38, Bratislava, Slovakia. Communicated by Emília Breierová <[Emilia.Breierova@savba.sk](mailto:Emilia.Breierova@savba.sk)>.**

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Recent publication.

- 1 Márová I, Čárnecká M, Halienová A, Breierová E and Kočí R 2010 Production of carotenoid-/ergosterol-supplemented biomass by red yeast *Rhodotorula glutinis* grown under external stress. *Food Technol Biotechnol* 48:56–61.

The aim of this study is to compare the production of biomass enriched with carotenoids and ergosterol by yeast strain *Rhodotorula glutinis* CCY 20-2-26 grown under optimal growth conditions and in the presence of exogenous stress factors. *R. glutinis* cells were exposed to UV irradiation, oxidative stress (2–10 mmol/L H<sub>2</sub>O<sub>2</sub>) and osmotic stress (2–10 % NaCl). During the experiment, growth characteristics and the production of biomass, carotenoids and ergosterol were evaluated. Experiments were carried out in Erlenmeyer flasks and in laboratory fermentor. First, *R. glutinis* cells were exposed to higher concentration of stress factors added into the production medium. Further, low concentrations of NaCl and H<sub>2</sub>O<sub>2</sub> were added to the inoculum medium or to both inoculum and production media. Exposure of red yeast cells to all tested stress

factors resulted in higher production of carotenoids as well as ergosterol, while biomass production was changed only slightly. Under high stress, 2–3 times increase of β-carotene was observed. The addition of low salt or peroxide concentration into the inoculation media led to about 2-fold increase of carotenoid production. In Erlenmeyer flasks the best effect on the carotenoid and ergosterol production (3- to 4-fold increase) was exhibited by the combined stress: the addition of low amount of NaCl (2 mmol/L) into the inoculum medium, followed by the addition of H<sub>2</sub>O<sub>2</sub> (5 mmol/L) into the production medium. The production of ergosterol in most cases increased simultaneously with the production of carotenoids. Cultivation of *R. glutinis* carried out in a 2-litre laboratory fermentor was as follows: under optimal conditions about 37 g/L of yeast biomass were obtained

containing approx. 26.30 mg/L of total carotenoids and 7.8 mg/L of ergosterol. After preincubation with a mild stress factor, the yield of biomass as well as the production of carotenoids and ergosterol substantially increased. The best production of enriched biomass was obtained in the presence of peroxide in the inoculation medium (52.7 g/L of biomass enriched with 34 mg/L

of carotenoids) and also under combined salt/peroxide or salt/salt stress (about 30–50 g/L of biomass enriched with 15–54 mg/L of total carotenoids and about 13–70 mg/L of ergosterol). *R. glutinis* CCY 20-2-26 could be used as a potential biotechnological producer of carotenoid-rich biomass.

Book chapter.

- 2 Čertík M, Hanusová V, Breierová E, Márová I and Rapta P 2009 Biotechnological production and properties of carotenoid pigments. Chapter 25. In *Biocatalysis and Agricultural Biotechnology*, edited by CT Hou and JFu Shaw. Taylor & Francis Group, LLC

Carotenoids represent one of the broadest groups of natural antioxidants (over 600 characterized structurally) with significant biological effects and numerous industrial applications. Lycopene is a typical acyclic carotene that serves as a starting metabolite for formation of carotenoid derivatives via specific routes ( $\beta$ -carotene, torulene, etc.). Xanthophylls include hydroxy-, methoxy- oxo-, epoxy-, carboxy-, and aldehydic groups (torularhodin, zeaxanthin, astaxanthin, etc.), which results in a broad structural variety of carotenoid compounds. Commercially, carotenoids are used as food colorants and nutritional supplements, with an estimated global market of some \$935 million by 2005 (Fraser and Bramley 2004). They are present in all photosynthetic organisms and responsible for most of the yellow-to-red colors of fruits and flowers. Because animals lack the ability to synthesize carotenoids, the

characteristic colors of many birds, insects, and marine invertebrates are due to the presence of carotenoids that originate in the diet. Carotenoid pigments have also been found in various microorganisms, including bacteria, algae, yeasts, and fungi. There is an increased interest in carotenoids as natural antioxidants and free radical scavengers for their ability to reduce and alleviate chronic diseases, various pathological stages, and aging. However, the application of chemical synthetic methods to prepare carotenoid compounds as food additives has been strictly regulated in recent years. Therefore, attention is paid to finding suitable natural methods for their production. One possibility lies in biotechnological techniques potentially employing microorganisms that are able to convert various substrates into carotenoid pigments.

- 3 Bauerová K, Paulovičová E, Mihalová D, Švík K. and Poništ S 2009 Study of new ways of supplementary and combinatory therapy of rheumatoid arthritis with Immuno-modulators. Glucmannan and Imunoglukán® in adjuvant arthritis. *Toxicol Indust Health* 25:329–335.

We studied the anti-arthritic activity of glucmannan (GM) isolated from *Candida utilis* and of Imunoglukán®, a  $\beta$ -(1,3/1,6)-D-glucan (IMG) isolated from *Pleurotus ostreatus*. Adjuvant arthritis (AA) was induced intradermally by the injection of *Mycobacterium butyricum* in incomplete Freund's adjuvant to Lewis rats. Blood for biochemical and immunological analysis was collected on experimental days 1, 14, 21, and 28. A clinical parameter – hind paw volume (HPV) – was also measured. The detection of IL-1  $\alpha$ , IL-4, TNF  $\alpha$ , and MCP-1 was done by immuno-flow cytometry. On day 28 – the end of the experiment – we determined spectrophotometrically: the total anti-oxidant status (TAS) of plasma samples along with thiobarbituric acid-reacting substances (TBARS) levels in plasma and we assessed the activity of gamma-glutamyl transferase

(GGT) in hind paw joint homogenate. The experiments included healthy animals, arthritic animals without treatment, and arthritic animals with administration of glucmannan (GM-AA) in the oral daily dose of 15 mg/kg b.w. and of IMG (IMG-AA) in the oral daily dose of 2 mg/kg b.w. The progress of AA was manifested by all parameters monitored. Both substances had beneficial effects on HPV, TBARS levels, GGT activity, and TAS levels. For cytokine assessment, only IMG-AA samples were selected, considering the significant HPV improvement accompanied with the observed anti-oxidant action. IMG administration had a positive immunomodulating effect on all cytokine plasma levels measured, changed markedly due to arthritis progression. Thus, IMG may be considered as a candidate for combinatorial therapy of rheumatoid arthritis.

- 4 Bujdaková H, Paulovičová E, Paulovičová L and Šimová Z 2010 Participation of the *Candida albicans* surface antigen in adhesion, the first phase of biofilm development. *FEMS Immunol Med Microbiol* 59:485–492.

The lack of work dealing with possible ways of reducing biofilm production via inhibiting *Candida albicans* adherence in the first stage of biofilm formation was a motivation for this study. The study was focused on two questions: (1) can a decrease in adherence affect the quantity of mature biofilm and (2) can blocking the surface *C. albicans* complement receptor 3-related protein (CR3-RP) with polyclonal anti-C3-RP antibody or

monoclonal antibody OKM1 significantly contribute to a reduction in adherence during biofilm formation? The presence and quantity the CR3-RP expressed in the biofilm was confirmed by immunofluorescence, immunocytometry and enzyme-linked immunosorbent assay. To determine the changes in adherence of *C. albicans* CCY 29-3-162 and *C. albicans* catheter isolate, 30-, 60-, 90- and 120-min time points were selected and viability was

determined by XTT assay. The strains were preincubated with both antibodies to block CR3-RP, which proved to be effective at reducing adhesion and the formation of a mature biofilm (64.1–74.6%). The duration of adhesion, between 30 and 120

min, seems to have a significant effect on the mature biofilm. The blocking of CR3-PR by antibodies before adherence affected the fitness of biofilm, which was not able to revitalize in the later stages.

- 5 Paulovičová L., Bystrický S., Paulovičová E, Karelin AA, Tsvetkov YE, and Nifantiev NE 2010 Model  $\alpha$ -mannoside conjugates: immunogenicity and induction of candidacidal activity. *FEMS Immunol Med Microbiol* 58:307–313.

The effect of *Candida* cell wall mannan-derived  $\alpha$ -oligomannoside structural components on the modulation of the immune system and their role in protective immunity are studied here. Semi-synthetic  $\alpha$ -mannoside-bovine serum albumin conjugates were used for immunization of rabbits. Dimeric  $\alpha$ -mannoside, representing *Candida* antigenic factor 1, was used as a model of linear  $\alpha$ -mannoside, and pentameric  $\alpha$ -mannoside was used as a model of branched oligomannoside side chain structure. The induction of humoral immune response and the functionality

of the serum tested by induction of peripheral blood leukocyte (PBL) candidacidal activity are documented. Anti-*Candida albicans* serotype B immunoglobulins (IgG and IgM) levels were higher than anti-serotype A following immunization with both conjugates. Dimer-conjugate postimmunization sera evidently enhanced *C. albicans* killing activity of PBLs in candidacidal assay. The study shows the importance of  $\alpha$ -mannoside structures in perspective anti-*Candida* vaccine with a broad spectrum of effectiveness.

- 6 Karelin AA, Tsvetkov YE, Paulovičová L, Bystrický S, Paulovičová E and Nifantiev NE 2010 Synthesis of 3,6-branched oligomannoside fragments of the mannan from *Candida albicans* cell wall corresponding to the antigenic factor 4. *Carbohydrate Res* 345:1283–1290.

3-Aminopropyl glycosides of 3,6-branched penta- and hexamannoside fragments of the cell wall mannan from *Candida albicans*, corresponding to the antigenic factor 4, have been

synthesized. Subsequent coupling of both oligosaccharides with BSA using the squarate procedure provided corresponding neoglycoconjugates.

- 7 Dzugasova V, Borecka S, Bařova M, Pilisiova R, Hervayova N and řubik J 2010 Site-directed mutagenesis of Asp853 in Pdr3p transcriptional activator from *Saccharomyces cerevisiae*. *Yeast* 27:277–284.

The *PDR3* gene encodes one of the main transcriptional activators involved in the control of multidrug resistance in the yeast *Saccharomyces cerevisiae*. Recently, it has been demonstrated that a specific D853Y mutation results in the loss of transactivation activity of Pdr3p and its conversion to multicopy suppressor of multidrug resistance. In this study, the Asp853 in Pdr3p was replaced by eight different amino acids and the function of mutated proteins was analysed. Different levels of complementation of cycloheximide hypersensitivity and expression of autoregulated *PDR3* and its *PDR5* target in the

*pdr1\_pdr3\_* mutant strain, ranging from that of the wild-type to loss-of-function alleles, were observed in *pdr3* mutants containing Pro, Glu, Arg, Asn, Ser, Leu, Phe, Ile or Tyr instead of Asp853 in Pdr3p. The introduction of the D853Y mutation into gain-of-function Pdr3p suppressed the transcription of the *PDR3* and *PDR5* genes and reduced both the rhodamine 6G efflux rate and the drug resistance level in corresponding double mutants. The results indicate that, while Pdr3p can tolerate several substitutions of Asp853, the occurrence of a hydrophobic amino acid at this position has an adverse effect on its function.

- 8 Karelin AA, Tsvetkov YE, Paulovičová L, Bystrický S, Paulovičová E and Nifantiev NE 2009 Synthesis of a heptasaccharide fragment of the mannan from *Candida guilliermondii* cell wall and its conjugate with BSA. *Carbohydrate Res* 344:29–35.

The 3-aminopropyl glycoside of a heptasaccharide fragment of the cell wall mannan from *Candida guilliermondii* 18, which corresponds to the antigenic Factor 9, has been synthesized by a convergent approach based on glycosylation of

a tetrasaccharide acceptor with a trisaccharide donor as the key step to give a protected heptasaccharide 17. Subsequent two-step deprotection of 17 afforded the heptamannoside 18, which was then conjugated with BSA using the squarate procedure.

- 9 Sláviková E, Vadkertiová R, Vránová D 2009 Yeasts colonizing the leaves of fruit trees. *Ann Microbiol*, 59:419-424.

The yeasts were isolated from the leaf surfaces of ten species of trees. The study site was a forest park (Železná Studnička) of the Small Carpathians mountain range. One hundred and thirty seven yeast strains belonging to 13 genera

were isolated from 320 samples of leaves and needles. Seventeen yeast species were isolated, but only seven occurred regularly: *Aureobasidium pullulans*, *Cryptococcus laurentii*, *Pichia anomala*, *Metschnikowia pulcherrima*, *Saccharomyces*

*sp.*, *Lachancea thermotolerans*, and *Rhodotorula glutinis*. The remaining species were isolated from the leaves and needles of three or less tree species. *A. pullulans*, *Cr. laurentii*, and *P. anomala* were the most frequently found species and they occurred on leaves and needles of all ten tree species. *Saccharomyces sp.* occurred in leaf samples collected from eight kinds of trees. *M. pulcherrima* and *L. thermotolerans* were found in samples collected from six species of trees. Both these species

occurred almost always on the leaves of deciduous trees. *Rh. glutinis* was the most frequently isolated carotenoids producing species. We have found out that the ascomycetous and basidiomycetous species were present in the leaf samples in approximately equal frequency, contrary to the soil samples taken from this forest park, where the ascomycetous species were found rarely.

- 10 Sulo P, Laurenčík M, Poláková S, Minárik G, Sláviková E 2009 *Geotrichum bryndzae* sp. nov., a novel asexual arthroconidial yeast species related to the genus *Galactomyces*. *Int J Syst Evol Microbiol* 59:2370-2374.

Ten strains of an asexual arthroconidial yeast species were isolated from Bryndza, a traditional Slovak artisanal sheep cheese, which was manufactured from raw milk during a 4-month summer production period at two Slovakian sites (the northern Ružomberok and the central-southern Tisovec areas). Sequence comparison of the D1/D2 domains of the large-subunit rRNA gene revealed that this yeast represents a novel species of

the genus *Geotrichum*, which contains anamorphs of the ascogenous genus *Galactomyces*, for which the name *Geotrichum bryndzae* sp. nov. is proposed (type culture CCY 16-2-1T = NRRL Y-48450T = CBS 11176T). The novel species is most closely related to *Geotrichum silvicola* NRRL Y-27641T, although yeasts with identical or very similar sequences have been found throughout the world.

- 11 Košíková B, Sláviková E, Lábaj J 2009 Affinity of lignin preparations towards genotoxic compounds. *BioResources* 4:72-79.

The carcinogenicity and mutagenicity of chemicals may be modulated by other chemicals, including those prepared by organic synthesis. Considering the several drawbacks of synthetic compounds vis-a-vis the human organism, the lignin biomass component was examined for this purpose. The binding affinity of lignin samples prepared by chemical and biological modification of lignin products derived from chemical wood treatment towards for N-nitrosodiethylamine (NDA) was

examined. The protective role of the lignin samples against carcinogenesis was tested on a well-known model carcinogen, N-methyl-N-nitro-N-nitrosoguanidine (MNNG). The observed ability of a series of lignin preparations to reduce alkylation damage of deoxyribonucleic acid (DNA) on hamster cells in vitro could be explained by their affinity to bind N-nitrosamines. The results indicate that lignin has potential to protect living organisms against damaging effects of different genotoxicants.

- 12 Košíková B, Sláviková E 2010 Use of lignin products derived from wood pulping as environmentally desirable additives of polypropylene films. *Wood Res* 55:87-92.

The blending of polypropylene with lignin preparations obtained from by-products of wood prehydrolysis and kraft pulping allows preparing optically transparent films (thickness 50-60 µm) with acceptable mechanical properties in the absence of commercial stabilizers. They exhibit strength properties comparable with those of lignin-free PP films. The lignin

preparations in the concentration 1-2 wt % possess the ability to act as processing stabilizers. The changes of mechanical properties during biodegradation and long-term artificial weathering indicate that the prepared lignin-polypropylene films are potentially non-persisting.

- 13 Farkaš V, Takeo K, Maceková D, Ohkusu M, Yoshida S, Sipiczki M 2009 Secondary cell wall formation in *Cryptococcus neoformans* as a rescue mechanism against acid-induced autolysis. *FEMS Yeast Res* 9:311-320.

Growth of the opportunistic yeast pathogen *Cryptococcus neoformans* in a synthetic medium containing yeast nitrogen base and 1.0–3.0% glucose is accompanied by spontaneous acidification of the medium, with its pH decreasing from the initial 5.5 to around 2.5 in the stationary phase. During the transition from the late exponential to the stationary phase of growth, many cells died as a consequence of autolytic erosion of their cell walls. Simultaneously, there was an increase in an ecto-glucanase active towards β-1,3-glucan and having a pH optimum between pH 3.0 and 3.5. As a response to cell wall degradation, some cells developed an unusual survival strategy

by forming ‘secondary’ cell walls underneath the original ones. Electron microscopy revealed that the secondary cell walls were thicker than the primary ones, exposing bundles of polysaccharide microfibrils only partially masked by an amorphous cell wall matrix on their surfaces. The cells bearing secondary cell walls had a three to five times higher content of the alkali-insoluble cell wall polysaccharides glucan and chitin, and their chitin/glucan ratio was about twofold higher than in cells from the logarithmic phase of growth. The cell lysis and the formation of the secondary cell walls could be suppressed by buffering the growth medium between pH 4.5 and 6.5.

- 14 Hanusová V, Čertík M, Breierová E, Márová I 2010 Effect of metals on metabolisms of pigment-forming yeasts – Proceedings, 37th International Conference of Slovak Society of Chemical Engineering, Tatranské Matliare, Slovakia, May 24-28, 2010. ISBN 978-80-227-3290-1.

Environmental pollution caused by toxic heavy metals in industrial waste is one of the most essential problems. Yeasts are eventual bioremediations, removing metals via active or passive uptake mechanisms. The environmental stress caused by heavy metals could influence the profile of carotenoid pigments in pigment-forming yeast. In order to improve the yield of carotenoid pigments and subsequently decrease the cost of this biotechnological process, various experiments have been performed by optimizing the culture conditions including nutritional and physical factors. In the present work, effect of metal ions (copper, zinc, iron, calcium, cobalt, selenium) on metabolisms of pigment-forming yeast *Rhodotorula glutinis* CCY 20-2-26 (the strain synthesizes  $\beta$ -carotene, torulene and torularhodin as major pigments) has been studied. Trace elements have been shown as a selective pressure on the carotenoid profile in the yeast. The heavy metals stress considerably changed of morphology of the strain. All metals caused enlargement of cells, the shape of yeast cells was more elongated when zinc ions occurred in the medium. It was found that zinc maximally stimulated accumulation of both  $\beta$ -carotene and torulene in the strain while copper enhanced formation of torularhodin. On the other hand, production of carotenoid pigments was suppressed by selenium. Regarding to lipids, copper enhanced linoleic acid formation and selenium increased

levels of both linoleic and linolenic acids in phosphatidylcholine and phosphatidyl-ethanolamine in the yeast. In addition, extracellular glycoproteins have been formed by the strain as adaptable response to heavy metal presence. Exoglycoproteins effectively captured heavy metals from media (e.g. up to 80% of zinc) and reduced their penetration into the cells. The ratio of protein/saccharide was also modified according to applied metals. Since heavy metals may generate various radicals (reactive oxygen species especially), antioxidant and radical-scavenging properties were also examined. Antioxidants present in fibrillar part of cell walls showed much higher ability to scavenge free radicals than those from cells. Zinc ions induced changes in yeast leading to more efficient scavenging and antioxidant capacities compare with copper and nickel ions. Completely different reactive radicals were detected when the yeast was stressed by copper ions in comparison to nickel and zinc ions. It could be summarized that alternations in pigment production by heavy metals might be explained by two hypotheses: a) possible activation/inhibition of specific enzymes involved in carotenoid biosynthesis, and b) presence of heavy metals results in formation of various active oxygen radicals what, in a turn, induces generation of protective carotenoid metabolites reducing negative behavior of free radicals.

- 15 Mészárosová Cs, Kolarova N 2009 Induction of surface glycosidases of yeast *Cryptococcus laurentii* during budding. IV<sup>th</sup> Scientific Conference of Students (with international participation), 5 November 2009, Nitra, Slovakia. ISBN 978-80-552-0280-8, p. 226-228.

Surface glycosidases of yeast *Cryptococcus* can play an important role in releasing or rearrangement its polysaccharide capsule. The polysaccharide capsule is an important virulent factor of pathogenic fungus *Cryptococcus neoformans*. The steps in capsule biosynthesis, releasing, degradation or rearrangement pose fascinating questions of enzymology, metabolism and cell biology; the answers have potential

application to treatment of cryptococcosis. This work is about induction of surface glycosidases of yeast *C. laurentii*. In our laboratory we found  $\alpha$ -galactosidase,  $\alpha$ -glucosidase and  $\beta$ -glucosidase that were induced with lactose in different phase of the growth. We compared these activities of glycosides with activities induced by glucose.

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**V Dipartimento di Biologia Applicata - Microbiologia, University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. Communicated by P. Buzzini <[pbuzzini@unipg.it](mailto:pbuzzini@unipg.it)>.**

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Recent publications.

- 1 Thomas-Hall SR, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K 2010 Cold adapted yeasts from Antarctica and the Italian Alps. Description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. Extremophiles 14:47-59.
- 2 Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P 2010 Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). FEMS Microbiol Ecol 72:354-369.
- 3 P Buzzini, M Goretti, E Branda, B Turchetti 2010 Basidiomycetous yeasts for production of carotenoids. In: Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology (Flickinger MC Ed.), Vol. 1, Wiley & Sons, New York, USA, 469-481.

- 4 Ricchi M, Goretti M, Branda E, Cammi G, Garbarino CA, Turchetti B, Moroni P, Buzzini P 2010 Molecular characterization of *Prototheca* strains isolated from Italian dairy herds. J Dairy Sci 93:4625-4631.
- 5 Raimondi S, Roncaglia L, Amaretti A, Leonardi A, Buzzini P, Forti L, Rossi M 2010 Rapid method for screening enoate reductase activity in yeasts. J Microb Methods 83:106-110.
- 6 Di Cagno R, Cardinali G, Minervini G, Antonielli L, Rizzello CG, Ricciuti P, Gobbetti M 2010 Taxonomic structure of the yeasts and lactic acid bacteria microbiota of pineapple (*Ananas comosus* L. Merr.) and use of autochthonous starters for minimally processing. Food Microbiol 27:381-389.
- 7 Corte L, Rellini P, Roscini L, Fatichenti F, Cardinali G 2010 Development of a novel, FTIR (Fourier Transform InfraRed spectroscopy) based, yeast bioassay for toxicity testing and stress response study. Analytical Chimica Acta 659:258-265.
- 8 Roscini L, Corte L, Antonielli L, Rellini P, Fatichenti F, Cardinali G 2010 Influence of cell geometry and number of replicas in the reproducibility of whole cell FTIR analysis. Analyst 135:2099-2105.

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**VI BCCM/MUCL Culture Collection, Earth and Life Institute – Mycology, Université catholique de Louvain, Louvain-la-Neuve, Belgium. Communicated by Heide-Marie Daniel**  
[<heidemarie.daniel@uclouvain.be>](mailto:heidemarie.daniel@uclouvain.be).

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Researchers working on different aspects of the species *Wickerhamomyces anomalus* (*Pichia anomala*, *Hansenula anomala*) have met for the 1<sup>st</sup> International *Pichia anomala* mini-Symposium held in Uppsala from 10th to 12th February 2010 following the initiative of Professor Johan Schnürer (Swedish University of Agricultural Sciences, Uppsala, Sweden). This small, but highly interesting meeting has led to a Special Issue of the Antonie van Leeuwenhoek Journal of Microbiology that will appear as first issue in 2011. The manuscripts listed below are available online already and can be accessed freely. Some more are still in production and will become available soon.

- 1 CP Kurtzman - Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. <http://www.springerlink.com/content/m1516772042254r7/>
- 2 A Vohra, P Kaur and T Satyanarayana - Production, characteristics and applications of the cell-bound phytase of *Pichia anomala*. <http://www.springerlink.com/content/b781x0505t72uk72/>
- 3 M Olstorpe and V Passoth - *Pichia anomala* in grain biopreservation. <http://www.springerlink.com/content/2325287m37313m68/>
- 4 L Polonelli, W Magliani, T Ciociola, L Giovati and S Conti - From *Pichia anomala* killer toxin through killer antibodies to killer peptides for a comprehensive anti-infective strategy. <http://www.springerlink.com/content/nu91866323156100/>
- 5 GM Walker - *Pichia anomala*: cell physiology and biotechnology relative to other yeasts. <http://www.springerlink.com/content/rq5v77526k763081/>
- 6 V Passoth, M Olstorpe and J Schnürer - Past, present and future research directions with *Pichia anomala*. <http://www.springerlink.com/content/j430183889x6t222/>
- 7 A Laitila, T Sarlin, M Raulio, A Wilhelmson, E Kotaviita, T Huttunen and R Juvonen - Yeasts in malting, with special emphasis on *Wickerhamomyces anomalus* (synonym *Pichia anomala*). <http://www.springerlink.com/content/42870681m718gmx8/>
- 8 J Schnürer and A Jonsson - *Pichia anomala* J121: a 30-year overnight near success biopreservation story. <http://www.springerlink.com/content/c633755863562373/>

- 9 HM Daniel, MC Moons, S Huret, G Vrancken and L De Vuyst - *Wickerhamomyces anomalus* in the sourdough microbial ecosystem. <http://www.springerlink.com/content/uw33277665652m15/>
- 10 P Melin, J Schnürer and S Håkansson - Formulation and stabilisation of the biocontrol yeast *Pichia anomala*. <http://www.springerlink.com/content/46q6275q1q244428/>

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**VII Rosenstiel School of Marine and Atmospheric Science/Marine Biology and Fisheries, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149. Communicated by J.W. Fell <[jfell@rsmas.miami.edu](mailto:jfell@rsmas.miami.edu)>.**

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The following references are recent publications from our laboratory.

- 1 Statzell-Tallman A, Scorzetti G & Fell JW 2010 *Candida spencermartinsiae* sp.nov., *Candida taylori* sp.nov. and *Pseudozyma abaconensis* sp. nov., novel yeasts from mangrove and coral reef ecosystems. Int J Syst Evol Microbiol 60:1978-1984

Three species of yeasts are taxonomically described for strains isolated from marine environments. *Candida spencermartinsiae* sp. nov. (type strain CBS 10894<sup>T</sup>, NRRL Y-48663<sup>T</sup>) and *Candida taylorii* sp. nov. (type strain CBS 8508<sup>T</sup>, NRRL Y-27213<sup>T</sup>) are anamorphic ascomycetous yeasts in a phylogenetic cluster of marine yeasts in the *Debaryomyces/Lodderomyces* clade of the Saccharomycetales.

The two species were isolated from multiple locations among coral reefs and mangrove habitats. *Pseudozyma abaconensis* sp. nov. (type strain CBS 8380<sup>T</sup>, NRRL Y-17380<sup>T</sup>) is an anamorphic basidiomycete that is related to the smut fungi of the genus *Ustilago* in the Ustilaginales. *P. abaconensis* was collected from waters adjacent to a coral reef.

- 2 Fell, JW, Statzell-Tallman S, Scorzetti G & Gutiérrez MH 2010 Five new species of yeasts from fresh water and marine habitats in the Florida Everglades. Antonie van Leeuwenhoek DOI: 10.1007/s10482-010-9521-6

Yeast populations in the Shark River Slough of the Florida Everglades, USA, were examined during a 3-year period (2002-2005) at six locations ranging from fresh water marshes to marine mangroves. Seventy-four described species (33 ascomycetes and 41 basidiomycetes) and an approximately equal number of undescribed species were isolated during the course of the investigation. Serious human pathogens, such as *Candida tropicalis*, were not observed, which indicates that their presence in coastal waters is due to sources of pollution. Some of the observed species were widespread throughout the fresh water and marine habitats, whereas others appeared to be habitat restricted. Species occurrence ranged from prevalent to rare. Five

representative unknown species were selected for formal description. The five species comprise two ascomycetes: *Candida sharkiensis* sp. nov. (CBS 11368<sup>T</sup>) and *C. rhizophoriensis* sp. nov. (CBS 11402<sup>T</sup>) (Saccharomycetales, Metschnikowiaceae), and three basidiomycetes: *Rhodotorula cladiensis* sp. nov. (CBS 10878<sup>T</sup>) in the *Sakaguchia* clade (Cystobasidiomycetes), *Rhodotorula evergladiensis* sp. nov. (CBS 10880<sup>T</sup>) in the *Rhodospiridium toruloides* clade (Microbotryomycetes, Sporidiobolales) and *Cryptococcus mangaliensis* sp. nov. (CBS 10870<sup>T</sup>) in the *Bulleromyces* clade (Agaricomycotina, Tremellales).

The technology described in the following reference targeted a dinoflagellate. However, the method is equally useful to enhance the signal for high through-put yeast detection and identification.

- 3 Diaz MR, JW Jacobson, KD Goodwin, SA Dunbar, and JW Fell 2010 Molecular detection of harmful algal blooms (HABs) using locked nucleic acids and bead array technology. Limnol Oceanogr Methods 8:269-284.

Harmful algal blooms (HABs) are a serious public health risk in coastal waters. As the intensity and frequency of HABs continue to rise, new methods of detection are needed for reliable identification. Herein, we developed a high-throughput, multiplex, bead array technique for the detection of the dinoflagellates *Karenia brevis* and *Karenia mikimotoi*. The method combined the Luminex detection system with two novel technologies: locked nucleic acid-modified oligonucleotides (LNA) and Mirus Label IT® nucleic acid technology. To study the feasibility of the method, we evaluated the performance of

modified and unmodified LNA probes with amplicon targets that were biotin labeled with two different strategies: direct chemical labeling (Mirus Label IT) versus enzymatic end-labeling (single biotinylated primer). The results illustrated that LNA probes hybridized to complementary single-stranded DNA with better affinity and displayed higher fluorescence intensities than unmodified oligonucleotide DNA probes. The latter effect was more pronounced when the assay was carried out at temperatures above 53°C degree. As opposed to the enzymatic 5' terminal labeling technique, the chemical labeling method enhanced the

level of fluorescence by as much as ~83%. The detection limits of the assay, which were established with LNA probes and Mirus Label IT system, ranged from 0.05 to 46 copies of rRNA. This high-throughput method, which represents the first molecular

detection strategy to integrate Luminex technology with LNA probes and Mirus Label IT, can be adapted for the detection of other HABs and is well suited for the monitoring of red tides at pre-blooming and blooming conditions.

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**VIII "Stefan cel Mare" University of Suceava, Faculty of Food Engineering, 13 Universitatii St., 720229, Suceava, Romania. Communicated by C.G. Gabriela <[codina@usv.ro](mailto:codina@usv.ro)>.**

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Recent publication.

- 1 CG Gabriela & V Daniela 2010 The influence of different forms of bakery's yeast *Saccharomyces cerevisiae* type strain on the concentration of individual sugars and their utilization during fermentation. Romanian Biotechnol Lett 15:5417-5422.

Research was conducted on the fermentation dynamics of carbohydrates in dough by three commercial forms of the yeast *Saccharomyces cerevisiae* produced by S.C. Rompak S.A. Pascani Romania (compressed yeast, instant active dry yeast and active dry yeast) at 0, 60, 120 and 180 minutes of dough fermentation. Experiments were performed using high-performance liquid chromatography for the analysis of sugars during dough fermentation. A Chopin rheofermentometer was

also used to analyze gas production and dough height. Using HPLC, a variation of sugars quantity was observed on the entire fermentation duration depending on the form of the yeast used in the following ascending order: dry instant active yeast > compressed yeast > dry active yeast. From the point of view of the results obtained with the Chopin rheofermentometer device, it was observed that the dough fermented with the compressed yeast has the highest released quantities of carbon dioxide.

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**IX Microbial Genomics and Bioprocessing Research, National Center for Agricultural Utilization Research, ARS-USDA, 1815 N. University Street, Peoria, IL 61604, USA. Communicated by C.P. Kurtzman <[Cletus.Kurtzman@ars.usda.gov](mailto:Cletus.Kurtzman@ars.usda.gov)>.**

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Recent publications.

- 1 Kurtzman CP, Robnett CJ and Basehoar-Powers E 2008. Relationships among species of *Pichia*, *Issatchenkia* and *Williopsis* determined from multigene phylogenetic analysis and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. FEMS Yeast Res 8:939-954.

Relationships among species assigned to the yeast genera *Pichia*, *Issatchenkia* and *Williopsis*, which are characterized by the ubiquinone CoQ-7 and inability to utilize methanol, were phylogenetically analyzed from nucleotide sequence divergence in the genes coding for large and small subunit rRNAs and for translation elongation factor-1 alpha. From this analysis, the species separated into five clades. Species of *Issatchenkia* are members of the *Pichia membranifaciens* clade and are proposed for transfer to *Pichia*. *Pichia dryadoides* and

*Pichia quercuum* are basal members of the genus *Starmera*. *Williopsis* species are dispersed among hat-spored taxa in each of the remaining three clades, which are proposed as the new genera *Barnettozyma*, *Lindnera* and *Wickerhamomyces*. Lineages previously classified as varieties of *Pichia kluyveri*, *'Issatchenkia'scutulata'*, *Starmera amethionina* and *'Williopsis'saturnus'* are elevated to species rank based on sequence comparisons.

- 2 Bishop JA, Chase N, Magill SS, Kurtzman CP, Fiandaca MJ and Merz WG 2008 *Candida bracarensis* detected among isolates of *Candida glabrata* by peptide nucleic acid fluorescence in situ hybridization: susceptibility data and documentation of presumed infection. J Clin Microbiol. 46:443-446.

Molecular taxonomic studies have revealed new *Candida* species among phenotypically delineated species, the best example being *Candida dubliniensis*. This study was designed to determine the occurrence of two new molecularly defined species, *Candida bracarensis* and *Candida nivariensis*, which are closely related to and identified as *Candida glabrata* by phenotypic assays. A total of 137 recent clinical isolates of *C. glabrata* identified by phenotypic characteristics was tested with *C. bracarensis* and *C. nivariensis* species-specific peptide nucleic acid fluorescence in situ hybridization probes. Three of

137 (2.2%) isolates were positive with the *C. bracarensis* probe, whereas the control strain, but none of the clinical isolates, was positive with the *C. nivariensis* probe. D1/D2 sequencing confirmed the identification of the three isolates as representing *C. bracarensis*. Clinically, one *C. bracarensis* isolate was recovered from a presumed infection, a polymicrobial pelvic abscess in a patient with perforated diverticulitis. The other two isolates were recovered from two adult oncology patients who were only colonized. *C. bracarensis* was white on CHROMagar Candida, had variable API-20C patterns that overlapped with

*C. nivariensis* and some *C. glabrata* isolates, and had variable results with a rapid trehalose assay. Interestingly, an isolate from one of the colonized oncology patients was resistant to fluconazole, itraconazole, voriconazole, and posaconazole in vitro. In summary, *C. bracarensis* was detected among clinical

isolates of *C. glabrata*, while *C. nivariensis* was not. One *C. bracarensis* isolate causing a presumed deep infection was recovered, and another isolate was azole resistant. Whether clinical laboratories should identify *C. bracarensis* will require more data.

- 3 Bishop JA, Chase N, Lee R, Kurtzman CP and Merz WG 2008 Production of white colonies on CHROMagar Candida medium by members of the *Candida glabrata* clade and other species with overlapping phenotypic traits. J Clin Microbiol. 46:3498-3500.

We hypothesized that species of the *Candida glabrata* clade and species with phenotypic traits that overlap those of *C. glabrata* would produce white colonies on CHROMagar Candida medium. Of 154 isolates (seven species) tested, *C. bracarensis*, *C. nivariensis*, *C. norvegensis*, *C. glabrata*, and

*C. inconspicua* produced white colonies; the *Pichia fermentans* group and *C. krusei* did not. Many of these species are difficult to identify phenotypically; white colonies may signal the need for the use of molecular approaches.

- 4 Kurtzman CP 2009 Biotechnological strains of *Komagataella (Pichia) pastoris* are *Komagataella phaffii* as determined from multigene sequence analysis. J Ind Microbiol Biotechnol 36:1435-1438.

*Pichia pastoris* was reassigned earlier to the genus *Komagataella* following phylogenetic analysis of gene sequences. Since that time, two additional species of *Komagataella* have been described, *K. pseudopastoris* and *K. phaffii*. Because these three species are unlikely to be resolved

from the standard fermentation and growth tests used in yeast taxonomy, the identity of biotechnologically important strains of *K. pastoris* was determined from multigene sequence analyses. Results from this study show that the strain of '*Pichia pastoris*' commonly used in gene expression studies is actually *K. phaffii*.

- 5 Kuo TM, Kurtzman CP and Levinson WE 2009 Production of itaconic acid by *Pseudozyma antarctica*. U.S. Patent 7,479,381 B1.

Itaconic acid may be produced in high yields by fermentation with a yeast, *Pseudozyma antarctica* NRRL Y-30980.

- 6 Manitchotpisit P, Leathers TD, Peterson SW, Kurtzman CP, Li XL, Eveleigh DE, Lotrakul P, Prasongsuk S, Dunlap CA, Vermillion KE and Punnapayak H 2009 Multilocus phylogenetic analyses, pullulan production and xylanase activity of tropical isolates of *Aureobasidium pullulans*. Mycol Res 113:1107-1120.

*Aureobasidium pullulans* is the source of the commercially valuable polysaccharide pullulan and the enzyme xylanase. Isolates are typically off-white to pale pink or black on solid media, while some tropical isolates have been described as 'color variants' with bright pigments of red, yellow or purple. We sequenced 5 loci (internal transcribed spacer, intergenic spacer 1, translation elongation factor-1 alpha, beta tubulin, and RNA polymerase II) from 45 new isolates from Thailand. Based on the

phylogenetic analyses, isolates were classified into 12 clades. Each clade showed different colors on different culture media including two clades with 'color variants' and some clades exhibited high levels of pullulan production or xylanase activity. Colony characteristics do not correlate perfectly with DNA sequence phylogeny or the physiological characters, but DNA sequence differences rapidly identify isolates with genetic novelty.

- 7 Kurtzman CP and Robnett CJ 2010 Systematics of methanol assimilating yeasts and neighboring taxa from multigene sequence analysis and the proposal of *Peterozyma* gen. nov. FEMS Yeast Res 10:353-361.

The relatedness among methanol-assimilating yeasts assigned to the genus *Ogataea* and neighboring taxa (Phylum Ascomycota, Subphylum Saccharomycotina, Class Saccharomycetes, Order Saccharomycetales) was determined from phylogenetic analyses of gene sequences for nuclear large and small subunit (SSU) rRNAs, translation elongation factor-1alpha and mitochondrial SSU rRNA. On the basis of the

analyses, *Williopsis salicorniae* and seven species of *Pichia* are proposed for transfer to the genus *Ogataea*, which has been emended, and *Pichia angophorae*, a nonhyphal species, is proposed for transfer to the mycelium forming genus *Ambrosiozyma*. *Pichia toletana* and *Pichia xylosa* form an independent lineage and are assigned to the genus *Peterozyma*, which is newly proposed.

- 8 Kurtzman CP and Suzuki M 2010 Phylogenetic analysis of ascomycete yeasts that form coenzyme Q-9 and the proposal of the new genera *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces* and *Scheffersomyces*. *Mycoscience* 51:2-14.

Species assigned to the genera *Debaryomyces*, *Lodderomyces*, *Spathaspora*, and *Yamadazyma*, as well as selected species of *Pichia* and *Candida* that also form coenzyme Q-9, were phylogenetically analyzed from the combined sequences of the D1/D2 domains of the large subunit and the nearly complete small subunit rRNA genes. Species assigned to *Debaryomyces* partitioned into three clades and species assigned to *Pichia* were distributed among six clades. These well-

supported clades were interpreted as genera, and from this analysis, the following new genera are proposed: *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces*, and *Scheffersomyces*. The genus *Schwanniomyces* was reinstated and emended, and the genus *Yamadazyma* was phylogenetically defined. From this study, 23 new combinations and 3 new ranks are proposed. The preceding genera are members of a single, large clade.

- 9 Janisiewicz WJ, Kurtzman CP and Buyer JS 2010 Yeasts associated with nectarines and their potential for biological control of brown rot. *Yeast* 27:389-398.

Resident fruit microflora has been the source of biocontrol agents for the control of postharvest decay of fruits and the active ingredient in commercialized biocontrol products. With the exception of grapes and apples, information on the resident microflora of other fruits is only fragmentary, but greater knowledge in this area can be very helpful in developing biocontrol strategies. We characterized the yeast microflora of nectarines ('Croce del Sud') from the early stages of fruit development until harvest. The fruit samples were collected from trees in an unmanaged orchard. The resident fruit microflora was separated from the occasionally deposited microorganisms by discarding initial fruit washings before the final wash, followed by sonication and plating on NYDA medium. The isolated yeasts

were identified by BIOLOG and by sequencing the D1/D2 domain of a large subunit of the rRNA gene and, where available, the ITS sequence. BIOLOG identified 19 and the genetic analysis 23 species of yeasts. Although the identification by these two systems was not always the same, the predominant yeasts were *Rhodotorula* spp., *Sporidiobolus* spp., *Cryptococcus* spp., *Pichia* spp., *Candida* spp. and yeast-like *Aureobasidium pullulans*. Several of the taxa appear to represent new species. The preliminary biocontrol tests against brown rot of nectarine fruit caused by *Monilinia fructicola* indicates significant decay control potential of some of the identified yeast species, namely *Cryptococcus magnus*, *Cryptococcus* sp. nov., *Sporidiobolus pararoseus*, *A. pullulans* and *Rhodotorula* sp. nov.

- 10 Kurtzman CP, Price NP, Ray KJ, Kuo TM 2010 Production of sophorolipids biosurfactants by multiple species of the *Starmerella (Candida) bombicola* yeast clade. *FEMS Microbiol Lett* 311: 140-146.

Sophorolipids are carbohydrate-based, amphiphilic biosurfactants that are of increasing interest for use in environmentally benign cleaning agents. Sophorolipid production was tested for 26 strains representing 19 species of the *Starmerella* yeast clade, including *Starmerella bombicola* and *Candida apicola*, which were previously reported to produce sophorolipids. Five of the 19 species tested showed significant production of sophorolipids: *S. bombicola*, *C. apicola*, *Candida riodocensis*, *Candida stellata* and a new species, *Candida* sp.

NRRL Y-27208. A high-throughput matrix-assisted laser desorption/ionization-time of flight MS assay was developed that showed *S. bombicola* and *C. apicola* to produce a lactone form of sophorolipid, whereas *C. riodocensis*, *C. stellata* and *Candida* sp. NRRL Y-27208 produced predominantly free acid sophorolipids. Phylogenetic analysis of sequences for the D1/D2 domains of the nuclear large subunit rRNA gene placed all sophorolipid-producing species in the *S. bombicola* subclade of the *Starmerella* clade.

- 11 Peter G, Dlauchy D, Tornai-Lehoczki J, Suzuki M, Kurtzman CP 2010 *Spencermartinsiella* gen. nov. and *Spencermartinsiella* sp. nov., a new member of the family Trichomonascaceae. *International Journal of Systematic and Evolutionary Microbiology*. In press.

Ten strains of a novel heterothallic yeast species were isolated from rotten wood collected at different locations in Hungary. Analysis of gene sequences for the D1/D2 domain of the large subunit ribosomal RNA, as well as analysis of concatenated gene sequences for the nearly complete nuclear large subunit rRNA, nuclear small subunit rRNA, and translation elongation factor 1-alpha, placed the novel species in the family Trichomonascaceae, but showed that it is distinct from all

currently recognized genera. The name *Spencermartinsiella europaea* is proposed to accommodate the new genus and species. *Spencermartinsiella europaea* can also be distinguished from currently recognized species of neighbouring genera on the basis of standard phenotypic characters. The type and isotype strains of *Spencermartinsiella europaea* are NCAIM Y.01817T (NRRL Y-48265T, CBS 11730T) and NCAIM Y.01819I (NRRL Y-48266I, CBS 11731I), respectively.

- 12 Kurtzman CP 2010 Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. Antonie van Leeuwenhoek. In press.

In this review, the phylogeny of the ascomycetous yeasts is discussed, with emphasis on the genus *Pichia* and its synonym *Hansenula*. The genus *Pichia*, as defined from phenotype, had nearly 100 assigned species, but the number of species has been reduced to 20 following phylogenetic

circumscription on *Pichia membranifaciens*, the type species of the genus. The remaining species of *Pichia* have been reassigned to 20 different genera, many of which are newly described, such as *Wickerhamomyces*. The reason for reclassification of *Pichia anomala* in the genus *Wickerhamomyces* is discussed.

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**X Yeast Group, Department of Microbiology, Faculty of Science, Kasetsart University, Jatujak, Bangkok 10900, Thailand. Communicated by Savitree Limtong <[fscistl@ku.ac.th](mailto:fscistl@ku.ac.th)>.**

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New yeast species in Thailand proposed by our group since 2004.

- 1 Nitiyon S, C Boonmak, S Am-In, S Jindamorakot, H Kawasaki, W Yongmanitchai and S Limtong 2010 *Candida saraburiensis* sp. nov. and *Candida prachuapensis* sp. nov., two xylose-utilizing yeast species isolated in Thailand. Int J Syst Evol Microbiol - doi: 10.1099/ijms.0.023317-0
- 2 Am-In S, S Limtong, W Yongmanitchai and S Jindamorakot 2010 *Candida andamanensis* sp. nov., *Candida laemsonensis* sp. nov., and *Candida ranongensis* sp. nov., three anamorphic yeast species isolated from estuarine waters in a mangrove forest in Ranong Province, Thailand. Int J Syst Evol Microbiol doi:ijms.0.022038-0
- 3 Limtong S, R Kaewwichian, S Am-In, T Nakase, CF Lee and W Yongmanitchai 2010 *Candida asiatica* sp. nov., anamorphic ascomycetous yeast species isolated from natural samples in Thailand, Taiwan and Japan. Antonie van Leeuwenhoek 98:475-481.
- 4 Limtong S and W Yongmanitchai 2010 *Candida chanthaburiensis* sp. nov., *Candida kungkrabaensis* sp. nov. and *Candida suratensis* sp. nov., three novel yeast species from decaying plant materials submerged in water of mangrove forest. Antonie van Leeuwenhoek. 98(3): 379-388.
- 5 Keawwichian R, W Yongmanitchai, N Srisuk, K Fujiyama and S Limtong 2010 *Geotrichum siamensis* sp. nov. and *Geotrichum phurueaensis* sp. nov., two asexual arthroconidial yeast species isolated in Thailand. FEMS Yeast Res.10:212-220.
- 6 Limtong S, R Kaewwichian, S Am-In, C Boonmark, S Jindamorakot, W Yongmanitchai, N Srisuk, H Kawasaki and T Nakase 2010 Three anamorphic yeast species *Candida sanitii* sp. nov., *Candida sekii* sp. nov. and *Candida suwanaritii*, three novel yeasts in the *Saturnispora* clade isolated in Thailand. FEMS Yeast Res 10:114-122.
- 7 Limtong S, W Yongmanitchai, H Kawasaki and K Fujiyama 2009 *Wickerhamomyces edaphicus* sp. nov. and *Pichia jaroonii* sp. nov., two ascomycetous yeast species isolated from forest soil in Thailand. FEMS Yeast Research. 9(3): 504-510.
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- 10 Limtong S, W Yongmanitchai, H Kawasaki and T Seki 2008 *Candida phangngensis* sp. nov., an anamorphic yeast species in the *Yarrowia* clade, isolated from water in mangrove forests in Phang-Nga Province, Thailand. Int J Syst Evol Microbiol 58:515-519.

- 11 Limtong S, N Srisuk, W Yongmanitchai, H Yurimoto and T Nakase 2008 *Ogataea chonburiensis* sp. nov. and *Ogataea nakhonphanomensis* sp. nov., two thermotolerant, methylotrophic yeast species isolated in Thailand and the transfer of *Pichia siamensis* and *Pichia thermomethanolica* to the genus *Ogataea*. Int J Syst Evol Microbiol 58:302-307.
- 12 Limtong S, Y. Imanishi S, Jindamorakot S, Ninomiya W, Yongmanitchai, T Nakase 2008 *Torulasporea maleeae* sp. nov., a novel ascomycetous yeast species from Japan and Thailand. FEMS Yeast Res 8:337-343.
- 13 Limtong S, W Yongmanitchai, H Kawasaki and T Seki 2007 *Candida thaimueangensis* sp. nov., an anamorphic yeast species from estuarine water in mangrove forest, Thailand. Int J Syst Evol Microbiol 57:650-653.
- 14 Limtong S, W Yongmanitchai, MM Tun, H Kawasaki and T Seki 2007 *Kazachstania siamensis* sp. nov., an ascomycetous yeast species from forest soil in Thailand. Int J Syst Evol Microbiol . 57:419-422.
- 15 Limtong S, N Srisuk, W Yongmanitchai, H Yurimoto, T Nakase and N Kato 2005 *Pichia thermomethanolica* sp. nov., a novel thermotolerant, methylotrophic yeast isolated in Thailand. Int J Syst Evol Microbiol 55:2225-2229.
- 16 Sumpradit T, S Limtong, W Yongmanitchai, H Kawasaki and T Seki 2005 *Tetrapisispora namnaoensis* sp. nov., a novel ascomycetous yeast species isolated from soil of Nam Nao national Park, Thailand. Int J Syst Evol Microbiol 55:1735-1738.
- 17 Limtong S, N Srisuk, W Yongmanitchai, H Kawasaki, H Yurimoto, T Nakase and N Kato 2004 Three new species of thermotolerant methylotrophic yeasts, *Candida krabiensis* sp. nov., *Candida sithepensis* sp. nov., *Pichia siamensis* sp. nov., isolated in Thailand. J Gen Appl Microbiol 50:119-127.

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**XI 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](mailto:mmalfeito@isa.utl.pt)>.**

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Recent publications.

- 1 Tristezza M, Lourenço A, Barata A, Brito L, Malfeito-Ferreira M, Loureiro V 2010 Susceptibility of wine spoilage yeasts and bacteria in the planktonic state and in biofilms to disinfectants. Ann Microbiol 60:549–556.

The aim of this work was to determine the ability of six yeast and two bacterial species associated with wine spoilage to form biofilm in mono- or co-culture using the *Calgary Biofilm Device* (CBD). Moreover, the efficacy of several disinfectants was evaluated against these spoilage microorganisms, both in planktonic and in biofilm state. Results showed that *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe* and *Acetobacter aceti* formed biofilm both in wine and in synthetic medium. *Zygosaccharomyces bailii* formed biofilm only in wine and *Pichia guilliermondii* and *Lactobacillus hilgardii* formed biofilm only in synthetic medium. In wine, *D. bruxellensis* presented the same biofilm population when grown in pure culture or in mixed culture with acetic acid bacteria. There was a 3 log increase in biofilm formed by *A. aceti* in mixed culture with *L. hilgardii*.

Alkaline chlorine-based disinfectant was the most effective in decontaminating spoilage yeast and bacteria both in planktonic and biofilm tests. Sodium hydroxide-based detergents and peracetic-based disinfectant were also efficient against suspended cells but at least 10-fold more concentrated solutions were needed to remove biofilm. Furthermore, the results showed that, except for the neutral detergent VK10, the tested agents were actually effective when used under the conditions recommended by manufacturers. In any case, biofilms showed greater tolerance to biocides when compared to the same microorganisms in planktonic state. To our knowledge, this is the first study in which the CBD is used to assess the ability of wine spoilage microorganisms to form biofilm and their susceptibilities to disinfectant agents.

- 2 Malfeito-Ferreira M 2010 Yeasts and wine off-flavours: a technological perspective. *Ann Microbiol - In press*.

In wine production, yeasts have both beneficial and detrimental activities. *Saccharomyces cerevisiae* is the main responsible for turning grape juice into wine but this species and several others may also show undesirable effects in wines. Among these, technologists are particularly concerned with the production of off-flavours that may occur during all stages of winemaking. Typical spoiling activities include the production of ethyl acetate by apiculate yeasts before fermentation, of hydrogen sulphide by *S. cerevisiae* during fermentation phases, of acetaldehyde by film-forming yeasts during bulk storage, and of volatile phenols by *Dekkera bruxellensis* during storage or after bottling. The occurrence of these hazards depends on the

technological operations designed to obtain a given type of wine and most of them may be avoided by current preventive or curative measures. On the contrary, good manufacturing practices must be strengthened to deal with the problem of volatile phenol production in red wines. Appropriate monitoring of *D. bruxellensis* populations and quantification of 4-ethylphenol is advised during storage, particularly when oak barrels are used, and absence of viable cells must be guaranteed in bottled wines. This work is based on our experience at winery level, aiming to provide adequate technological strategies to deal with the problem of off-flavours produced by yeasts.

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**XII State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <[gnaumov@yahoo.com](mailto:gnaumov@yahoo.com)>.**

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We are grateful to Dr. Ching-Fu Lee (National Hsinchu University, Taiwan) for collaboration during our stay in his lab and yeast expeditions in October-November 2010. Many thanks to Kyria Boundy-Mills (Davis, USA) and Justin Fay (St. Louis, USA) for the possibility to visit their labs in May 2010 and fruitful discussions.

The following are papers for 2010 or in press.

- 1 Naumoff DG, Naumov GI 2010 Discovery of novel family of  $\alpha$ -glucosidase genes *IMA* genes in yeast *Saccharomyces cerevisiae*. *Doklady Biochem Biophys* 432:114-116.
- 2 Naumova ES, Naumov GI, Barrio E, Querol A 2010 Mitochondrial DNA polymorphism of the yeast *Saccharomyces bayanus* var. *uvarum*". *Microbiology (Moscow)* 79:520-527.

Genetic relationships among forty-one strains of *Saccharomyces bayanus* var. *uvarum* isolated in different wine regions of Europe and four wild isolates were investigated by restriction analysis (RFLP) of mitochondrial DNA (mtDNA) with four restriction endonucleases, *AluI*, *DdeI*, *HinfI* and *RsaI*. No clear correlation between origin and source of isolation of *S. bayanus* var. *uvarum* strains and their mtDNA restriction

profiles was found. On the whole, the mtDNA of *S. bayanus* var. *uvarum* is much less polymorphic than that of *S. cerevisiae*. This observation is in good agreement with results obtained by electrophoretic karyotyping. Unlike wine *S. cerevisiae*, strains of *S. bayanus* var. *uvarum* display a low level of chromosome length polymorphism.

- 3 Naumova ES, Naumov GI, Michailova YuV, Martynenko NN, Masneuf-Pomarède I 2010 Genetic diversity study of the yeast *Saccharomyces bayanus* var. *uvarum* reveals introgressed subtelomeric *Saccharomyces cerevisiae* genes. *Research in Microbiology* (in press).

Intraspecies polymorphism of the yeast *S. bayanus* var. *uvarum* was studied using polymerase chain reaction with a microsatellite primer (GTG)<sub>5</sub>. Sixty-nine strains of different origins were analyzed. There is a correlation between PCR patterns of the strains and source of their isolation: type of wine and particular winemaking region. Southern hybridization

analysis revealed introgression between *S. cerevisiae* and *S. bayanus* var. *uvarum*, for the first time. Two strains isolated from alcoholic beverage in Hungary and identified by genetic analysis as *S. bayanus* var. *uvarum* were found to harbor a number of *S. cerevisiae* subtelomeric sequences: *Y'*, *SUC*, *RTM* and *MAL*.

- 4 Naumov GI, Naumova ES 2010 Comparative genetics of *Saccharomyces cerevisiae* yeasts. Chromosomal translocations carrying the *SUC2* marker. *Russian J Genet* 46(11) (in press).
- 5 Naumov GI, Naumova ES 2010 Genetic identification of cultured *Saccharomyces* yeasts from Africa. *Microbiology (Moscow)* (in press).

On the basis of genetic hybridization analysis and molecular karyotyping, 11 strains isolated from various

fermentations in different sites of Africa were reidentified as *S. cerevisiae*. Pronounced chromosome length polymorphism

was found in the yeasts. The fertile genetic lines created are of interest for evolutionary genetics and breeding of *Saccharomyces*

yeasts..

- 6 Naumova ES, Serpova EV, Korshunova IV, Naumov GI 2010 Molecular polymorphism of  $\alpha$ -galactosidase *MEL* genes of *Saccharomyces* yeasts. Microbiology (Moscow) (in press).

To infer the molecular diversity of *Saccharomyces* yeasts and finding new  $\alpha$ -galactosidase *MEL* genes we conducted molecular genetic analysis of melibiose fermenting *Saccharomyces* strains isolated from fermentative processes and natural sources in different world regions. *S. bayanus*, *S. mikatae* and *S. paradoxus* were shown to have only one *MEL* copy and

do not accumulate polymeric genes as some *S. cerevisiae* populations. Polymeric genes *MELp1* and *MELp2* are identified in *S. paradoxus*, for the first time. The genes having 98.7% of identity are located on chromosomes X and VI, respectively. Phylogenetic analysis indicates that *MEL* genes of *Saccharomyces* yeasts are species specific.

- 7 Naumov GI 2010 Molecular and genetic differentiation of small-spored species of the genus *Metschnikowia* Kamienski. Microbiology (Moscow) (in press).

Literature data on molecular reidentification of the rejected genus *Chlamydozyma* are summarized. The yeasts *Chl. pulcherrima* Wickerham (1964) and *Chl. reukaufii* Wickerham (1964) were shown to represent sibling species *Metschnikowia pulcherrima* Pitt et Miller (1968) and

*M. reukaufii* Pitt et Miller (1968), respectively. It is proposed to reinstate the species *M. zygota* (Wickerham) Fell et Hunter (1968). Parasexual cycle of the yeast *Metschnikowia* is discussed.

- 8 Kondratieva VI, Naumov GI 2010 Population antagonism in the yeasts *Schizosaccharomyces pombe*. Ecological genetics (St. Petersburg) (in press) (in Russian).

Using the new yeast *Schizosaccharomyces kambucha* nom. nud. and genetic lines, widely explored in different laboratories, we continue the investigation of the phenomenon of ascospore death in interstrain hybrids of *Sch. pombe*. All interstrain hybrids were sterile when analyzed by a micromanipulator. However random spore analysis revealed

recombination of control markers, suggesting assignment of the strains studied to the same biological species *Sch. pombe*. Possible causes of hybrid ascospores death are discussed. The population antagonism of the yeast *Sch. pombe* should be taken into account in taxonomic studies.

- 9 Naumoff DG, Naumov GI 2010 New family of isomaltase genes *IMA* in *Saccharomyces cerevisiae*. Proceedings of the 3rd International Conference on Mathematical Biology and Bioinformatics, Pushchino, Russia, 2010, pp. 135-136.

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**XIII Laboratoire INRA, MICALIS UMR, 1319 Micalis, Domaine de Vilvert, F-78352 Jouy-en-Josas, and BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.-V.**  
<[nguyenhv@grignon.inra.fr](mailto:nguyenhv@grignon.inra.fr)>

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Recent publication.

- 1 M Cornet, B Sendid, C Fradin, C Gaillardin, D Poulain, HV Nguyen - In press - Molecular identification of closely related *Candida* species using two ribosomal intergenic spacer fingerprinting methods. J Molec Diagnostics.

Recent changes in the epidemiology of candidiasis highlighted an increase in non-*albicans* *Candida* species emphasizing the need for reliable identification methods. Molecular diagnostics in fungal infections may improve species characterization, particularly in cases of the closely related species in the *Candida* complexes. With a panel of 271 isolates using the IGSAF method<sup>1</sup> developed for the identification of species of the genus *Debaryomyces* we identified *C. parapsilosis*, *C. metapsilosis* and *C. orthopsilosis* and differentiated correctly *C. guilliermondii* (*Pichia guilliermondii*) which was frequently confused with *C. famata* (*Debaryomyces hansenii*)<sup>2</sup>, a yeast present in cheese because used as starter. Species *C. carpphila*, *C. fermentati* and *C. xestobi* of the complex *Pichia guilliermondii*

can also be identified by IGSAF pattern recognition. For *C. albicans*, *C. dubliniensis* and complex *C. glabrata/C. bracarensis/C. nivariensis* the whole IGS could not be amplified so we selected primers to amplify the IGS2 (from the 5S to the 18S); restriction of the amplicons with *NlaIII* gave specific patterns to identify and grouped *C. albicans*, *C. dubliniensis*, *C. glabrata/C. bracarensis/C. nivariensis* and *C. tropicalis*. Sharing similar biochemical patterns, *Pichia norvegensis* and *C. inconspicua* exhibited specific IGSAF profiles. *C. zeylanoides* exhibits a profile clearly different from *C. norvegensis*, these two species have been confused by the use of a wrong strain CBS 1922 type strain of *C. norvegensis*.<sup>3</sup> These two PCR/RFLP methods are quick, in-house and cheap. The time to identify 20

strains is a working day (from an overnight culture), each identification costs around \$4 compared with \$15, the cost to sequence D1/D2. They may be used as reference tools, either alternatively or adjunctively to the existing rDNA (26S or ITS) sequence comparisons.

References:

<sup>1</sup> Nguyen HV, Gaillardin C, Neuveglise C 2000 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*:

## Commentary

*“False facts are highly injurious to the progress of Sciences for they often endure long; but false views, if supported by some evidence, do little harm for every one takes a salutary pleasure in proving their falseness, and when it is done, one path towards error is closed and the road to the truth is often at the same time open.”*

This quote from C. Darwin was cited by A. Panek on one of her numerous publications. In yeast taxonomy or related fields, false facts and false views are frequently induced by misuse of yeast names and or yeast strains. I mentioned in the abstract above the use of the wrong strain CBS 1922 (that we renamed CBS 1922<sup>HF</sup> to distinguish it from CBS 1922<sup>T</sup> and DBVPG 6371<sup>T</sup>). CBS 1922<sup>HF</sup> was in fact a strain of *C. zeylanoides*; its use in the study cited above in reference 3 led the author to conclude that “a discrepancy of minor importance involves the placement of *P. norvegensis*...” For people working with *Saccharomyces*, strain MCYC 623 regarded as *S. bayanus* but we have renamed it *S. uvarum* (Beijerinck). The strain was described by Santa Maria as *S. abuliensis* (or *S. abulensis*) and was deposited first in the MCYC Spanish collection (Microbiology Collection of Yeasts Cultures, Madrid, Spain) and at the same time in the CBS as CBS 7001. The MCYC has since cease to exist and strains of that collection were integrated into the CECT collection (Spanish Type Culture Collection (for details see <http://www.springerlink.com/content/h001360t42735684/>). Curiously strain MCYC 623 is not in the CECT and so can no longer be ordered under that accession number. In this case, MCYC was thought in fact to be NCYC, the acronym of the National Collection of Yeast Cultures Norwich, England. But there is no strain NCYC 623. So it is necessary to refer to the strain as CBS 7001 instead of MCYC 623 so that researchers can obtain the strain from the CBS in order to study it or attempt to reproduce the published results. The erroneous citation of MCYC as NCYC has occurred in publication 3 (above) as well as in Gonzalez et al., 2008<sup>4</sup>. In the Materials and Methods one can read *S. bayanus* (NCYC 623 ; alternatively, CBS 7001, p. 2315) but in the Results MCYC 623 (CBS 7001) reappeared (p. 2317). The change from NCYC to MCYC apparently escaped the three authors of the article and probably the same number of reviewers! As the MCYC collection no longer exists, it would be appropriate to use strain CBS 7001 in all future publications. Moreover, *S. uvarum* (Beijerinck) has been reinstated<sup>5</sup> because

description of *D. vietnamensis* sp. nov. closely related to *D. nepalensis*. FEMS Yeast Res 9:641-662.

<sup>2</sup> Desnos-Ollivier M, Ragon M, Robert V, Raoux D, Gantier JC, Dromer F 2008 *Debaryomyces hansenii* (*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). J Clin Microbiol 46:3237-3242.

<sup>3</sup> Diezmann S, Cox CJ, Schönián G, Vilgalys RJ, Mitchell TG 2004 Phylogeny and evolution of medical species of *Candida* and related taxa: a multigenic analysis. J Clin Microbiol. 42:5624-35.

it is distinct from *S. bayanus* (Saccardo). This proposal is recent and separate from an older proposal to consider *S. uvarum* as a variety of *S. bayanus*. The new valid name should be used, as in a publication Sampaio & Gonçalves (2008),<sup>6</sup> who deposited many more relevant D1/D2 sequences. Resistance to or lack of awareness of this proposal<sup>5</sup> can be seen in use of *S. bayanus* by Liti et al. (2009)<sup>7</sup> and the varieties *uvarum* and *bayanus* by Naumov et al. (2010).<sup>8</sup> In the latter publication, the authors omitted to cite another work of Liti et al. (2006)<sup>9</sup> showing that *S. cariocanus* is a strain of *S. paradoxus* with two translocations that prevent it from being inter-fertile with *S. paradoxus*, illustrating a shortcoming of the Biological Species Concept (BSC). The use of the name *S. cariocanus* should have been discontinued, but it continues to reappear.

References cited

<sup>4</sup> Gonzalez SS, Barrio E, and Querol A 2008 Molecular characterization of new natural hybrids of *Saccharomyces cerevisiae* and *S. kudriavzevii* in Brewing. Appl Environ Microbiol 74:2314–2320.

<sup>5</sup> Nguyen HV and Gaillardin C 2005 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 Res 5:471-483.

<sup>6</sup> Sampaio JP, Gonçalves P 2008 Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. Appl Environ Microbiol. 74:2144-2152.

<sup>7</sup> Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, Davey RP, Roberts IN, Burt A, Koufopanou V, Tsai IJ, Bergman CM, Bensasson D, O’Kelly MJ, van Oudenaarden A, Barton DB, Bailes E, Nguyen AN, Jones M, Quail MA, Goodhead I, Sims S, Smith F, Blomberg A, Durbin R, Louis EJ. 2009 Population genomics of domestic and wild yeasts. Nature 458:337-341.

<sup>8</sup> Naumov GI, Naumova ES, Masneuf-Pomarède I 2010 Genetic identification of new biological species *Saccharomyces arboricolus* Wang et Bai. Antonie van Leeuwenhoek 98:1-7.

<sup>9</sup> Liti G, Barton DB, Louis EJ 2006 Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. Genetics 174:839-850.

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**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 J. P. Sampaio <[jss@fct.unl.pt](mailto:jss@fct.unl.pt)>.**

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The following papers were recently published:

- 1 Coelho MA, Almeida JMF, Martins IM., Jorge da Silva A and Sampaio JP 2010 The dynamics of the yeast community of the Tagus river estuary – testing the hypothesis of the multiple origins of estuarine yeasts. *Antonie van Leeuwenhoek* 98:331-342.
- 2 Coelho MA, Sampaio JP and Gonçalves P 2010 A deviation from the bipolar-tetrapolar mating paradigm in an early diverged basidiomycete. *PLoS Genetics* 6 (8): e1001052.

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**XV Canadian Institute of Fisheries Technology, Dalhousie University, P.O. Box 1000; Halifax, Nova Scotia, Canada B3J 2X4. Communicated by R.A. Speers <[Alex.Speers@dal.ca](mailto:Alex.Speers@dal.ca)>.**

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Recent publications.

- 1 Speers RA, Bendiak D, Caudill J, Cook D, Eck E, Fernandez S, Gibson B, MacLeod A, Parker N, Porter A, Schuba R, Walker S, White L, Voetz M and Powell C 2010 Technical Committee Report: Mini-Fermentation Method. *J ASBC*. 68:231-237.

This collaborative study to develop a small-scale fermentation assay is continuing into its 2nd year. Researchers interested in collaborating should contact <[Alex.Speers@dal.ca](mailto:Alex.Speers@dal.ca)>.

- 2 Patel JK, Speers RA and Lake JC 2010 Colloidal examination of worts associated with premature yeast flocculation. Accepted Oct. *J ASBC*.

This research reports on a study of colloidal properties of yeast fermented in control and premature yeast flocculation ‘PYF’ worts. A lager yeast strain was fermented in both control and PYF worts and analyzed for cell wall properties. Yeast from the PYF wort exhibited more flocculation than the control. The PYF fermentation yielded yeast with less negative zeta potential than the control ( $p < 0.001$ ). When both yeasts were resuspended in beer (filtered through a 10 kDa filter), there was no difference in surface charge of the control or PYF yeast ( $p > 0.05$ ). This implied that wort colloids/trub larger than 10 kDa caused a reduction in surface charge. Cell separation forces estimated by

the floc breakup through a capillary indicated PYF yeast flocs showed higher apparent separation force. The orthokinetic capture coefficients of control and PYF yeast showed significant difference in capture coefficient values between both the yeast suspensions, with the PYF capture coefficient values being higher from 72 h of fermentation onward ( $p < 0.05$ ). A physical mechanism of premature yeast flocculation has been proposed involving electrostatic interaction between wort particles (positively charged towards end of fermentation) and yeast (negatively charged through fermentation).

- 3 Lake JC and Speers RA 2010 Investigations of malt causing PYF. In press: *International Brewers Symposium: Yeast Flocculation, Vitality and Viability*. In Press. R.A. Speers (Ed). Master Brewers Association of the America’s, St. Paul, MN.

Premature Yeast Flocculation (PYF) is loosely defined as the early flocculation of yeast, during brewing, leading to an undesirable end product. Although there is widespread debate the cause and mechanism of PYF remain elusive. In general, PYF is believed to be caused by a polysaccharide with an acidic component. Reported sizes of the compound range from 40 kDa

to greater than 100 kDa. How the PYF inducing compound interacts with the yeast cell remains unknown. In this report a filtration technique used to fractionate wort is explored as a means to isolate and purify PYF inducing compounds naturally without degradation that may occur in other methods of purification such as chromatography methods.

- 4 Porter AV, Lake JC, Gill TA and Speers RA 2010 A method to detect anti-metabolic factors in fermentations. Accepted *J Inst Brew*

Premature yeast flocculation (PYF) has been described as the rapid settling of yeast cells during fermentation despite the presence of sufficient nutrients. PYF can cause negative impacts on beer quality and thus be quite costly to brewers and maltsters.

To investigate the causative agent of PYF, small-scale fermentations were undertaken in both test tubes and cuvettes (15 and 3.5 mL respectively) using worts prepared from PYF-positive and PYF-negative malt samples. Fermentations were carried out

using six malts, for up to seven days. Turbidity and extract values were monitored for all samples. The small-scale (test tube) assay exhibited clear yeast cell flocculation differences between malts. In the cuvette assay the wort fermented, but the yeast cells settled out of suspension rapidly. While this property made the cuvette assay unsuitable for detecting PYF malt, it did allow for measurement of impaired sugar uptake by the yeast independent

of yeast in suspension effects. All wort samples fermented in the cuvette assay showed a similar decline in apparent extract ( $p > 0.05$ ), indicating that (at least in the samples studied) premature yeast flocculation was not caused by a decline in yeast activity. We believe the simple cuvette assay reported here could have application in the measurement of anti-metabolic factors in fermenting media.

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**XVI Food Microbiology Laboratory, Sikkim University, Gangtok 737102, India. Communicated by Jyoti Prakash Tamang <jyoti\_tamang@hotmail.com>.**

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The following papers and books were published during 2010.

- 1 Yonzan H and Tamang JP 2010 Microbiology and nutritional value of *selroti*, an ethnic fermented cereal food of the Himalayas. *Food Biotechnology* 24:227-247.

*Selroti* is an ethnic fermented rice food of the Himalayas. A total of 125 samples of *selroti* batters were collected from different villages and markets of the Himalayas. The microbial population of *selroti* batters showed that lactic acid bacteria (LAB) were present in viable numbers above 108 cfu/g, followed by yeasts at 105 cfu/g. LAB *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Pediococcus pentosaceus* and *Lactobacillus curvatus* and yeasts *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Debaryomyces hansenii*, *Pichia burtonii*, and *Zygosaccharomyces rouxii* were identified. The most prevalent LAB and yeasts in *selroti* batters were *Leuc. mesenteroides* (42.9%) and *S. cerevisiae* (35.6%). Molds and

pathogenic bacteria were not detected. It was observed that seasons affect the development and prevalence of microorganisms in the fermented batters. LAB and yeast strains were screened for their acidifying and coagulating capacity, and it was found that most of the LAB strains acidified with lowering of pH up to 4.3. These strains showed a wide spectrum of enzymatic profiles in commercial API-zym kits. All strains of LAB showed antimicrobial activities under the applied condition. The nutritional value of fermented batters was found to be increased. This is the first report on *selroti* concerning its microbiology and nutritional value.

- 2 Rai AK, Tamang JP and Palni U 2010 Microbiological studies of ethnic meat products of the Eastern Himalayas. *Meat Science* 85:560–567.

Native microorganisms from some ethnic meat products of the Eastern Himalayas such as lang kargyong, yak kargyong, faak kargyong, lang satchu, yak satchu and suka ko masu were isolated and characterized. The bacterial isolates included *Lactobacillus sake*, *Lactobacillus curvatus*, *Lactobacillus divergens*, *Lactobacillus carnis*, *Lactobacillus sanfrancisco*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Enterococcus faecium*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Bacillus subtilis*, *Bacillus mycoides*,

*Bacillus thuringiensis*, *Bacillus lentus* and *Bacillus licheniformis*, *Micrococcus* and *Staphylococcus*. Yeast isolates included *Debaryomyces hansenii*, *Debaryomyces polymorphus*, *Debaryomyces pseudopolymorphus*, *Pichia burtonii*, *Pichia anomala*, *Candida famata* and the mould *Rhizopus* was also identified. Many of the LAB isolates demonstrated some antimicrobial activity, enzymatic activity and a few showed a high degree of hydrophobicity. None of the strains produced biogenic amines.

- 3 Tamang JP 2010 Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values. CRC Press, Taylor & Francis Group, New York, pages 295. ISBN: 9781420093247.

The magnificent Himalayan Mountains, the highest in the world and home to the famed Mount Everest and K2, are also imbued with a rich diversity of ethnic fermented foods. Dr. Jyoti Prakash Tamang, one of the leading authorities on food microbiology, has studied Himalayan fermented foods and beverages for the last twenty-two years. His comprehensive volume, *Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values* catalogs the great variety of common as well as lesser-known fermented foods and beverages in the Himalayan region. This volume begins with an introduction to the Himalayan and the Himalayan food culture. Using a consistent format throughout the book, Dr. Tamang discusses fermented

vegetables, legumes, milk, cereals, fish and meat products, and alcoholic beverages. Each chapter explores indigenous knowledge of preparation, culinary practices, and microorganisms for each product. Additional information on microbiology and nutritive value supplements each section, and discussions on ethnic food history and values as well as future prospects for these foods complete the coverage. Dr. Tamang demonstrates that fermentation remains an effective, inexpensive method for extending the shelf life of foods and increasing their nutritional content through probiotic function, and therefore remains a valuable practice for developing countries and rural communities with limited facilities.

- 4 Tamang JP and Kailasapathy K, Editors 2010 Fermented Foods and Beverages of the World. CRC

Press, Taylor & Francis Group, New York, pages 448. ISBN: 9781420094954.

Did you know? It's estimated that fermentation practices have been around since as early as 6000 BC, when wine was first being made in Caucasus and Mesopotamia. Today, there are roughly 5000 varieties of fermented foods and beverages prepared and consumed worldwide, which accounts for between five and forty percent of daily meals. *Fermented Foods and Beverages of the World* is an up-to-date review on fermentation practices, covering its storied past, cultural aspects, microbiology, biochemistry, nutrition, and functionality. With contributions from 24 seasoned fermentation authorities, this book begins with a concise introduction to food fermentation – one of the oldest biotechnological processes – including its history and global varieties. After covering the various preparation techniques and culinary methods, the book addresses the microbiology-phenotypic and genotypic characterizations, the identifications of functional microorganisms, the functional and technological properties, and issues related to food safety. The book also explores the functional properties of fermentation, how it improves product shelf life, ensures food safety, enriches nutritional supplements, and increases the probiotic functions in some foods. The rising popularity of probiotic and prebiotic foods and the health benefits they are known for are also discussed. Covering many undocumented minor or lesser-known ethnic fermented products, *Fermented Foods and Beverages of the World* is an all-in-one guide to global fermentation practices and consumption behaviors. The book has 16 chapters:

1 Dietary Cultures and Antiquity of Fermented Foods and Beverages: Jyoti Prakash Tamang and Delwen Samuel

2 Diversity of Fermented Foods: Jyoti Prakash Tamang  
3 Diversity of Fermented Beverages and Alcoholic Drinks: Jyoti Prakash Tamang  
4 Functional Yeasts and Molds in Fermented Foods and Beverages: Kofi E. Aidoo and M. J. Robert Nout  
5 Fermented Vegetable Products: Carmen Wachter, Gloria Diaz-Ruiz, and Jyoti Prakash Tamang  
6 Fermented Legumes: Soybean and Non-Soybean Products: Toshirou Nagai and Jyoti Prakash Tamang  
7 Fermented Soybean Pastes Miso and Shoyu with Reference to Aroma: Etsuko Sugawara  
8 Fermented Cereal Products: Jean-Pierre Guyot  
9 Fermented Milk Products: Baltasar Mayo, Mohammed Salim Ammor, Susana Delgado, and Ángel Alegria  
10 Fermented Fish Products: Junus Salampessy, Kasipathy Kailasapathy, and Namrata Thapa  
11 Fermented Meat Products: Martin Adams  
12 Ethnic African Fermented Foods: N. A. Olasupo, S. A. Odunfa, and O. S. Obayori  
13 Tea, Coffee, and Cacao: Ulrich Schillinger, Louis Ban-Koffi, and Charles M. A. P. Franz  
14 Probiotic and Prebiotic Fermented Foods: Kasipathy Kailasapathy  
15 Health Aspects of Fermented Foods: Mariam Farhad, Kasipathy Kailasapathy, and Jyoti Prakash Tamang  
16 Packaging Concepts for Enhancing Preservation of Fermented Foods: Kasipathy Kailasapathy

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

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Recent publications.

1 Golubev WI 2010 A new myo-inositol-positive species of the genus *Rhodotorula*. *Mykologia i Phytopathologia* 44:310-313.

A new species of anamorphic basidiomycetous yeast *Rhodotorula pinalis* was isolated from dead needles of *Pinus sylvestris* L. collected in Moscow region (Russia). Its cultures are non-pigmented, nitrate- and myo-inositol-positive,

ballistoconidia are not formed. This species belongs to the Microbotryales and the closest species was *Sporobolomyces inositophilus*.

2 Golubev WI 2010 *Mastigobasidium* Golubev (1999). In: *The Yeasts, a Taxonomic Study*. 5<sup>th</sup> ed. (C.P. Kurtzman et al., eds.) Elsevier Sci. Publ. B.V., Amsterdam, Vol. 3, 1495-1497.

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**XVIII Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by MA Lachance** <[lachance@uwo.ca](mailto:lachance@uwo.ca)>.

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The following lectures were presented recently on the topic of sequence-based species delineation.

1 Lachance MA Yeast biodiversity and the species concept. 28th International Specialized Symposium on Yeasts, Bangkok, Thailand, September 2010.

The current effort to discover yeast biodiversity involves as a first step the characterization of isolates and the description of new species. One of the many objectives of this

activity is to determine the number of yeast species that exist on our planet. This in turn is predicated on the availability of a clear concept of species. I shall first review how yeast systematists

have perceived and defined species, contrasting various metaphysical viewpoints on the very nature of species. I shall then review the criterion that is currently applied broadly, namely the extent of variation in barcoding sequences such as the D1/D2 variable domains of the LSU rRNA gene. Kurtzman and Robnett (1998) observed that members of well-defined ascomycetous yeast species rarely differ by more than three substitutions in D1/D2 sequence and that representatives of different species tend to show at least 1% divergence in that sequence. Their empirical observation has seldom if at all been discussed in a theoretical framework, in particular the impact of sampling effort on the amount of sequence diversity that can be detected within a species. Most biologists, including yeast systematists, probably agree that a nomenclatural yeast species should correspond to a real evolutionary unit consisting of individuals that share a

common gene pool. As Darwin put it in 1859, the task of systematists is to recognize species from mere varieties. Hennig (1962) provided a solution to this challenge by distinguishing between two forms of variation, namely phylogenetic variation, which accompanies speciation, and tokogenetic variation, more commonly known as polymorphism. Templeton et al. (1992) developed a statistical test which, when applied to suitable DNA sequences, allows one to draw meaningful boundaries between species in the absence of more objective criteria such as the ability to form fertile progeny. I shall show how haplotype parsimony network analysis can be used to interpret sequence variation. My examples will be drawn from analyses of ITS and D1/D2 sequences of well-sampled species such as *Candida azyma*, *Candida apicola*, *Metschnikowia agaves*, *Starmerella bombicola*, and others.

2 Lachance MA Evolution of the yeast species concept in the age of sequencing. World Federation for Culture Collections 12th conference, Florianopolis, Brazil, September 2010.

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## Essay

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### Molecular Events in Osmoadaptation of *Saccharomyces cerevisiae*

J. Geraldine Sandana Mala, M. Gayathiri, J. Vandhana and C. Rose

Department of Biotechnology, Central Leather Research Institute,

Council of Scientific and Industrial Research (CSIR), Chennai-600020, Tamilnadu, India

Corresponding author: Dr. C. Rose <chellanrose@yahoo.co.uk>

#### Introduction

Osmotic stress is a universal phenomenon from bacteria and plants to humans. Osmotic stress factors tend to destabilize the cellular homeostasis in the organism. Osmoadaptation is a remarkable cellular response to restore the normalcy of living cells. Yeast cells also sense and respond to hypertonicity. We describe here the associated cellular events that are upregulated

insights at any point of time. Osmotic stress factors are multifold, primarily, Na<sup>+</sup>, sorbitol and sucrose etc. performing a direct effect or various pathological conditions that have an indirect mode of action. Sodium chloride affects many parameters of yeast growth including growth rate, yield of biomass, lag phase of growth and cell composition. Suspension of yeasts in hypertonic solutions induces shrinkage of the entire cell envelope (the cell wall and plasma membrane, Figure 1). The earliest event

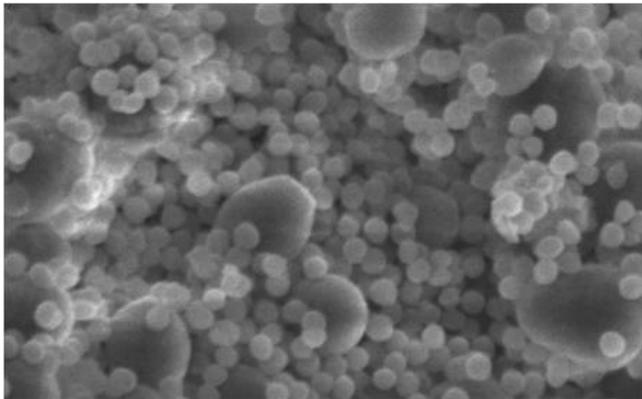


Figure 1A. SEM photograph (5K) of yeast cells at 72h of growth phase in the absence of NaCl. Cells show proliferative growth in peak budding stage.

to overcome the metabolic and cellular disturbances arising from osmotic stress. *Saccharomyces cerevisiae* is a unicellular model system of higher eukaryotes and is a GRAS organism (Generally Regarded As Safe). Osmoadaptation in *S.cerevisiae* is a highly complex and an orchestrated flow of events that require keen

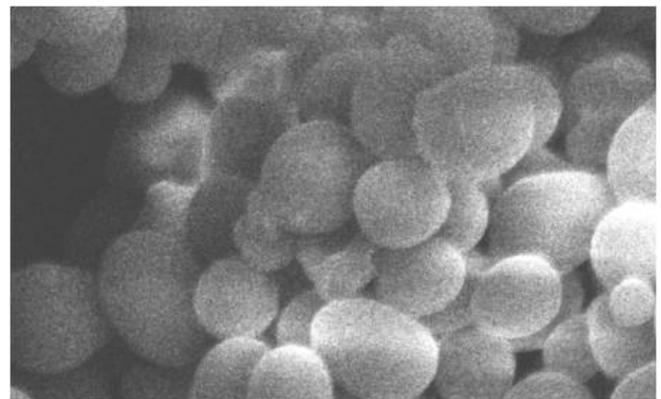


Figure 1B. SEM photograph (8K) of yeast cells at 72h of growth phase in 1M NaCl. Cells show increased cell shrinkage and porosity and aggregation. Budding stage is inhibited.

upon transfer of yeast cells to conditions of high osmolarity is the instantaneous loss of cytoplasmic water, partially compensated by a rapid influx of water from the vacuole. Cells temporarily arrest growth at G1 and G2/M phases of the cell cycle. At a later stage,

glycerol accumulates in the cytoplasm up to molar concentrations in order to counteract dehydration. Depolarization of actin occurs which collapse the cytoskeleton. At physiologically acceptable solute concentrations, cell proliferation resumes after a period of adaptation, the adaptive phase; its duration is dependent on the type and nature of the stress solute, the strain of yeast and the state of growth. Different species of yeast employ different physiological strategies in the adaptive response. These strategic events occur for proper adjustment to new growth conditions. The accumulation of glycerol is a result of the prevention of glycerol efflux out of the cell as well as the synthesis of intracellular glycerol by an increased transcription of the *GPD1* gene which is regulated by the mitogen-activated protein kinase (MAPK) pathway. Gene expression resets to a new steady state and ultimately to the intake of water and swelling of the cell to a critical size which remains smaller than before the osmotic challenge. Growth resumes and cells continue to divide however at a slower rate that is dependent on the effect of the DNA strand breaks. Cell wall organization is adjusted to the new environmental circumstances. Cytoskeleton is repaired and display actin repolarization. The cellular response to osmotic stress is also produced as a cross-effect induced by heat stress or oxidative stress. Hence, osmoadaptation is related to the overall functioning of a cell and induces a cascade of metabolic events or the signaling pathways that generate a wide variety of client proteins which play a protective role in maintenance of cellular homeostasis.

#### Increased osmolyte production

Glycerol and trehalose are two major compatible solutes that account for the adaptation to osmotic stress. We hereby discuss the roles of these osmolytes in osmotic adaptation of yeast.

**Glycerol:** Glycerol is an osmoregulator in *S.cerevisiae* by its accumulation in the cytosol by mechanisms which prevent its efflux from the cell and by increased glycerol synthesis. It is involved in controlling intracellular water activity in osmotically stressed cells. As an immediate response to hyperosmosis, the yeast vacuole participates in an immediate osmoregulatory process permitting survival until osmoadaptive glycerol accumulation occurs. Glycerol accumulation appears to start immediately after exposure to hyperosmolarity, but high levels of intracellular glycerol reach during the late phase of adaptation. The increased glycerol content is a result of enhanced production, increased retention by the cytoplasmic membrane, decreased dissimilation or uptake of external glycerol from the media. By accumulating internal glycerol, cells accomplish regain of turgor. Glycerol metabolism plays an important role in redox balancing and cellular functioning and in growth at elevated temperatures. Salt-stimulated glycerol content leads to lowered osmotic potential of the cytoplasm and re-entry of some of the lost water. The turgor and/or volume parameters of the cell therefore increase toward growth promoting levels. Hence, it is evident that high glycerol concentration at intracellular levels should be sustained for yeast growth.

**Trehalose:** Trehalose accumulates during salt adaptation. Though its primary role is lacking in hyperosmolarity, it assumes the role of glycerol as a compatible solute under metabolic conditions suboptimal for glycerol production. In the absence of glycerol synthesis, trehalose plays a unique role in the regulation

of glycolysis by enabling the yeast to grow on fermentable sugars.

#### High osmolarity glycerol pathway

In eukaryotes, the hyperosmolarity signal is relayed to the transcriptional machinery through regulation of a protein cascade. Yeast cells adapt to hyperosmotic stress by accumulating glycerol by an increased activity of glycerol-3-phosphate dehydrogenase encoded by the *GPD1* gene and by *HOG1* encoding a member of MAPK family and *PBS2* for the MAPK kinase (MAPKK) family. Activation of these protein kinases regulate glycerol synthesis by a signal transduction pathway known as the High Osmolarity Glycerol (HOG) pathway. Mager & Siderius (2002) have reviewed the HOG MAPK pathway in *S.cerevisiae*. Sln1p and Sho1p are upstream receptor proteins which act as osmosensors to activate the HOG pathway. Sln1p is autophosphorylated at an internal His. This phosphate is intramolecularly transferred to Asp residue and then to His in Ypd1p. Subsequent phosphotransfer to Asp in Ssk1p activates the functionally redundant MAPKK kinases (MAPKKKs) namely, Ssk2/Ssk22p, which in turn activates the MAPKK Pbs2p. By a dual phosphorylation MAPK Hog1p is activated. The rapid phosphorylation of Hog1p aids in nuclear translocation and mediates regulation of gene expression by repression as well as activation. Hog1p inactivates the transcriptional repressor Sko1p by phosphorylation which induces the transcription of the sodium pump encoding *ENAI* gene. Simultaneous activation of the transcriptional activator Hot1p results in transcription of the *GPD1* gene. Sho1p is an adaptor protein which has a membrane-docking rather than an osmosensing function. This signal transduction pathway recruits the osmosignaling complex (Cdc42p, Ste20p, Ste50p and Ste11p) to the plasma membrane. The small GTPase Cdc42p recruits Pbs2p complex to the site where the cell wall/membrane structure is most susceptible to osmotic stress. Ste50p kinase activates Ste20p kinase and is therefore a MAPKKK kinase (MAPKKKK) of the HOG pathway since it phosphorylates and activates Ste11p, the MAPKKK leading to the release of the inhibitory N-terminal domain from its C-terminal catalytic domain resulting in Hog1 activation. Fps1p prevents the efflux of glycerol to the extracellular space. The HOG pathway is deactivated by dephosphorylation of Hog1p by the Ser/Thr phosphatases Ptc1p, Ptc2p and Ptc3p and the Tyr phosphatases Ptp2p and Ptp3p. This deactivation is essential under physiological conditions since constitutive Hog1p is lethal.

#### Cell wall integrity

HOG pathway through its MAPK signaling also displays cell wall integrity during osmo-stress conditions. The osmotic shock perturbs the yeast cell wall which leads to the activation of Slt2 MAPK pathway. This results in the upregulation of cell wall related genes which aids in chitin synthesis (Gfa1 and Chs3),  $\beta$ 1,3-glucan synthesis (Gsc2/Fks2) and those of the cell wall proteins present in the outer cell wall. Cell integrity pathway orchestrates changes in cellular morphology by controlling the expression of genes encoding enzymes involved in cell wall metabolism. Control of gene expression is mediated by the MAP kinase cascade.

## Heat shock protein expression

The molecular chaperone HSP90 along with its co-chaperones represents a unique way of controlling cellular signaling by mediating the stability and activation of client proteins involved in signal transduction. HSP90 is required for high osmotic stress response in *Saccharomyces cerevisiae*. In addition to the MAP kinases, other types of kinases also play a role in osmoadaptation. Several kinases and transcription factors require the augmentation of HSP90 (Hsp82p in *S.cerevisiae*) for their folding and activation towards efficient cell signaling. HSP82p functions in association with its co-chaperones such as Sti1p, Sba1p and Cdc37p. The Hsp82 chaperone and its Cdc37p co-chaperone are required for the activation of Ste11p involved in the HOG MAPK pathway. Cdc37p targets protein kinases to Hsp82 and is significant in osmoadaptation of the yeast by control of high osmolarity via the MAPK Kss1p. This is important for the cell wall organization in yeast by a stress responsive MAPK cascade signaling.

## Cell cycle

Cell cycle is temporarily blocked at G1 by a downregulation of Cln3p-Cdc28p kinase, while at the G2/M transition Clb2p-Cdc28p kinase is inhibited. Thereby, yeast cells need to attain proper growth requirements by factors that are favourable for progression of the cell cycle.

## Protein synthesis

During hyperosmotic conditions, protein synthesis is transiently repressed. This is accounted by a cytoplasmic role of Hog1p. It activates Rck2p, a putative calmodulin protein kinase that is responsible for the inhibition of protein synthesis.

## Cellular ATP

Yeast cells exposed to severe osmotic stress in their active proliferation state face rapid growth retardation with an increased ATP demand from biosynthesis. Osmotic stress imposes the activation of ATP futile cycles via trehalose and glycerol turnover to avoid substrate-accelerated death. This salt stress-activated ATP futile cycles hold importance in cellular stress adaptation.

## DNA damage

DNA damage is caused by an increased intracellular ionic strength condition of hyperosmolarity resulting in strand breaks. Such damage is not repaired completely and adapted cells retain the DNA breaks although the cells restore their proliferation rates. DNA damage is a sensor of hyperosmotic stress and is proposed to initiate adaptive signaling cascades. Cell cycle arrest persists until the DNA is repaired. Any failure of DNA repair causes apoptosis to eliminate malignant cells.

## Actin recovery

Osmotic stress induces disassembly of the actin cytoskeleton. Actin depolarization results in osmosensitivity. The conserved actin-interacting MAPKKK Ssk2p/MEKK4 mediates the actin recovery following osmotic stress. Ssk2p requires polarisome proteins to promote efficient polarized actin reassembly and this requirement can be bypassed by overexpression of Ssk2p. It is also understood that Ssk2p acts upstream of tropomyosin and drives actin recovery by an upregulation of actin nucleation activity of the formins.

## Intracellular calcium release

Intracellular  $\text{Ca}^{2+}$  levels act as second messengers in the proper relay of stress signals to cellular responses in unicellular eukaryotes. An hypertonic condition causes a release of the vacuolar  $\text{Ca}^{2+}$  into the cytoplasm by the opening of a mechanosensitive channel in the vacuolar membrane, the Yvc1 (TRP1) transient receptor potential channel (TRP) that senses chemical, thermal or mechanical stimuli. As described earlier, water is deported from the cytoplasm followed by its efflux from the vacuole for compensation to regain turgor. The deformation of the vacuolar membrane is a direct cause of opening of the Yvc1 channel to release of  $\text{Ca}^{2+}$ . Thereby the stress on the cell wall induces  $\text{Ca}^{2+}$  accumulation in anticipation of the need in defense or repair against future osmotic stress suggesting its role in osmoadaptation in yeast.

## Protein expression

Adaptation to salt stress is accompanied by a massive change in global protein synthesis; 8 proteins have been induced 8-fold. A prominent feature of these highly salt-responsive proteins for osmoadaptation is their transient response, exhibiting maximal rate of synthesis during the adaptation period. Glycerol-3-phosphate dehydrogenase was highly induced, while glyceraldehyde-3-phosphate dehydrogenase and enolase decreased during growth in NaCl medium, all related to the flux of glycerol. A comparative study of hyperosmotic stress and oxidative stress indicated the induction of seven new proteins in 1% KCl treatment and three new proteins induced by 0.2 M  $\text{H}_2\text{O}_2$  treatment and seven new proteins in both hyperosmotic and oxidative stress conditions suggesting cross-adaptation.

## Potassium content

Transport of  $\text{Na}^+$  and  $\text{K}^+$  determine the cytoplasmic concentration of  $\text{Na}^+$ . The source of  $\text{Na}^+$  is external depending upon its net uptake, i.e., its influx and efflux. The capacity to transport  $\text{K}^+$  affects  $\text{Na}^+$  tolerance in *S.cerevisiae*. Yeast cells with high-affinity  $\text{K}^+$  transport are more tolerant to high  $\text{Na}^+$  accumulating more potassium and less sodium. Hence, there exists an inverse relationship transport system to maintain  $\text{Na}^+$  content and tolerate sodium-induced metabolic stress.

## Conclusion

The field of osmoadaptation in *Saccharomyces cerevisiae* has received tremendous interests in view of its potential roles in baking and brewery applications. The underlying molecular mechanisms for cell survival in hyperosmotic shock by the consequent adaptation response towards hyperosmolarity are highly significant and conserved across eukaryotes. Osmoadaptation involves a cascade of events for cellular reorganization to overcome osmotic stress factors. The MAPK signaling pathway is of utmost importance in the activation of osmosignaling complex components that serve to induce and upregulate cellular metabolites for the culmination of osmotic stress effects. Thereby, in this context, we have described the associated molecular events that occur and regulate the osmoadaptation to hyperosmolarity in yeast for restoration of cellular function.

## Acknowledgements

The authors thank Dr. A. B. Mandal, Director, CLRI (CSIR), India, for his kind permission to publish this article. The financial assistance extended by CSIR, India, to J. Geraldine Sandana Mala is gratefully acknowledged.

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## Obituary

### Dr. Frank Spencer (1922-2010)

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John Francis Theodore (Frank) Spencer passed away at the Chinook Regional Hospital on Sunday, May 23, 2010 at the age of 88 years. He is the loving father of Carla (Nels) Anderson, Catherine (Ronn) Quinton, Margaret (Richard) Powell, John (Carole) Spencer, Jane (Kevin) O'Brien, and Hugh (Helen) Spencer. He also leaves many grandchildren, great-grandchildren, and great-great-grandchildren. As well, Frank is survived by his brother, Geoffrey (Betty) Spencer, and his sisters, Amy Hatch, Margreta Spencer, and Esther Orchard. He was predeceased by his parents John Arthur and Olga Spencer, his wife Dorothy Higgins Spencer, his former wife Lila Bennett Spencer Huber, his sister Louise Cahoon, grandson Clayton O'Brien and great-grandson Eric Kilistoff.



Frank was born January 18, 1922 in Magrath, Alberta. He had a fine, bright mind and a photographic memory. This served him well as he studied in the Magrath school system where he earned an entrance scholarship to Olds Agricultural School. His studies were interrupted by World War II and he enlisted first in the army, then the air force, serving as a navigational instructor.

Near the end of the war, he married Lila Bennett and together they raised six children. During their marriage, Frank earned a BSc (Agriculture) from the University of Alberta, and an MSc and Ph.D from the University of Saskatchewan.

He became a research microbiologist, working for many years at the Prairie Regional Laboratory of the National Research Council in Saskatoon and then at Goldsmith's College in London, England, and PROIMI in Tucuman, Argentina. He is the author of over 200 scholarly papers and numerous books, the last 3 published in 2004. Perhaps his best known academic writing was the entry on yeasts found in the Canadian Encyclopedia ([www.thecanadianencyclopedia.com](http://www.thecanadianencyclopedia.com)). He is renowned for his substantial contributions to the field of yeast taxonomy and genetics. In addition to his achievements in science Frank had a keen interest in music, the arts and politics, believing that scientific knowledge and discovery were relevant to all areas of society. He also enjoyed working with his hands and built many things including a cedar canoe and several period instruments such as harpsichords, clavichords and spinets.

In 2006 ill health forced Frank to move to Lethbridge, Alberta where he could be close to family. He lived at the Good Samaritan Society West Highland Centre and developed a close friendship with Lorraine Nickel, his constant companion during his last years.

Frank will be greatly missed by his family, friends and colleagues. He also leaves many dear friends in Argentina, especially Eric and Norma Fengler, who supported him through difficult times. All will mourn his loss.

Courtesy of Dr. Spencer's family.

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## **Forthcoming Meetings**

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### **39<sup>th</sup> Annual Conference on Yeasts of the Czech and Slovak Commission on Yeasts 3-6 May 2011, Smolenice Castle, Slovakia**

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The 39<sup>th</sup> Annual Conference on Yeasts is still being planned for 3-6 May 2011 at Smolenice Castle, Slovakia. On-line registration will be opened in December. All information will be updated on the following website: <http://www.chem.sk/yeast>.

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### **Yeasts as Models and Tools, Madrid May 10-11, 2011**

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A symposium entitled “Yeasts as Models and Tools”, sponsored by the Fundación Ramón Areces and coordinated by C. Gancedo will take place in Madrid May 10-11, 2011. The following speakers are scheduled: A. Aguilera, Sevilla (Spain), J. Ariño, Barcelona (Spain), U. Brandt, Frankfurt am Main (Germany), C.L. Flores, Madrid (Spain), J.M.gancedo, Madrid (Spain), D.G. Hardie, Dundee (United Kingdom), E. Herrero, Lleida (Spain), M. Kaeberlein, Seattle (USA), K. Natter, Graz (Austria), E. Lesuisse, Paris (France), S.E. Mole, London (United Kingdom), M. Molina, Madrid (Spain), F. Moreno, Oviedo (Spain), J.M. Siverio, La Laguna (Spain), D.J. Thiele, Durham, (USA), I. van der Klei, Groningen (the Netherlands).

For further information, please contact: [jrvillanueva@fundacionareces.es](mailto:jrvillanueva@fundacionareces.es)

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### **ISSY 29 - The Relevance of Yeasts and Microbial Consortia in Traditional and Industrial Fermentations Guadalajara, México, August 29-September 2 2011**

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The 29th International Specialized Symposium of Yeasts will take place at the Hotel El Camino Real in Guadalajara, México, August 29 to September 2, 2011. The meeting will be organized by Dr. Patricia Lappe. A website is in preparation. A poster announcement is available on the YNL website: <http://publish.uwo.ca/~lachance/ISSY29small.jpg>

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### **Non-Conventional Yeasts in the Postgenomic Era Lviv, Ukraine, September 11-14 2011**

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For information on this meeting, please consult [www.ncy2011.org](http://www.ncy2011.org)

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For up-to-date announcements on other forthcoming meetings, please see the YNL website

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

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## Positions Available

### Eleven early stage researchers/PhD positions and two experienced researchers/postdoctoral positions within the Marie Curie Initial Training Network (ITN) *Cornucopia*

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*Cornucopia* is the acronym for the EU Marie Curie Initial Training Network: “Yeast biodiversity as a source of innovations in food and health”. The Network is coordinated by Lund University, Sweden, and the participating institutions are as follows. Positions available are given in brackets:

Lund University, Sweden (2 PhD)  
Copenhagen University, Denmark (1 PhD)  
Flanders Institute of Biotechnology, Leuven, Belgium (2 PhD)  
University of Milan, Italy (1 PhD)  
Institute of Physiology, Prague, Czech Republic (1 PhD)  
CBS Fungal Biodiversity Centre, Utrecht, The Netherlands (1 PhD)  
Spanish National Research Council, Valencia (1 PhD)  
Carlsberg A/S, Copenhagen, Denmark (1 PhD; 1 PostDoc)  
Christian Hansen A/S, Hoersholm, Denmark (1 PhD)  
NIZO, Ede, The Netherlands (1 PostDoc)

Each position will be advertised at the Institution’s web page at the European Commission-*Euraxess* Jobs Portal. A majority of the positions have a deadline before January 31, 2011. Candidates may apply for one or more of the positions with individual preferences of the choices. The positions will also be advertised through the ten *Cornucopia* partner web pages.

#### Job summary

For the eleven selected Marie Curie early stage fellows the *Cornucopia* Consortium will organize academic training in the form of a research project within the scope of the *Cornucopia* research focus (see below), consortium courses and workshops, short visits at different member institutions, as well as institutional post-graduate courses, with the objective to gain a PhD degree. For the two selected Marie Curie postdoctoral Fellows the *Cornucopia* Consortium will organize academic training in the form of a research project within the scope of the *Cornucopia* research focus, consortium courses and workshops, short visits at different member institutions, as well as institutional post-graduate courses, with the objective to promote their career within biological sciences and related industry. For all Marie Curie Fellows EU restrictions

#### Project summary

*Cornucopia* will train a new generation of young scientists focusing on less studied yeasts with interesting traits, which could be applied in the food and health sectors. Yeasts are a divergent group of fungi that predominantly exist as unicellular organisms. The baker's yeast *Saccharomyces cerevisiae* is by far the best known because of its role in producing beverages, baking and recombinant drugs, such as insulin. *S. cerevisiae* is also the main model for the analysis of

regarding mobility apply. Applicants for the PhD positions must hold a MSc degree or equivalent, and for the postdoctoral positions, a PhD degree or equivalent. The previously obtained degrees should be in the area of Biological Sciences, Biotechnology, Chemistry and Chemical Engineering, Medical Sciences or equivalent. The PhD applicants should have less than 4 years of research experience and the postdoctoral candidates should be in the possession of a Doctoral degree and be within their first five years of research experience. We seek talented, motivated, enthusiastic, creative and mobile young scientists with a strong commitment to research. Excellent communication skills, both oral and written, are important. Candidates should seek further information from the provided contacts for each consortium partner, as detailed below.

common features of all eukaryotic cells, and has been used in pioneering the development of several molecular biology, genomics and post-genomic tools. However, the yeast kingdom includes more than 1,500 other species that display a variety of unusual characteristics, and play an important role in their natural environments, but have so far been only poorly studied. These yeasts represent a large untapped potential to develop novel food and health related processes and products. We will

make use of thousands of strains available within *Cornucopia* to screen, using a variety of microbiological, analytical chemistry and bioinformatics techniques, for traits of interest to industry, such as ethanol-, acid- and osmo-tolerance, aromatic and off-flavor compounds and probiotic properties. Our young researchers will develop novel species-specific molecular, genetic and post-genomic tools to find out which genes determine the superior traits. They will “domesticate” new isolates so that they can be easily

#### **Further requirements**

Applications should contain the following a letter of motivation, a CV, a list of publications, copies of the university degree(s) and courses taken, letters of reference from former supervisors. Indications/Ranking

#### **Further information about the available positions**

For each position please contact the provided e-mail address. Each position will also be advertised on the participating consortium member institution web page and other public sources. A majority of the positions have a dead-line before 31<sup>st</sup> January, 2011, but find out about the details on the detailed advertisement for each

#### **Two PhD positions at Lund University, Sweden**

Project: Investigation of genes behind carbon metabolism, aromas, and probiotic properties in nonconventional yeasts, using molecular biology, *in vitro* evolution and genetic tools. Experience with yeast

#### **One PhD position at Copenhagen University, Denmark**

Project: Identification of new probiotic yeasts, which have an inhibitory effect on enteropathogens, and investigations of yeast interactions with bacteria. Candidates should have experience with microbial cultivation, molecular biology methods, and food

#### **Two PhD positions at the Flanders Institute of Biotechnology, Belgium**

Project: Investigation of the molecular basis of superior stress tolerance in nonconventional yeast species and transfer of the genetic elements to industrial *Saccharomyces cerevisiae* strains. Applicants should have experience with recombinant DNA technology and molecular biology methods. Experience with yeast

#### **One PhD position at the University of Milan, Italy**

Project: Investigation on carbon metabolism in nonconventional yeast species in relation to extreme physiological traits and aroma characteristics, and development of fermentation processes. Applicants should have experience with microbial cultivation and molecular biology methods. Experience with yeast

handled in the lab, and develop scale-up cultivations for applied purposes. We will benefit from yeast biodiversity and open new avenues within fundamental and applied research. *Cornucopia* consists of seven leading yeast academic laboratories and three leading European industry partners, will provide a unique environment to develop strong academia and industry oriented careers, in-depth training in major experimental technologies used in yeast research and the industrial application of innovative ideas.

of the favorite institution within the Consortium Appointments is made in accordance with the current regulations and supplementary rules with guidelines for the employment as Research Fellows.

position by contacting the relevant consortium participant or by reading his/her contact home page. Further information is also available from: [Jure.Piskur@cob.lu.se](mailto:Jure.Piskur@cob.lu.se) and each responsible contact person (see below).

molecular genetics and/or fermentation techniques is an advantage. Further information is available from Jure Piskur at [Jure.Piskur@cob.lu.se](mailto:Jure.Piskur@cob.lu.se) and further details how to apply will also be at the job portal of [www.lu.se](http://www.lu.se).

microbiology. In addition, experience with yeast fermentation, immunology or bioimaging are an advantage. Further information is available from Lene Jespesen at [lj@life.ku.dk](mailto:lj@life.ku.dk) or Nils Arneborg at [na@life.ku.dk](mailto:na@life.ku.dk)

molecular genetics is an advantage. Further information is available from Johan Thevelein at [johan.thevelein@bio.kuleuven.be](mailto:johan.thevelein@bio.kuleuven.be) and further details how to apply will also be available at the job portal of KULeuven Arenberg Doctoral School (<http://phd.kuleuven.be/set/>).

fermentation is an advantage. Further information is available from Concetta Compagno at [concetta.compagno@unimi.it](mailto:concetta.compagno@unimi.it) and further details how to apply will also be available at the job portal of [www.unimi.it](http://www.unimi.it).

### **One PhD position at the Institute of Physiology, Czech Republic**

Project: Investigation of the molecular basis of transport-related high osmotolerance in nonconventional yeast species and transfer of the responsible genetic elements to industrial yeast strains. Applicants should have experience with recombinant DNA technology and other molecular biology methods. Experience with yeast

molecular genetics is an advantage. Further information is available from Hana Sychrova at [sychrova@biomed.cas.cz](mailto:sychrova@biomed.cas.cz) and further details how to apply will also be available at the job portal of <http://www.biomed.cas.cz/fgu/en/index.php>.

### **One PhD position at the CBS Fungal Biodiversity Centre, Utrecht, The Netherlands**

Project: search for nonconventional yeasts with extreme traits. Applicants should have a strong interest in functional aspects of yeast biodiversity. Experience in yeast physiology, microbial screenings, genome data mining and management, bioinformatics and yeast

molecular biology are an advantage. Further information is available from Teun Boekhout at [t.boekhout@cbs.knaw.nl](mailto:t.boekhout@cbs.knaw.nl) and further details how to apply will also be available at the job portal of [www.cbs.knaw.nl](http://www.cbs.knaw.nl).

### **One PhD position at the Spanish National Research Council, Valencia**

Project: Screening for yeast species/strains exhibiting traits/properties, like aromas and high ethanol production relevant for wine industry. Investigation of the molecular basis for the appearance of aromatic compounds and glycerol metabolism in nonconventional yeasts, and transfer of the corresponding genes into industrial strains. The applicant should have experience

with DNA recombinant methods. Experience with yeast physiology and molecular genetics is an advantage. Further information is available from Amparo Querol at [aquerol@iata.csic.es](mailto:aquerol@iata.csic.es) and further details how to apply will also be available at the job portal of <https://sede.csic.gov.es/servicios/formacion-y-empleo/convocatorias/personal-laboral>.

### **One PhD position at Carlsberg A/S, Copenhagen, Denmark**

Project: Genetic analysis of specific yeast traits includes a focus on transcriptional circuits and signal transduction pathways to monitor growth and proliferation. Comparative biology will be used to specify particular genetic traits, like cell-cell adhesion/flocculation. Applicants should have

experience with fluorescence microscopy and image analysis. Computational skills in this area are beneficial and experience with fungal molecular genetics is an advantage. Further information is available from Juergen Wendland at [jww@crc.dk](mailto:jww@crc.dk).

### **One postdoctoral position at Carlsberg A/S, Copenhagen, Denmark**

Project: Investigations of microbial physiology and fermentations with non-brewing yeast strains. Experience in a brewing related subject is an advantage. Further information is available from Behnam Taidi at

[bta@crc.dk](mailto:bta@crc.dk) or from Michael Katz at [mka@crc.dk](mailto:mka@crc.dk) and further details how to apply will also be available at the job portal of [www.carlsberg.com](http://www.carlsberg.com).

### **One PhD position at Christian Hansen A/S, Hoersholm, Denmark**

Project: Investigation of nonconventional yeast cell wall components as regulators of immune system markers. Preferred applicants will have experience with microbial cultivation, mammalian cell culture and immunoassay techniques. Experience with macromolecule

purification and/or molecular biology techniques is an advantage. Further information is available from Jeffrey Earl Christensen at [DKJyC@chr-hansen.com](mailto:DKJyC@chr-hansen.com) and details on how to apply will be available at the Chr. Hansen job portal: [www.chr-hansen.com](http://www.chr-hansen.com).

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## Publication of Interest

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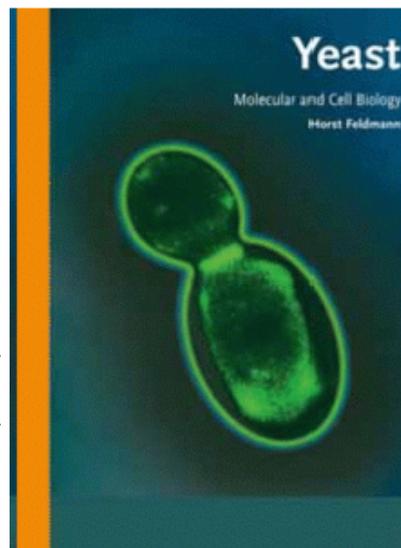
### Yeast Molecular and Cell Biology

Horst Feldmann, Adolf-Butenandt-Institute, Molecular Biology, University of Munich, Germany

Yeast is one of the oldest domesticated organisms and has both industrial and domestic applications. In addition, it is very widely used as a eukaryotic model organism in biological research and has offered valuable knowledge of genetics and basic cellular processes. In fact, studies in yeast have offered insight in mechanisms underlying ageing and diseases such as Alzheimers, Parkinsons and cancer.

Yeast is also widely used in the lab as a tool for many technologies such as two-hybrid analysis, high throughput protein purification and localization and gene expression profiling. The broad range of uses and applications of this organism undoubtedly shows that it is invaluable in research, technology and industry.

This book is an up-to date resource providing a comprehensive account of yeast biology and its use as a tool and model organism for understanding cellular and molecular processes of eukaryotes. Topics covered range from the fundamentals of yeast biology such as cell structure, biochemistry, genetics and signaling, to current approaches and applications such as metabolomics, disease models and uses in biotechnology. Written by a top expert in the field, this book offers an invaluable companion to beginners and experts in yeast research.



Publisher: John Wiley

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**Fifty Years Ago in “YEAST: A News Letter for Persons Interested in Yeast”  
(Volume IX Number 2, November 1960)**

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Editor: H. J. Phaff, University of California, Davis; Associate Editors L. R. Hedrick, Illinois Institute of Technology and C. Dunn, Massachusetts Institute of Technology).

**Mrs. N. J. W. Kreger-van Rij** (Centraal Bureau voor Schimmelcultures, the Netherlands) reported that type strains of 10 newly described species were received at CBS.

**Dr. D. W. R. Mackenzie** (Queen’s University of Belfast, Northern Ireland) reported publication of “Yeasts isolated from Man” in “Sabouraudia”. In a survey of yeasts isolated from human sources, *Torulopsis glabrata* was the most common species isolated from urine, and the second most commonly isolated species second to *Candida albicans*.

**Professor O. Verona** (Universita di Pisa, Italy) “emphasized that species containing large oil droplets are assimilating atmospheric nitrogen. Such was found in *Candida pulcherrima*.”

**Dr. N. van Uden** (University of Lisbon, Portugal) “returned last September from a nine months’ trip to the U.S.A., which he describes in high tones. He spent most of his time fishing yeasts from the Pacific and the Atlantic. The well remembered bases of operation were Dr. Claude E. ZoBell’s laboratory (Scripps Institution of Oceanography, La Jolla, Calif.) And Dr. Samuel P. Meyer’s laboratory (Marine Laboratory, Miami, Florida.”

**H. Saëz** (Parc Zoologique, Paris) described publications on saprophytic and parasitic fungi occurring on the animals living in the Zoological Garden of Paris.

**Dr. H. J. Phaff** reported that **Dr. Yoneyama** “will return to Hiroshima University [...] after a 15 month stay at Davis. A paper describing a new species of yeast, *Endomycopsis scolyti* will be published in Antonie van Leeuwenhoek.”

**Dr. S. Windisch** (Institute für Garungsgewerbe, Berlin) used a mass isolation technique to assess the ploidy of polyploid yeasts.

**Dr. Y. Oshima** (Osaka University, Japan) described biochemical genetics of alpha-glucosidase formation in *Saccharomyces*.

**Dr. K. Kodama** (Kodama Brewing Co., Japan) summarized a lecture on ecology of film-forming yeasts in the sake brewing process, given at the symposium on “Ecology of Microorganism”, Institute of Applied Microbiology, University of Tokyo, November 1960. Dr. Kodama also informed readers that Dr. Kendo Saito, emeritus professor of Osaka University, passed away in October 1960.

**Dr. James A. Barnett**, Low Temperature Research Station, Downing Street, Cambridge, England, “writes: [...] My article, Comparative studies of yeasts - Nature, Lond. 186, 449 (1960) - has evoked some controversy which is going to be published in Nature.”

Kyria Boundy-Mills & M.A. Lachance

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