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Editorials

Alessandro Martini

It is with much regret that I must announce the recent death of Prof. Alessandro Martini. A dear friend to many of us, Sandro was a strong advocate of ICY and will be remembered, amongst many other things, for having hosted the 1988 International Symposium on Yeasts in Perugia, Italy. On behalf of the yeast researcher community I extend my condolences to Ann (Vaughan-Martini) and her children. An account of Sandro's life will be presented in the December issue.

MA Lachance, Editor

I Mycology Program, American Type Culture Collection (ATCC), 10801 University Blvd, Manassas, Virginia 20110, USA. Communicated by SO Suh <ssuh@atcc.org>.

Recent publications.

1 Gujjari P, Suh SO, Coumes K, Zhou JJ 2011 Characterization of oleaginous yeasts revealed two novel species: *Trichosporon cacaoliposimilis* sp. nov. and *Trichosporon oleaginosus* sp. nov. Mycologia, in press, 2011.

Two new species in the anamorphic basidiomycetous genus *Trichosporon* (Tremellomycetes, Agaricomycotina) were uncovered in a DNA sequence-based molecular analysis of oleaginous yeasts maintained in the ATCC Mycology Collection. One yeast is named as *Trichosporon cacaoliposimilis* sp. nov. for its capability of synthesizing and accumulating a large amount of lipids having a composition equivalent to that of natural cacao butter. The type strain is ATCC 20505^T, originally deposited as *Trichosporon* sp. The other can utilize food industry wastes and agro byproducts as the substrate for growth and accumulation of a high level of oil, and accordingly is named as *Trichosporon oleaginosus* sp. nov. The type strain is ATCC 20509^T, previously identified as *Cryptococcus curvatus*. Molecular phylogenetic analyses indicate that *T. cacaoliposimilis* is a novel taxon in the Gracile clade of the genus, close to *T. gracile* and *T. dulcitum*, and that *T. oleaginosus* belongs to the Cutaneum clade, with *T. jirovecii* as the closest sister taxon. Other oleaginous yeasts were identified as new strains of known taxa: *T. insectorum*, *Candida orthopsilosis* and *C. palmioleophila*.

2 Suh SO, Zhou JJ 2011 *Kazachstania intestinalis* sp. nov., an ascosporogenous yeast from the gut of passalid beetle *Odontotaenius disjunctus*. Antonie van Leeuwenhoek, in press, DOI: 10.1007/s10482-011-9569-y.

Three ascosporogenous yeast strains were isolated from the gut of passalid beetle, *Odontotaenius disjunctus*, inhabiting on rotten oak trees. DNA sequence comparison and other taxonomic characteristics identified the strains as a novel species in the genus *Kazachstania*. The name *Kazachstania intestinalis* sp. nov. (type strain EH085^T = ATCC MYA-4658^T = CBS 11839^T) is proposed for the strains. The yeast is homothallic, producing persistent asci with 1-4 spheroidal ascospores. Molecular phylogeny from ribosomal RNA gene sequences placed this novel species on the basal lineage of a clade including *K. lodderae*, *K. exigua*, *K. martiniae*, and other related *Kazachstania* spp., but none of those species was a close sister to *K. intestinalis*.

II School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England. Communicated by J.A. Barnett <j.barnett@uea.ac.uk>.

Current publication.

1 Barnett JA & Barnett L 2011 Yeast Research: a Historical Overview. American Society for Microbiology. http://estore.asm.org/viewItemDetails.asp?ItemID=960

III Food Science and Technology, Oregon State University, Corvallis, OR 97331-6602. Communicated by A. Bakalinsky <alan.bakalinsky@oregonstate.edu>.

Recent publications.

1 Rowe JD, Harbertson JF, Osborne JP, Freitag M, Lim J, Bakalinsky AT 2010 Systematic identification of yeast proteins extracted into model wine during aging on the yeast lees. J Ag Food Chem 58:2337-2346.

Total protein and protein-associated mannan concentrations were measured and individual proteins were identified during extraction into model wines over 9 months of aging on the yeast lees following completion of fermentations by 7 wine strains of *Saccharomyces cerevisiae*. In aged wines, protein-associated mannan increased about 6-fold (± 66%), while total protein only increased 2-fold (± 20%), which resulted in a significantly greater protein-associated mannan/total protein ratio for 3 strains. A total of 219 proteins were identified among all wine samples taken over the entire time course. Of the 17 "long-lived" proteins detected in all 9-month samples, 13 were cell wall mannoproteins and 4 were glycolytic enzymes. Most cytosolic proteins were not detected after 6 months. Native mannosylated yeast invertase was assayed for binding to wine tannin and was found to have a 10-fold lower affinity than non-glycosylated bovine serum albumin. Enrichment of mannoproteins in the aged model wines implies greater solution stability than other yeast proteins and the possibility that their contributions to wine quality may persist long after bottling.

2 Zara S, Gross MK, Zara G, Budroni M, Bakalinsky AT 2010 Ethanol-independent biofilm formation by a flor wine yeast. Appl Environ Microbiol 76:4089-4091.

Flor strains of *Saccharomyces cerevisiae* form a biofilm on the surface of wine at the end of fermentation, when sugar is depleted and growth on ethanol becomes dependent on oxygen. Here, we report greater biofilm formation on glycerol and ethyl acetate and inconsistent formation on succinic, lactic, and acetic acids.

3 Hadduck AN, Hindagolla V, Contreras A, Li Q, Bakalinsky AT 2010 Does aqueous fullerene inhibit growth of yeast or *E. coli*? Appl Environ Microbiol 76:8239-8242.

Studies reporting on potentially toxic interactions between aqueous fullerene nanoparticles (nC_{60}) and microorganisms have been contradictory. When known confounding factors were avoided, growth yields of *Saccharomyces cerevisiae* and *E. coli* cultured in the presence and absence of independently prepared lots of underivatized nC_{60} were not found to be significantly different.

IV Geobotany, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany - http://www.ruhr-uni-bochum.de/geobot/en/. Communicated by Dominik Begerow <dominik.begerow@rub.de> and Andrey Yurkov <andrey.yurkov@rub.de>.

Recent publications.

1 Yurkov A, Schäfer AM & Begerow D (2011) *Leucosporidium drummii* sp. nov. a new member of Microbotryomycetes isolated from soil. International Journal of Systematic and Evolutionary Microbiology, DOI: 10.1099/ijs.0.027102-0.

Two strains of a new teleomorphic basidiomycete were isolated from grassland soil. Standard phenotypic tests and phylogenetic analyses of 26S rRNA gene (D1/D2 domains) and ITS region sequences showed that the species belongs to the core group of the genus *Leucosporidium*. We describe it as *Leucosporidium drummii* and designate type culture SEG-3-2-AY220^T (= CBS11562^T = MUCL52878^T) as the type strain. Additionally, phylogenetic analysis revealed great genetic variability of the *Leucosporidium scottii* complex.

2 Yurkov A, Kemler M & Begerow D (2011) Assessment of yeast diversity in soils under different management regimes. Fungal Ecology, SI: Fungi and global change.

Human activities, land management and climate change all have great impact on soil biology, but our knowledge on biodiversity of soil organisms is still very limited. Therefore, we wanted to assess responses of soil yeasts to land management and analysed 57 soils showing different land use from three distinct localities. We isolated and identified molecularly a total of 40 yeast fungi including several new species. Overall, species composition of different localities was very heterogeneous and nearly half of the species were found in a single site only. The analysis of species abundance and community composition revealed a strong long-term effect of forest replacement by grassland vegetation. Unlike forests, grasslands harbour predominantly ascomycetous yeasts and their proportion increases with management intensity. In forests, evenness of yeast communities follows the gradient of land management intensity and natural beech forests harboured the most unevenly structured community, thereby mirroring the evenness of plant communities.

V Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Wolczanska 171/173, 90-924 Lodz, Poland. Communicated by D. Kriegel <dorota.kregiel@p.lodz.pl>.

The following posters were presented on 39th Annual Conference on Yeasts, 3-7 May 2011.

1 Rajkowska K, Rygala A - Characterization of *Candida* strains isolated from human faeces. 39th Annual Conference on Yeasts, Smolenice, Slovakia, 2011, L12.

The aim of this study was to characterize morphology, reproduction, physiological and biochemical features of yeasts isolated from human faeces. Identification was performed on the basis of these traits and karyotypes obtained by the method of pulsed field gel electrophoresis PFGE. Faecal isolates were identified according to their ability to assimilate carbon and nitrogen compounds using API 20 C AUX test (BioMerieux). Chromosomes were separated with a CHEF-DR II apparatus (Bio-Rad) in TBE buffer (Sigma) at 10 °C and an interpolation of pulsed time of 110 – 120 s for 26 hours. After electrophoresis the gel was stained with ethidium bromide for visualization of chromosomal DNA. A standard commercial set of *S. cerevisiae* YNN 295 chromosomes (Bio-Rad) was used for comparison. Cluster analysis of biochemical profiles and karyotypes was

obtained by the unweighted pair-group average method (Statistica 6.0, StatSoft). Pursuant to phenotypic and genotypic traits yeasts isolated from human faeces were classified to six species belonging to *Candida* genus, ie., *C. inconspicua*, *C. parapsilosis*, *C. tropicalis*, *C. famata*, *C. lusitaniae* and *C. albicans*. Electrophoretic profiles of chromosomal DNA *Candida* sp. consisted of 1 – 8 bands sized 705 – 2368 kb. For examined isolates length polymorphism of chromosomal DNA, reflected in differences in both number and length of bands, was observed. According to cluster analysis of karyotypes, similarity levels among *Candida* strains amounted from 63 to 91%. Dendrogram of biochemical profiles similarity grouped examined isolates differently and biochemical similarity of isolated strains was in range 32 – 100%.

2 Kregiel D - Monitoring ATP status in cells of different yeast strains from LOCK105 Collection. 39th Annual Conference on Yeasts, Smolenice, Slovakia, 2011, P13.

The yeast culture processes seeks high efficiency and reproducibility. The cells must be in good physiological state during each industrial process. In recent years, considerable effort has been made to establish suitable online process analytical tools for cell culture control. The classic method – plate count - allows only a post facto assessment of their state because cell numbers simply reflect an existing physiological state. Cell viability reveals nothing about the state of nonviable cells. The aim of this study was to establish and evaluate an assay for rapid and reliable measurement of intracellular ATP (adenoside-5'-triphosphate) to monitor the physiological state of different yeast cells in combination with other parameter - cell number - obtained by direct microscopic count. Determination of cell numbers in prepared suspensions was performed with microscope Olympus BX41 with digital camera and WinMeasue (v.1.1) computer program. For ATP content in yeast cells, the luciferin–luciferase bioluminescent assay was used. Because of its high stability and ease of automation, the luminometer Hy-Lite2 with special bioassay kits (Merck) were used. For standard curve ATP disodium salt (Fluka) was applied. Five different strains *Saccharomyces cerevisiae* from Collection LOCK105 for industrial applications (distillery, bakery, feed) were used in this study. They were stored on wort agar slants in standard laboratory conditions. Yeast cells used in experiments were cultivated in 50 ml of worth broth (Merck) at 30ºC during the 7 days on rotary shaker at 20 rpm/min. Yeast cells were collected from young (24 –hrs) and old (7-days) cultures in wort broth. Significant decreasing of ATP content were seen with the stage of cells aging. For young cultures *S. cerevisiae* ATP level was 100÷200-fold higher than in old yeast cells. The results have shown that ATP assay conducted on-line can be powerful research tool for evaluation of physiological state of tested yeast cells.

3 Kregiel D, Ambroziak W - Co-immobilization of industrial yeast strains encapsulated in foamed alginate beads for fermentation of starchy materials. 39th Annual Conference on Yeasts, Smolenice, Slovakia, 2011, P11.

Starch is one of the major fermentable sugar presents in plant biomass. However, Saccharomyces sp., the best fermenting microorganisms, are not able to metabolize this polysaccharide. Yeasts *Debaryomyces* sp. show these abilities. We developed system of immobilized different yeasts: amylolytic strain *Debaryomyces occidentalis* Y500/5 and distillery strain *Saccharomyces cerevisiae* Bc16a, that can transform starch into ethanol. The properties of strains in this system, make it able to efficiently hydrolize of starch and ethanol production in the same medium simultaneously. We used a new yeast cell immobilization technique with foamed alginate and check the effect of yeast entrapment in multichamber cores on the growth of yeasts inside gel stucture. This research was also focused on the determination of cell viability and their growth in the multichamber cores. The initial cell concentration in the bead was $10^3 \div 10^4$ cfu/bead and after cultivation of the beads in the growth medium increased to 5×10^7 cfu/bead. In the multichamber cores the further colonization with the formation of yeast microcolonies was observed. Fermentative activity of co-immobilized yeasts was measured in minimal medium with 5% starch using three yeast systems: immobilized cells Bc16a and immobilized cells Y500/5 in separate cores (E+E), immobilized cells Y500/5 and free cells Bc16a (E+F) and mixed immobilized yeast cells Bc16a and Y500/5 inside the same cores (MIX). The best fermentative activity was observed in the case system E+E. The practical yield of ethanol Y_p was 4-fold higher than in other yeast systems.

4 Kregiel D, Rygala A, Libudzisz Z - Yeasts and *Asaia* sp. in mixed populations as spoilage of fruit-flavoured bottled waters. 39th Annual Conference on Yeasts, Smolenice, Slovakia, 2011, P12.

The examination was performed on defective fruit-flavored bottled waters to determine the casual spoilage microorganisms. The spoiled products were turbid and had a characteristic flocks, but sanitary indicators were not detected. The presence of microorganisms in twenty samples of drinks was investigated by membrane filtration (250 ml) through sterile membrane filters with 0,45 ìm pore diameter (Millipore), followed by incubation on specified selective nutrient media. Colonies on agar media were subcultured in proper medium to isolate a pure, single colony for identification. Yeast isolates were identified using the AUXACOLOR (Sanofi Diagnostics Pasteur), and the API 20C AUX (bioMerieux) yeast identification systems. For bacterial strains biochemical API tests and PCR techniques were applied. Quantitative studies on different agar media demonstrated the presence of various microflora (>10^7 CFU/ml) consisting of yeasts and bacteria.

Preliminary diaganostic results showed that the isolated red yeasts belonged to genera *Rhodotorula: Rh. glutinis, Rh. mucilaginosa and Rh. bacarum*, but the spoilage bacterial organisms were extremely difficult to identify phenotypically employing commercial API20 E and API20NE tests. As the results of genetic identification were unusual acetic acid bacteria *Asaia* sp. The isolated bacteria exhibited the exopolysaccharide production and all microorganisms showed ability to growth during first two weeks after production of fruit-flavored bottled waters. Yeasts and bacteria were integral part of flocks and, perhaps, constituted specific consortium, that will be the subject of future studies.

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

Recent publications.

1 Golubev WI 2010. Tacrolimus-sensitive yeasts. Immunologiya, Allergologiya, Infectologiya N 1, 219.

Twenty-four yeast species of 22 genera were screened for sensitivity to tacrolimus (FK506). Sensitive yeasts were found in genera *Cryptococcus, Filobasidiella*, and *Saccharomyces*. The species *Cr. amylolentus* (= *Tsuchiyaea wingfieldii*) is most sensitive but generally sensitivity to FK506 is strain but not a specific property.

2 Golubev WI 2011 Differentiation between aquatic and terrestrial *Metschnikowia* species of based on their sensitivity to *Pichia membranifaciens* mycocins. Microbiology, 80:166-168 (English translation: 154–157).

Aquatic *Metschnikowia* species (*M. australis, M. bicuspidata, M. krissii,* and *M. zobellii*) are sensitive to 10 out of 12 mycocins secreted by *Pichia membranifaciens* strains. Terrestrial species *M. pulcherrima* and *M. reukaufii* are resistant to all these mycocins, while *M. gruessii* and *M. lunata* are sensitive to one of them. The yeast described as *Torula rubifaciens* is also sensitive to this mycocin. There are reasons to think that the genus *Metschnikowia* Kamienski with the type species *M. bicuspidata* should be restricted to aquatic species.

VII Department of Biology, Faculty of Medicine Masaryk University, Kamenice 5, Building A6, 62500 Brno, Czech Republic - http://www.med.muni.cz/~mkopecka/. Communicated by Marie Kopecká <mkopecka@med.muni.cz>.

Papers published in journals.

1 Yamaguchi M, Kopecká M 2010 Ultrastructural disorder of the secretory pathway in temperature-sensitive actin mutants of Saccharomyces cerevisiae. J Electron Microscopy 59:141–152.

Phenotypes of the two temperature-sensitive actin mutants of *Saccharomyces cerevisiae act1-1* and *act1-2* at permissive, restrictive and semi-restrictive temperatures were studied by freeze fracture and thin section electron microscopy, and fluorescent microscopy. In contrast to secretory mutants where accumulations of either secretory vesicles, Golgi apparatus, or endoplasmic reticulum were reported, *act1-1* and *act1-2* mutants revealed accumulation of all the three components, even at permissive temperature. However, more distinct accumulation of secretory organelles was evident during cultivation at sub-restrictive temperature of 30°C. At the restrictive temperature of 37°C, many cells died, and their empty cell walls remained. Some of the few living cells showed features of apoptosis. From the present study, actin cables are concluded to be necessary for *(i)* correct spatial positioning and orientation of secretary pathway to the bud and septum, and *(ii)* vectorial movement of vesicles of the secretory pathway along the actin cables to the bud and septum.

2 Kopecká M, Ilkovics L, Ramíková V, Yamaguchi M 2010 Effect of cytoskeleton inhibitors on conidiogenesis and capsule in the long neck yeast *Fellomyces* examined by scanning electron microscopy. Chemotherapy 56:197-202.

Background: The aim of this basic study was to investigate by scanning electron microscopy the effects of cytoskeleton inhibitors on conidiogenesis and capsule in the yeast *Fellomyces fuzhouensis* CBS 8243, related to *Cryptococcus neoformans*. **Methods**: Cells were treated by methyl benzimidazole-2-ylcarbamate (BCM) and latrunculin A (LAT) in yeast extract peptone dextrose medium and examined by scanning electron microscopy. **Results:** During conidiogenesis, mother cells covered by capsule formed hypha-like stalks and at the hyphal tip yeast-like conidium developed. LAT blocked both stages of conidiogenesis. Inhibited mother cells and conidia became spherical and their capsule disappeared. BCM did not block formation of conidia that were neckless, or affect capsule. Combined application of LAT and BCM blocked both stages of

conidiogenesis, cells became spherical and their capsule disappeared. **Conclusion**: Yeast cells with disrupted actin cytoskeleton do not reproduce by conidiogenesis and do not retain inherited cell shape and capsule.

Paper published in a monograph.

3 Kopecká M, Svoboda A 2010 Ten years of activity of the Laboratory for Cell Biology of Human Pathogenic Yeasts at the Department of Biology, Faculty of Medicine Masaryk University, Brno. Chapter in Monograph: "Fifty Years of Czech and Slovak Yeast Research Period 2000-2009", pp.44-46. Editors: E Breierová, V Farkaš, I Hapala, K Sigler. Bratislava, 2010. ISBN: 978-800-89257-20-1.

Papers sent to press in 2011.

4 Kopecká M, Yamaguchi M - Ultrastructural disorder of actin mutant suggests uncoupling of actin-dependent pathway from microtubule-dependent pathway in budding yeast. Sent to J Electron Microscopy.

5 Kopecká M, Golubev W, Ramíková V, Klemová D. and Ilkovics L - Ultrastructural characteristics and variability of vegetative reproduction in *Fellomyces penicillatus*. Sent to Fungal Biology.

Application for a new research grant, for the period 2012-2014, to the Czech Science Foundation GA CR, Prague, Czech Republic.

Title: The Cytoskeleton of Pathogenic Yeasts as a New Antifungal Target. **Applicants:** Marie Kopecká and Augustin Svoboda, Department of Biology Faculty of Medicine Masaryk University, Brno, Czech Republic in cooperation with Masashi Yamaguchi, Medical Mycology Research Center, Chiba University, Japan.

VIII Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39/I, HR-10000 Zagreb, Republic of Croatia. Communicated by Ivan Kosalec <ikosalec@pharma.hr>.

Recent publications.

1 Kosalec I, Ramiæ S, Jeliæ D, Antoloviæ R, Pepeljnjak S, Kopjar N 2011 Assessment of tryptophol genotoxicity in four cell lines in vitro: a pilot study with alkaline comet assay. Arh Hig Rada Toksikol 62:41-49.

Tryptophol is an aromatic alcohol and secondary metabolite of the opportunistic fungus *Candida albicans*. Although its toxicity profile at cell level has been poorly investigated, recent data point to cytotoxic, cytostatic, and genotoxic effects in lymphocytes and the induction of apoptosis in leukaemic blood monocytes. In this pilot study we evaluated the genotoxicity of tryptophol in vitro on four permanent cell lines of animal and human origin: ovary cells, alveolar epithelium, liver cells, and blood monocytes using the alkaline comet assay. We selected cells that might be principal targets of tryptophol and other low-molecular geno(toxins) secreted by *Candida albicans* during host invasion. Our results suggest that tryptophol applied in vitro at 2 mmol L-1 for 24 h damages DNA in HepG2, A549 and THP-1 cells, obviously due to bioactivation and/or decomposition of the parent compound, which results in the formation of more genotoxic compound(s) and production of reactive species that additionally damage DNA. On the other hand, notably lower levels of primary DNA damage were recorded in CHO cells, which lack metabolic activity. Future studies with tryptophol should look further into mechanisms involved in its toxic action and should focus on other cell types prone to infection with *Candida* spp. such as vaginal epithelial cells or keratinocytes of human origin.

IX Laboratorio de Microbiología Aplicada y Biotecnología (Applied Microbiology and Biotechnology Lab.), Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-UNComahue), Quintral 1250, (8400), Bariloche, Argentina. Communicated by Diego Libkind <libkindfd@comahue-conicet.gov.ar>.

Recent Publications.

1 Moliné M, Regina Flores M, Libkind D, Diéguez MC, Farías ME & van Broock M 2010 Photoprotection by carotenoid pigments in the yeast *Rhodotorula mucilaginosa*: the role of torularhodin. Photochem Photobiol Sci 9:1145–1151.

2 Libkind D, Moliné M, van Broock MR 2011 Production of the UVB absorbing compound Mycosporine-glutaminol-glucoside by *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). FEMS Yeast Res 11:52-59.

3 Moliné M, Arbeloa EM, Regina Flores M, Libkind D, Diéguez MC, Farías ME, Bertolotti SG, Churio MS. & van Broock M 2011 UV-B photoprotective role of mycosporines in yeasts: photostability and antioxidant activity of mycosporine-glutaminol-glucoside. Radiation Res 175:44-50.

4 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 2011 Proposal of *Holtermanniella takashimae* sp. nov., *Holtermanniella* gen. nov. and the new order Holtermanniales to accommodate Tremellomycetous yeasts of the *Holtermannia* clade. Int J Syst Evol Microbiol 61: 680 - 689.

5 Brandão LR, Libkind D, Vaz ABM, Espírito Santo L, Moliné M, de García V, van Broock M, Rosa CA 2011 Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photo-protective compounds and extracellular enzymes. FEMS Microbiol Ecol 76:1-13.

Papers in Press.

6 Vaz ABM, Rosa LH, Vieira MLA, de Garcia V, Brandão LR, Teixeira LCRS, Moliné M, Libkind D, van Broock M, Rosa CA 2011 The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Brazilian J Microbiol - BJM-2733.

7 Libkind D, Moliné M, Sommaruga R, Sampaio JS, van Broock M - Phylogenetic distribution of fungal mycosporines within Pucciniomycotina (Basidiomycota). Yeast - YEA-Jan-11-0006.

Book chapters.

8 Libkind D 2011 *Rhodotorula*. In: Molecular Detection of Human Fungal Pathogens. D Liu (Ed). Ch 58. CRC Press, Taylor & Francis Group. ISBN: 9781439812402. In press.

9 Libkind D, Moline M, Tognetti C 2011 Isolation and selection of new astaxanthin producing strains of *Xanthophyllomyces dendrorhous*. In: Microbial carotenoids: Methods and Protocols, Methods in Molecular Biology Series. Barredo, J.L. (Ed.). Chapter 13. Humana Press. In press.

10 Moliné M, Libkind D, van Broock MR 2011 Production of torularhodin, torulene and â-carotene by *Rhodotorula* yeasts. In: Microbial carotenoids: Methods and Protocols, Methods in Molecular Biology Series. Barredo, JL (Ed). Ch 38. Humana Press. In press.

X Industrial Biotechnology, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-41296 Gothenburg, Sweden.
http://www.chalmers.se/chem/EN/divisions/industrial-biotechnology.
Communicated by Maurizio Bettiga <maurizio.bettiga@chalmers.se>.

Professor Lisbeth Olsson was recruited to Chalmers in 2008, when the Industrial Biotechnology group was founded. Yeast research within the group is mostly focused on its use as cell factory for the production of fuels, chemicals and more complex molecules, such as bioactive molecules with high added value or pharmaceutical proteins. The research on lignocellulose fermentation is articulated along the biorefinery chain, where yeast is used as a catalyst for the conversion of the raw material. The group consists now of 5 senior staff members, 5 post docs and 10 PhD students. Two new doctoral students will join the group during the summer 2011.

Recently hired staff members.

Maurizio Bettiga - Maurizio joined the staff of Industrial Biotechnology at Chalmers in October 2010 as Assistant Professor. As part of Chalmers Energy Initiative, his research is focused on the study and improvement of the microbial robustness properties required for the fermentation of lignocellulosic raw materials. His studies aim at identifying the molecular targets of different inhibitory compounds and harness them to generate more robust and productive strains.

George Anastontzis - George started his post doc at Industrial Biotechnology at Chalmers in November 2010. His project, funded by the Wallenberg Wood Science Center, aims to identify and produce new enzymes for the modification of lignin and hemicellulose derivatives. In his work he will mainly use *Pichia pastoris* as expression host.

Elia Tomás-Pejó - Elia has started her post doc at Industrial Biotechnology at Chalmers in March 2011. Her project is part of a collaborative project between Chalmers, Lund University, and two industrial partners. Recently developed *Saccharomyces cerevisiae* strains will be evaluated to characterize improved lignocellulosic substrates fermentation performance. A strain development program will be defined to further improve important characteristics of the yeast, such as fermentative capacity, sugar consumption capacity, robustness, and inhibitor tolerance relevant for different types of lignocellulosic raw materials.

Guo Zhongpeng - Guo started his post doc at Industrial Biotechnology at Chalmers on May 2011. His project, funded by Vinnova and in collaboration with SCA, Södra and Domsjö Fabriker. His project aims to the bio-based production of industrial chemicals in yeast from industrial side streams from the paper and pulping industry.

Courses.

The Industrial biotechnology group is actively involved in the organization of post graduate courses. This year we will offer the course "Industrial Biotechnology for lignocellulose based processes", October 16th-21st 2011. The aim of the course is to introduce the students to production of biofuels and other chemicals using plant cell wall materials as the raw material with emphasis on the biotechnology aspect of the production process If you are interested in the course please contact Maurizio Bettiga (maurizio.bettiga@chalmers.se).

Recent Publications.

1 Piddocke MP, Fazio A, Vongsangnak W, Wong ML, Heldt-Hansen HP, Workman C, Nielsen J, Olsson L 2011 Revealing the beneficial effect of protease supplementation to high gravity beer fermentations using "-omics" techniques. Microb Cell Fact 10:27.

2 Mapelli V, Hillestrøm PR, Kápolna E, Larsen EH, Olsson L 2011 Metabolic and bioprocess engineering for production of selenized yeast with increased content of seleno-methylselenocysteine. Metab Eng 13:282-93.

3 Eliasson Lantz A, Gernaey KV, Franzén CJ, Olsson L 2010 On-line monitoring of fermentation processes in lignocellulose-to-bioalcohol production. Waldron KW (ed.) Bioalcohol production: Biochemical conversion of lignocellulosic biomass. 315-339. ISBN 1 84569 510 0

4 Krogh KBRM, Harris PV, Olsen CL, Johansen KS, Hojer-Pedersen, Börjesson J, Olsson L 2010 Characterization and kinetic analysis of a thermostable GH3 â-glucosidase from *Penicillum brasilianum*. Appl Microbiol Biotechnol 86:143-154.

5 Olivares Hernandez R, Sunner H, Frisvad JC, Olsson L, Nielsen J, Panagiotou G 2010 Combining substrate specificity analysis with support vector classifiers reveals feruloyl esterase as a phylogenetically informative protein group. PLoS ONE, 5 (9) e12781.

6 Otero JM, Papadakis MA, Udatha DBRKG, Nielsen J, Panagiotou G 2010 Yeast Biological Networks Unfold the Interplay of Antioxidants, Genome and Phenotype, and Reveal a Novel Regulator of the Oxidative Stress Response. PLoS ONE, 5 (10) e13606.

7 Panagiotou G, Topakas E, Olivares Hernandez R, Christakopoulos P, Olsson L 2010 Feruloyl esterases as biosyntetic tools for the organic synthesis of nutraceutical compunds. Encyclopedia of Biotechnology in Agriculture and Food.

8 Spadiut O, Olsson L, Brumer H 2010 A comparative summary of expression systems for the recombinant production of galactose oxidase. Microbial Cell Factories, 9 ISSN 1475-2859

9 Tomás-Pejó E, Ballesteros M, Oliva JM, Olsson L 2010 Adaption of the xylose fermenting yeast *Saccharomyces cerevisiae* F12 for improving ethanol production in different fed-batch SSF processes. J Indust Microbiol Biotechnol 37(11):1211-1220.

10 Udatha DBRKG, Olsson L, Panagiotou G, Kouskoumvekaki I 2010 The interplay of descriptor-based computational analysis with pharmacophore modeling builds the basis for a novel classification scheme for feruloyl esterases. Biotechnol Advances, ISSN 0734-9750

11 Zhang J, Olsson L, Nielsen J 2010 The b-subunits of the Snf1 kinase in *Saccharomyces cerevisiae*, Gal83 and Sip2, but not Sip1 are redundant in glucose derepression and regulation of sterol biosynthesis. Molec Microbiol 77(2):371-383.

12 Ödman P, Johansen CL, Olsson L, Gernaey KV, Lantz AE 2010 Sensor combination and chemometric variable selection for online monitoring of *Streptomyces coelicolor* fed-batch cultivations. Appl Microbiol Biotechnol 86(6):1745-1759.

XI Yeast Molecular Genetics Laboratory, Institute of Molecular Biology "Acad. Rumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev str., 1113 Sofia, Bulgaria. Communicated by G. Miloshev <miloshev@bio21.bas.bg>.

The following are summaries of our current projects.

1 Georgieva M. and Miloshev G - Hho1p, the linker histone of *Saccharomyces cerevisiae* - can yeast cells manage without it?

Linker histones have important functions in building up the higher-order chromatin structure. These functions are believed to maintain genome integrity and at the same time to provide conditions for proper gene expression. Higher eukaryotic cells have several tissue and cell specific subtypes of linker histones in contrast to *Saccharomyces cerevisiae*. These yeasts possess only one linker histone, Hho1p, which is coded by a single-copy gene. The deletion of the genes for three of the linker histone subtypes in higher eukaryotes leads to early stage embryonic developmental defects and lethality while *Saccharomyces cerevisiae* linker histone is not essential for yeast cells. Generally *S. cerevisiae* cells live quite well without a linker histone. No drastic morphological changes have been reported in *ÄHho1* cells, which raise questions about the functions of this linker histone. In search for ultimate Hho1p function we probed linker histoneless cells' potential to live under different stress conditions. Results show that these cells poorly survived temperature and replicative stress as well as irradiation. Therefore, our results unambiguously prove the significance of Hho1p for cell survival during stress conditions.

Current Project at the Bulgarian Science Fund DMU 02/8 (period 2010-2012).

2 Staneva D., Georgieva M., Miloshev G - Yeast *Kluyveromyces lactis* - a model system for studying human linker histone H1°.

Yeasts as single-celled eukaryotes have been widely used for elucidating of the basic molecular processes conserved in all eukaryotic cells. For example, yeast are used as a model system for studying the cell cycle as it turns out that many of the genes controlling the cell cycle in baker's yeast have their homologues in human cells and may malfunction in tumor cells. The linker histone H1° is a structural and functional component of chromatin. Its implication in changes of chromatin structure and function, accompanying fundamental cellular processes such as differentiation, aging/senescence and malignant transformation has been documented. However, its precise role and the molecular mechanisms via which it acts remains elusive. Our current work is focused on development of a model system for studying human H1° expressed in the yeast *K. lactis*. The yeast *K. lactis* possesses only one ORF which can represent a linker histone gene. Disruption of this ORF and the characterization of yeast strain without KlH1 encoding gene is necessary to obtain a "clean room" in which to investigate the functional role of human H1°. The expression of the human H1° in yeast *K. lactis ÄH1* represents a unique opportunity to assess the individual impact of this specific linker histone on chromatin organization, gene expression and cellular proliferation.

This work is supported by the Bulgarian Science Fund (Grant DMU 02/8) and the "Human Resources Development" Operational Programme (Grant BG051ÐÎ 001-3.3.04/58), co-financed by the European Union through the European Social Fund.

3 Peycheva E, Miloshev G - Application of silver staining on comets obtained in Yeast Comet Assay

Yeast *Saccharomyces cerevisiae* is a model system for investigation of different cellular processes. In the recent years, a method acquiring popularity for investigation of DNA damages is the Single Cell Gel Electrophoresis (SCGE) also known as Comet Assay. Several years ago, we applied the method on yeast developing Yeast Comet assay (YCA). According to our recent results, the YCA is more sensitive than standard Comet Assay. Therefore, we attempt to modify the technique in order to make it convenient for use in small local laboratories. As a first step, we looked for a new way to visualize the results. Considering the limitation of the YCA namely the fluorescent visualization, we tried to apply the silver staining, which was used before in the standard Comet Assay on higher eukaryotic cells. By modifying of the steps in staining, we were able to demonstrate that silver staining can be successfully applied for visualization of yeast comets in YCA.

This work is supported by the European Commission, European Social Found, Human Resources Development Operational Programme, Ministry of Labour and Social Policy; "Young scientist's support for career development", Grant number: BG051PO001-3.3.04/58.

4 Peycheva E, Vasilev A, Deligeorgiev T, Miloshev G - Assessment of the biological effect of the DNA fluorescent dyes

In the recent decade, with the fast optimization of the live imagining techniques a lot of attention has been paid to number of fluorescent agents capable to bind DNA. These DNA dyes play important role in investigation of different biological processes, however, simultaneously could execute unexpected effects on cells causing misleading interpretation of the obtained results. Four oxazolo[4,5-*b*]pyridinium cyanine dyes were synthesized and their spectral-luminescent characteristics were studied. In order to investigate the mechanisms by which these dyes could act on living organisms we studied their cytotoxic and cytostatic effect on yeast *Saccharomyces cerevisiae.* Additionally, the method Yeast Comet Assay was used for investigation of the genotoxic effect of the dyes. The results show that the studied dyes manifest cytotoxic and cytostatic effect when they are used in high concentrations. Genotoxic effect has been detected for two of the dyes.

This work was supported by the National Science Fund of Bulgaria to E.P. Grant number: MU-B 1512/2005.

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>.

We are grateful to Organizing Committee for the invitation to participate at the International DOM Symposium on Microbial Formulation, Safety and Commercialization on 11-13 April 2011, Uppsala, Sweden.

The following are papers for 2011 or in press.

1 Naumova ES, Naumov GI, Michailova YuV, Martynenko NN, Masneuf-Pomarède I 2011 Genetic diversity study of the yeast *Saccharomyces bayanus* var. *uvarum* reveals introgressed subtelomeric *Saccharomyces cerevisiae* genes. Res Microbiol 162:204-213.

2 Naumov GI, Naumova ES 2011 Comparative genetics of yeast *Saccharomyces cerevisiae*. Chromosomal translocations carrying the *SUC2* marker. Russian J Genet 47:147-152. © Pleiades Publishing, Ltd.

3 Naumov GI, Naumova ES 2011 Genetic identification of African cultured yeasts of the genus *Saccharomyces*. Microbiology (Moscow) 80:386–390. © Pleiades Publishing, Ltd.

4 Naumov GI 2011 Molecular and genetic differentiation of small-spored species of the yeast genus *Metschnikowia* Kamienski. Microbiology (Moscow) 80:135–142. © Pleiades Publishing, Ltd.

5 Kondratieva VI, Naumov GI 2011 Population antagonism in the yeasts *Schizosaccharomyces pombe*. Ecological genetics (St. Petersburg) 9:21-26 (in Russian).

6 Naumov GI, Naumova ES, Martynenko NN, Masneuf-Pomarède I 2011 Taxonomy, ecology and genetics of the yeast *Saccharomyces bayanus* – a new object for science and practice. Microbiology (Moscow, in press).

The review deals with different aspects of biology of the yeast *Saccharomyces bayanus*, which is distantly related to cultured yeast *S. cerevisiae*. Found in wine-making cryotolerant strains of *S. bayanus* become the second object for basic and applied studies. Introduction of natural and experimental hybrids of *S. cerevisiae* × *S. bayanus* in different fermentation processes is evidence of great breeding importance of the yeast *S. bayanus*. The biological species *S. bayanus* represents a new gene pool for scientific and breeding projects.

7 Naumov GI, Lee C.-F 2011 Species specificity action of killer toxins of *Zygowilliopsis californica* on *Saccharomyces* yeasts: study of Taiwanese populations. Mikologiya i Fitopatologiya (in Russian, in press).

8 Naumov GI, Kondratieva VI, Naumova ES, Chen G-Y, Lee C-F 2011 Polymorphism and species-specificity of killer activity formation in the yeast *Zygowilliopsis californica*. Biotechnologiya (in Russian, in press).

9 Naumov GI 2011 Are melibiose-fermenting intestinal and alpechin strains of *Saccharomyces cerevisiae* a new type of yeast probiotics? Dokl Biol Sci (Moscow, in press).

10 Naumova ES, Naumov GI, Nikitina TN, Sadykova AZh, Kondratieva VI 2011 Molecular-genetic and physiological differentiation of *Kluyveromyces lactis* and *Kluyveromyces marxianus* yeasts: analysis of strains from All-Russian collection of microorganisms (VKM). Microbiology (Moscow, submitted).

11 Naumov GI, Martynenko NN, Naumova ES 2011 Are melibiose-fermenting *Saccharomyces cerevisiae* yeasts natural human and animal probiotics? International DOM Symposium on Microbial Formulation, Safety and Commercialization on 11-13 April 2011, Uppsala, Sweden. p. 27.

Many plant substrates, in particular soybean, are known to contain alpha-galactosides (stachyose, raffinose, and melibiose) that humans and animals cannot consume. As a result, the growth of flatulence-causing bacteria promotes. The yeast *Saccharomyces cerevisiae* having alpha-galactosidase is able both to hydrolyze and to utilize raffinose family of oligosaccharids, and has to be classified as a probiotic microorganism. Owing to scientific activity of N. van Uden it was shown that some melibiose-fermenting *S. cerevisiae* strains (syn. *S. italicus* var. *melibiosi, S. oleaginosus* and *S. oleaceus*) can occur in human's and animal's (horse and pig) intestines. In particular, van Uden and Assis-Lopes [1] described 9 Mel^+ strains isolated from human's intestines. In such yeasts, different sets of polymeric subtelomeric alpha-galactosidase genes *MEL* scattered over genome. First, polymeric *MEL* genes have been found by Roberts et al. [2]. They showed that two strains CBS 2909 (=IGC 2613) and IGC 2624 isolated from intestines of man and horse, probably, contained three and six polymeric genes, respectively. However, the *MEL* genes have not been identified at that time. Using genetic analysis and molecular karyotyping we precisely identified five genes *MEL3–MEL7* in strain CBS 4411 (=N. van Uden, N° 2709) isolated from pig intestines (Naumov et al, 1990). According to Southern hybridization of chromosomal DNAs with the *MEL1* probe, strains CBS 2909 and CBS 2910 (=IGC 6683) isolated from intestines of man contain at least three and four *MEL* genes, respectively (Naumov et al, 1995). It should be noted that *S. cerevisiae* strains with multiple *MEL* genes can be also isolated from alpechin, waste of olive oil production (Naumov et al, 1991, 1995, 1996). Altogether, 11 polymeric genes *MEL1–MEL11* have been identified among European strains. Accumulation of polymeric *MEL* genes having cumulative effect [3] may result from natural selection of yeasts inhabiting mammal's intestines due to presence of plant origin alpha-galactosides. To our knowledge there are no such

oligosaccharides in alpechin. In this case, selection of Mel^+ strains can be connected with destruction of alpechin alpha-galactoside alkaloids inhibiting yeast growth. Probably the same selective process can take place in mammal's intestines, as well. The data presented suggest probiotic role of *S. cerevisiae* strains fermenting plant alpha-galactosides.

[1] van Uden N, Assis-Lopes L. Fermentation of raffinose in the absence of invertase by *Saccharomyces italicus Castelli* var. *melibiosi* nov. var. Portugaliae Acta Biologica, 1957. Sér. A. 4(4): p. 323-327.

[2] Roberts C, Ganesan A.T, Haupt W. Genetics of melibiose fermenttion in *Saccharomyces italicus* var. *melibiosi.* Heredity, 1959. 13(4): p. 499-517.

[3] Haupt W, Alps H. Über die Vergärung der Melibiose durch *Saccharomyces*-Stämme. Archiv für Mikrobiologie, 1963. 45: p. 179-187.

XIII Cell and Organism Biology, Lund University, Sölvegatan 35, S-22362 Lund, Sweden. Communicated by Jure Piškur <Jure.Piskur@cob.lu.se>.

A recent publication.

1 Rozpędowska E, Hellborg L, Ishchuk OP, Orhan F, Galafassi S, Merico A, Woolfit M, Compagno C & Piškur J 2011 Parallel evolution of the make–accumulate–consume strategy in *Saccharomyces* and *Dekkera* yeasts. Nature.

Saccharomyces yeasts degrade sugars to two-carbon components, in particular ethanol, even in the presence of excess oxygen. This characteristic is called the Crabtree effect and is the background for the 'make–accumulate–consume' life strategy, which in natural habitats helps *Saccharomyces* yeasts to out-compete other microorganisms. A global promoter rewiring in the *Saccharomyces cerevisiae* lineage, which occurred around 100 mya, was one of the main molecular events providing the background for evolution of this strategy. Here we show that the *Dekkera bruxellensis* lineage, which separated from the Saccharomyces yeasts more than 200 mya, also efficiently makes, accumulates and consumes ethanol and acetic acid. Analysis of promoter sequences indicates that both lineages independently underwent a massive loss of a specific cis-regulatory element from dozens of genes associated with respiration, and we show that also in *D. bruxellensis* this promoter rewiring contributes to the observed Crabtree effect.

XIV VTT Technical Research Centre of Finland, P.O.Box 1000, FI-02044 VTT, Finland. Communicated by John Londesborough <john.londesborough@vtt.fi>.

Publications reported since our last communication include the following.

1 Huuskonen A, Markkula T, Vidgren V, Lima L, Mulder L, Geurts W, Walsh M & Londesborough J 2010 A selection from industrial lager yeast strains of variants with improved fermentation performance in Very High Gravity worts. Appl Environ Microbiol 76:1563-1573.

There are economic and other advantages if the fermentable sugar concentration in industrial brewery fermentations can be increased from that of currently used high gravity (ca. 14-17°P) worts into the very high gravity (VHG; 18-25°P) range. Many industrial strains of brewer's yeasts perform poorly in VHG worts, exhibiting decreased growth, slow and incomplete fermentations and low viability of the yeast cropped for recycling into subsequent fermentations. A new and efficient method for selecting variant cells with improved performance in VHG worts is described. In the method, mutagenized industrial yeast was put through a VHG wort fermentation and then incubated anaerobically in the resulting beer whilst maintaining the á-glucoside concentration at about 10 to 20 $g \bullet l^{-1}$ by slow feeds of maltose or maltotriose until most of the cells had died. When survival rates fell to one to ten cells per 10^6 original cells, a high proportion (up to 30 %) of survivors fermented VHG worts 10 - 30% faster and more completely (residual sugars lower by 2 - 8 $g.l^{-1}$) compared to the parent strains, but the sedimentation behaviour and profiles of yeast-derived flavor compounds of the survivors were similar to thos of the parent strains.

2 Laitila A, Wilhelmson A, Kotaviita E, Olkku J, Home S and Juvonen R 2006 Yeasts in an industrial malting ecosystem. J Ind Microbiol Biotechnol 33:953–966.

The malting ecosystem consists of two components: the germinating cereal grains and the complex microbial community. Yeasts and yeast-like fungi are an important part of this ecosystem, but the composition and the effects of this microbial group have been largely unknown. In this study we surveyed the development of yeasts and yeast-like fungi in four industrial scale malting processes. A total of 136 malting process samples were collected and examined for the presence of yeasts growing at 15, 25 and 37°C. More than 700 colonies were isolated and characterized. The isolates were discriminated by PCR-fingerprinting with microsatellite primer (M13). Yeasts representing different fingerprint types were identified by

sequence analysis of the D1/D2 domain of the 26S rRNA gene. Furthermore, identified yeasts were screened for the production of α-amylase, β-glucanase, cellulase and xylanase. A numerous and diverse yeast community consisting of both ascomycetous (25) and basidiomycetous (18) species was detected in the various stages of the malting process. The most frequently isolated ascomycetous yeasts belonged to the genera *Candida*, *Clavispora*, *Galactomyces*, *Hanseniaspora*, *Issatchenkia*, *Pichia*, *Saccharomyces* and *Williopsis* and the basidiomycetous yeasts to *Bulleromyces*, *Filobasidium*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and *Trichosporon*. In addition, two ascomycetous yeast-like fungi (black yeasts) belonging to the genera *Aureobasidium* and *Exophiala* were commonly detected. Yeasts and yeastlike fungi produced extracellular hydrolytic enzymes with a potentially positive contribution to the malt enzyme spectrum. Knowledge of the microbial diversity provides a basis for microflora management and understanding of the role of microbes in the cereal germination process.

3 Laitila A, Sarlin T, Kotaviita E, Huttunen T, Home S, and Wilhelmson A 2007 Yeasts isolated from industrial maltings can suppress *Fusarium* growth and formation of gushing factors. J Ind Microbiol Biotechnol 34:701–713.

Fusarium infection of barley and malt can cause severe problems in the malting and brewing industry. In addition to being potential mycotoxin producers, *Fusarium* fungi are known to cause beer gushing (spontaneous overfoaming of beer). Cereal-derived bacteria and yeasts are potential biocontrol agents. In this study, the antifungal potential of selected yeasts (12 strains) derived from the industrial malting ecosystem was studied in vitro with a plate-screening assay. Several ascomycetous yeast strains showed antagonistic activity against field and storage moulds, *Pichia anomala* being the most effective strain. The effects of *P. anomala* VTT C-04565 (C565) were examined in laboratory scale malting with naturally contaminated barley exhibiting gushing potential. *P. anomala* C565 restricted *Fusarium* growth and hydrophobin production during malting and prevented beer gushing. Grain germination was not disturbed by the presence of yeast. Addition of *P. anomala* C565 into the steeping seemed to retard wort filtration, but the filtration performance was recovered when yeast culture was combined with *Lactobacillus plantarum* VTT E-78076. Well-characterized microbial cultures could be used as food-grade biocontrol agents and they offer a natural tool for tailoring of malt properties.

4 Laitila A, Sarlin T, Raulio M, Wilhelmson A, Kotaviita E, Huttunen T and Juvonen R 2011 Yeasts in malting with special emphasis on *Wickerhamomyces anomalus* (synonym *Pichia anomala*) Antonie van Leeuwenhoek 99:75-48.

Malted barley is a major raw material of beer, as well as distilled spirits and several food products. The production of malt (malting) exploits the biochemical reactions of a natural process, grain germination. In addition to germinating grain, the malting process includes another metabolically active component: a diverse microbial community that includes various types of bacteria and fungi. Therefore, malting can be considered as a complex ecosystem involving two metabolically active groups. Yeasts and yeast-like fungi are an important part of this ecosystem, but previously the significance of yeasts in malting has been largely underestimated. Characterization and identification of yeasts in industrial processes revealed 25 ascomycetous yeasts belonging to 10 genera, and 18 basidiomycetous yeasts belonging to 7 genera. In addition, two ascomycetous yeast-like fungi belonging to the genera *Aureobasidium* and *Exophiala* were commonly detected. Yeasts and yeast-like fungi produced extracellular hydrolytic enzymes with a potentially positive contribution to the malt enzyme spectrum. Several ascomycetous yeast strains showed strong antagonistic activity against field and storage moulds, *Wickerhamomyces anomalus* (synonym *Pichia anomala*) being the most effective species. Malting studies revealed that *W. anomalus* VTT C-04565 effectively restricted *Fusarium* growth and hydrophobin production during malting and prevented beer gushing. In order to broaden the antimicrobial spectrum and to improve malt brewhouse performance, *W. anomalus* could be combined with other starter cultures such as *Lactobacillus plantarum*. Well characterized microbial mixtures consisting of barley and malt-derived microbes open up several possibilities to improve malt properties and to ensure the safety of the malting process.

5 Nygård Y, Toivari MH, Penttilä M, Ruohonen L and Wiebe MG 2011 Bioconversion of D-xylose to D-xylonate with *Kluyveromyces lactis*. Metabolic Engineering Eng - doi:10.1016/j.ymben.2011.04.001

D-Xylonate was produced from D-xylose using *Kluyveromyces lactis* strains which expressed the gene for $NADP^+$-dependent D-xylose dehydrogenase from *Trichoderma reesei* (*xyd1*). Up to 19 ± 2 g D-xylonate l^{-1} was produced when *K. lactis* expressing *xyd1* was grown on 10.5 g D-galactose l^{-1} and 40 g D-xylose l^{-1}. Intracellular accumulation of D-xylonate (up to ~70 mg [g biomass]$^{-1}$) was observed. D-Xylose was metabolised to D-xylonate, xylitol and biomass. Oxygen could be reduced to 6 mmol O_2 l^{-1} h^{-1} without loss in titre or production rate, but metabolism of D-xylose and xylitol were more efficient when 12 mmol O_2 l^{-1} h^{-1} were provided. D-xylose uptake was not affected by deletion of either the D-xylose reductase (*XYL1*) or a putative xylitol dehydrogenase encoding gene (*XYL2*) in *xyd1* expressing strains. *K. lactis xyd1ΔXYL1* did not produce extracellular xylitol and produced more D-xylonate than the *xyd1* strain containing the endogenous *XYL1*. *K. lactis xyd1ΔXYL2* produced high concentrations of xylitol and significantly less D-xylonate than the *xyd1* strain with the endogenous *XYL2*.

6 Rintala E, Jouhten P, Toivari M, Wiebe MG, Maaheimo H, Penttilä M and Ruohonen L 2011 Transcriptional responses of *Saccharomyces cerevisiae* to shift from respiratory and respiro-fermentative to fully fermentative metabolism. OMICS: A Journal of Integrative Biology, 2011, Feb 24

In industrial fermentations of *Saccharomyces cerevisiae*, transient changes in oxygen concentration commonly occur and it is important to understand the behavior of cells during these changes. Glucose-limited chemostat cultivations were used to study the time-dependent effect of sudden oxygen depletion on the transcriptome of *S. cerevisiae* cells initially in fully aerobic or oxygen-limited conditions. The overall responses to anaerobic conditions of cells initially in different conditions were very similar. Independent of initial culture conditions, transient downregulation of genes related to growth and cell proliferation, mitochondrial translation and protein import, and sulphate assimilation was seen. In addition, transient or permanent upregulation of genes related to protein degradation, and phosphate and amino acid uptake was observed in all cultures. However, only in the initially oxygen-limited cultures was a transient upregulation of genes related to fatty acid oxidation, peroxisomal biogenesis, oxidative phosphorylation, TCA cycle, response to oxidative stress, and pentose phosphate pathway observed. Furthermore, from the initially oxygen-limited conditions, a rapid response around the metabolites of upper glycolysis and the pentose phosphate pathway was seen, while from the initially fully aerobic conditions, a slower response around the pathways for utilization of respiratory carbon sources was observed.

7 Toivari MH, Ruohonen L, Richard P, Penttilä M and Wiebe MG 2010 *Saccharomyces cerevisiae* engineered to produce D-xylonate. Appl Microbiol Biotechnol 88:751-760.

Saccharomyces cerevisiae was engineered to produce D-xylonate by introducing the *Trichoderma reesei xyd1* gene, encoding a D-xylose dehydrogenase. D-xylonate was not toxic to *S. cerevisiae*, and the cells were able to export D-xylonate produced in the cytoplasm to the supernatant. Up to 3.8 g of D-xylonate per litre, at rates of 25–36 mg of D-xylonate per litre per hour, was produced. Up to 4.8 g of xylitol per litre was also produced. The yield of D-xylonate from D-xylose was approximately 0.4 g of D-xylonate per gramme of D-xylose consumed. Deletion of the aldose reductase encoding gene *GRE3* in *S. cerevisiae* strains expressing *xyd1* reduced xylitol production by 67%, increasing the yield of D-xylonate from D-xylose. However, D-xylose uptake was reduced compared to strains containing *GRE3*, and the total amount of D-xylonate produced was reduced. To determine whether the co-factor $NADP^+$ was limiting for D-xylonate production the *Escherichia coli* transhydrogenase encoded by *udhA*, the *Bacillus subtilis* glyceraldehyde 3-phosphate dehydrogenase encoded by *gapB* or the *S. cerevisiae* glutamate dehydrogenase encoded by *GDH2* was co-expressed with *xyd1* in the parent and *GRE3* deficient strains. Although each of these enzymes enhanced NADPH consumption on D-glucose, they did not enhance D-xylonate production, suggesting that $NADP^+$ was not the main limitation in the current D-xylonate producing strains.

8 Verho R, Penttilä M and Richard P 2011 Cloning of Two Genes (LAT1,2) Encoding Specific L-Arabinose Transporters of the L-Arabinose Fermenting Yeast *Ambrosiozyma monospora*. Appl Biochem Biotechnol - DOI 10.1007/s12010-011-9161-y

We identified and characterized two genes, LAT1 and LAT2, which encode specific L-arabinose transporters. The genes were identified in the L-arabinose fermenting yeast *Ambrosiozyma monospora*. The yeast Saccharomyces cerevisiae had only very low Larabinose transport activity; however, when LAT1 or LAT2 was expressed, L-arabinose transport was facilitated. When the LAT1 or LAT2 were expressed in an *S. cerevisiae* mutant where the main hexose transporters were deleted, the L-arabinose transporters could not restore growth on D-glucose, D-fructose, D-mannose or D-galactose. This indicates that these sugars are not transported and suggests that the transporters are specific for L-arabinose.

9 Vidgren V, Multanen JP, Ruohonen L and Londesborough J 2010 The temperature-dependence of maltose transport in ale and lager strains of brewer's yeast. FEMS Yeast Res 10:402-411.

Lager beers are traditionally made at lower temperatures (6-14 °C) than ales (15-25 °C). At low temperatures, lager strains (*Saccharomyces pastorianus*) ferment faster than ale strains (*Saccharomyces cerevisiae*). Two lager and two ale strains had similar maltose transport activities at 20 °C, but at 0 °C the lager strains had 5-fold greater activity. *AGT1*, *MTT1* and *MALx1* are major maltose transporter genes. In 9 tested lager strains the *AGT1* genes contained premature stop codons. None of 5 tested ale strains had this defect. All tested lager strains, but no ale strain, contained *MTT1* genes. When functional *AGT1* from an ale strain was expressed in a lager strain, the resultant maltose transport activity had the high temperature-dependence characteristic of ale yeasts. Lager yeast *MTT1* and *MALx1* genes were expressed in a maltose-negative laboratory strain of *S. cerevisiae*. The resultant Mtt1 transport activity had low temperature-dependence and the Malx1 activity had high temperature-dependence. Faster fermentation at low temperature by lager strains than ale strains may result from their different maltose transporters. Loss of Agt1 transporters during evolution of lager strains may have liberated plasma membrane space for the Mtt1 transporters that perform better at low temperature.

10 Vidgren V, Kankainen M, Londesborough J and Ruohonen L 2011 Identification of regulatory elements in the AGT1 promoter of ale and lager strains of brewer's yeast. Yeast, in press.

Agt1 is an interesting α-glucoside transporter for the brewing industry as it efficiently transports maltotriose, a sugar often remaining partly unused during beer fermentation. It has been shown that on maltose the expression level of *AGT1* is much higher in ale strains than in lager strains, and that glucose represses the expression particularly in the ale strains. In the present study the regulatory elements of the *AGT1* promoter of one ale and two lager strains were identified by computational methods. Promoter regions up to 1.9 kbp upstream of the *AGT1* gene were sequenced from the three brewer's yeast strains and the laboratory yeast strain CEN.PK-1D. The promoter sequence of the laboratory strain was identical to the *AGT1* promoter of S288c strain of *Saccharomyces* Genome Database, whereas the promoter sequences of the industrial strains diverged markedly from the S288c strain. The *AGT1* promoter regions of the ale and lager strains were on most parts identical to each other except for one 22 bp deletion and two 94 and 95 bp insertions in the ale strain. Computational analyses of promoter elements revealed that the promoter sequences contained several Mig1p- and MAL-activator binding sites, as was expected. However, some of the Mig1p and MAL-activator binding sites were located on the two insertions of the ale strain, and thus offered a plausible explanation for the different expression pattern of the *AGT1* gene in the ale strains. Accordingly, functional analysis of A60 ale and A15 lager strain *AGT1* promoters fused to GFP (encoding the green fluorescent protein) showed a significant difference in the ability of these two promoters to drive GFP expression. Under the control of the *AGT1* promoter of the ale strain the emergence of GFP was strongly induced by maltose, whereas only a low level of GFP was detected with the construct carrying the *AGT1* promoter of the lager strain. Thus, the extra MAL-activator binding element, present in the *AGT1* promoter of the ale strain, appears to be necessary to reach a high level of induction by maltose. Both *AGT1* promoters were repressed by glucose but their derepression was different possibly due to a distinct distribution of MIG1 elements in these two promoters.

11 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 and Libkind D 2011 Description of *Holtermanniella* gen. nov., including *Holtermanniella takashimae* sp. nov. and four new combinations, and proposal of the order Holtermanniales to accommodate tremellomycetous yeasts of the *Holtermannia* clade. Int J Syst Evol Microbiol 61:680–689.

The novel genus *Holtermanniella* is proposed here to accommodate four *Cryptococcus* species closely related to *Holtermannia corniformis* that are included in the *Holtermannia* clade Basidiomycota, Agaricomycotina). Thus, four novel combinations are proposed: *Holtermanniella nyarrowii* comb. nov., *Holtermanniella festucosa* comb. nov., *Holtermanniella mycelialis* comb. nov. and *Holtermanniella wattica* comb. nov. In addition, a novel anamorphic yeast species was studied with 15 isolates obtained from different habitats around the world. Analysis of the sequences of the D1/D2 region of their large subunit rDNA showed that the novel species is placed phylogenetically within the *Holtermannia* clade of the Tremellomycetes (Agaricomycotina, Basidiomycota). PCR fingerprinting and sequencing of ITS1–5.8S–ITS2 showed genetic intraspecific variability among the strains: three groups were formed, which did not correlate with geographical origin or substrate. This novel species, designated the type species of *Holtermanniella* gen. nov., is described as *Holtermanniella takashimae* sp. nov.; the type strain is CBS 11174T (5HB 982T 5DBVPG 8012T). The order Holtermanniales ord. nov. is proposed here to include *Holtermannia* (the type genus) and *Holtermanniella*.

The following theses have been successfully defended:

12 Eija Rintala 2010 Effects of oxygen provision on the physiology of baker's yeast *Saccharomyces cerevisiae*. Doctoral thesis, University of Helsinki.

13 Virve Vidgren 2010 Maltose and maltotriose transport into ale and lager brewer's yeast strains. Doctoral thesis, University of Helsinki.

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

The new website of the Portuguese Yeast Culture Collection (PYCC) is now online at the following URL: **http://pycc.bio-aware.com/**.

Request for strains: An R&D project headed by Á. Fonseca on the molecular population genetics of *Cryptococcus* spp. is about to start; the target species of this study are *C. carnescens*, *C. flavescens* and *C. victoriae* (often phenotypically identified as *C. laurentii*); I'm interested in receiving yeast isolates belonging to any of the three target species, for which substrate and locality of origin are known and, if possible, also D1/D2 or ITS sequences; contact email: <amrf@fct.unl.pt>.

The following paper was recently accepted for publication.

1 Gonçalves P, Valério E, Correia C, Almeida JMGCF, Sampaio JP - Evidence for divergent evolution of growth temperature preference in sympatric *Saccharomyces* species PLoSOne.

The genus *Saccharomyces* currently includes eight species in addition to the model yeast *Saccharomyces cerevisiae*, most of which can be consistently isolated from tree bark and soil. We recently found sympatric pairs of *Saccharomyces* species, composed of one cryotolerant and one thermotolerant species in oak bark samples of various geographic origins. In order to contribute to explain the occurrence in sympatry of *Saccharomyces* species, we screened *Saccharomyces* genomic data for protein divergence that might be correlated to distinct growth temperature preferences of the species, using the dN/dS ratio as a measure of protein evolution rates and pair-wise species comparisons. In addition to proteins previously implicated in growth at suboptimal temperatures, we found that glycolytic enzymes were among the proteins exhibiting higher than expected divergence when one cryotolerant and one thermotolerant species are compared. By measuring glycolytic fluxes and glycolytic enzymatic activities in different species and at different temperatures, we subsequently show that the unusual divergence of glycolytic genes may be related to divergent evolution of the glycolytic pathway aligning its performance to the growth temperature profiles of the different species. In general, our results support the view that growth temperature preference is a trait that may have undergone divergent selection in the course of ecological speciation in *Saccharomyces*.

XVI Molecular Mycology Research Laboratory, Sydney Medical School - Westmead, University of Sydney, Centre for Infectious Diseases and Microbiology, ICPMR, Level 3, Room 3114A, Darcy Road, Westmead Hospital, Westmead, NSW 2145, Australia - www.mycologylab.org. Communicated by Wieland Meyer <w.meyer@usyd.edu.au>.

Recent publications.

1 Chayakulkeeree M, Johnston SA, Bijosono Oei J, Lev S, Williamson PR, Wilson CF, Zuo X, Leal AL, Henning Vainstein M, Meyer W, Sorrell TC, May RC, Djordjevic JT 2011 - in press - *SEC14* is a specific requirement for secretion of phospholipase B1 and pathogenicity of *Cryptococcus neoformans*. Mol Microbiol (accepted 9.3.2011)

2 Kronstad JW, D'Souza CA, Taylor G, Warren R, Yuen M, Hu G, Jung WH, Sham A, Kidd SE, Tangen K, Lee N, Zeilmaker T, Sawkins J, McVicker G, Shah S, Gnerre S, Griggs A, Zeng Q, Bartlett K, Li W, Wang X, Heitman J, Stajich JE, Fraser JA, Meyer W, Carter D, Schein J, Krzywinski M, Kwon-Chung KJ, Varma A, Wang J, Brunham R, Fyfe M, Ouellette BFF, Siddiqui A, Marra M, Jones S, Holt R, Birren BW, Galagan JE, Cuomo CA 2011 Genome variation in *Cryptococcus gattii*, an emerging pathogen of immunocompetent hosts. mBio 2(1) e00342-10

3 Ngamskulrungroj P, Price J, Sorrell T, Perfect JR, Meyer W 2011 *Cryptococcus gattii* virulence composite*:* Candidate genes revealed by microarray analysis of high and less virulent Vancouver Island outbreak strains. PLoS ONE 6(1) e16076; doi:10.1371/journal.pone.0016076

4 Carriconde F, Gilgado F, Arthur I, Ellis D, Malik R, van de Wiele N, Robert V, Currie BJ, Meyer W 2011 Clonality and alpha-a recombination in the Australian *Cryptococcus gattii* VGII population - an emerging outbreak in Australia. PLoS ONE 6(2): e16936

5 Sykes JE, Sturges BK, Cannon MS, Gericota B, Higgins RJ, Trivedi SR, Dickinson PJ, Vernau KM, Meyer W, Wisner ER 2010 Clinical signs, imaging features, neuropathology and outcome in cats and dogs with CNS cryptococcosis from California. J Vet Internal Med. 24:1427-1438.

6 Choi YH, Ngamskulrungroj P, Varma A, Sionov E, Hwang SM, Carriconde F, Meyer W, Litvintseva AP, Lee WG, Shin JH, Kim EC, Lee KW, Choi TY, Lee YS, Kwon-Chung KJ 2010 Prevalence of the VNIc genotype of Cryptococcus neoformans in non-HIV associated cryptococcosis in the Republic of Korea. FEMS Yeast Res 10:769-778.

7 Krockenberger M, Malik R, Nagamskulrungroj P, Trilles L, Escandon P, Dowd S, Allen C, Himmelreich U, Canfield PJ, Sorrell TC, Meyer W 2010 Pathogenesis of pulmonary *Cryptococcus gattii* infection: A rat model. Mycopathol 170 (5): 315-330.

8 Ngamskulrungroj P, Serena C, Gilgado F, Malik R, Meyer W 2010 Global VGIIa isolates are of comparable virulence to the major fatal *Cryptococcus gattii* Vancouver Island outbreak genotype. Clinical Microbiology and Infection. 17:252-258.

9 Meyer W, Trilles L 2010 Genotyping of the *Cryptococcus neoformans/C. gattii* species complex. Australian Biochemist 41 (1):11-15.

10 Lucas S, da Luz Martins M, Flores O, Meyer W, Spencer-Martins I, Inacio J 2010 Differentiation of *Cryptococcus neoformans* varieties and *Cryptococcus gattii* using *CAP59*-based loop-mediated isothermal DNA amplification. Clinical Microbiology and Infection. 16:711-714. PubMed PMID: 19694768.

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

Recent publications.

1 Lachance MA, Wijayanayaka TM, Bundus JD, Wijayanayaka DN 2011 Ribosomal DNA sequence polymorphism and the delineation of two ascosporic yeast species, *Metschnikowia agaves* and *Starmerella bombicola*. FEMS Yeast Res 11:324–333.

The relationship between mating success and sequence divergence in the internal transcribed spacer (ITS)/5.8S-D1/D2 rDNA region was examined in isolates tentatively assigned to *Metschnikowia agaves* and *Starmerella bombicola*. Both species are haplontic and heterothallic, such that the formation of mature asci can be used as a measure of genetic compatibility. Parsimony haplotype network analysis and mating success confirmed that all known isolates of *M. agaves* are conspecific. The previously reported D1/D2 polymorphism of five substitutions was not corroborated; the maximum divergence observed between any two strains was three substitutions, four with ITS. Of 39 putative *S. bombicola* strains, 36 formed an ITS-D1/D2 haplotype network using the 95% criterion. Thirty-five strains could mate with one or more compatible partner. The excluded strains did not mate. Mature asci arose from crosses between individuals differing by as many as five, but not six or seven substitutions in the D1/D2 domain. All strains capable of mating formed mature asci with at least one partner and all network members could be linked to another member by three or fewer substitutions. These results support the use of sequence divergence as a criterion for species delineation, but caution against describing poorly sampled species solely on the basis of that criterion.

2 Canelhas M, Barbosa M, Medeiros A, Lee CF, Huang LY, Lachance MA, Rosa CA 2011 *Saturnispora serradocipensis* sp. nov. and *Saturnispora gosingensis* sp. nov., two ascomycetous yeasts from ephemeral habitats. Antonie van Leeuwenhoek 99:241-247.

3 Barbosa AC, Morais CG, Morais PB, Rosa LH, Pimenta RS, Lachance MA, Rosa CA *Wickerhamiella pagnoccae* sp. nov. and *Candida tocantinsensis* sp nov., two ascomycetous yeasts from flower bracts of *Heliconia psittacorum* (Heliconiaceae) Int J Syst Evol Microbiol (Accepted April 2011).

Essay

Lipase production from yeast sp: novel resources with industrial potentials

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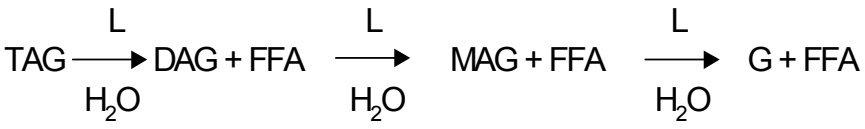
Communicated by C.Rose <chellanrose@yahoo.co.uk>.

Lipases (E.C.3.1.1.3) catalyse the hydrolysis of triacylglycerols with concomitant yield of free fatty acids and glycerol in an oil-water interface and exhibit multiplex reactions such as hydrolysis, esterification, interesterification and transesterification in aqueous/nonaqueous media. Lipases are, therefore, endowed with remarkable characteristics as promising biocatalysts suited for a wide range of industrial applications. The hydrolytic activity of lipases can be represented as:



TAG – Triacylglycerol; DAG – Diacylglycerol; MAG – Monoacylglycerol; G – Glycerol; FFA – Free Fatty acid; L – Lipase

Generally, lipases are 20-60 kDa in Molecular weight and do not require a cofactor for its reaction. These enzymes contain a catalytic triad consisting of Ser-Asp-His and a consensus sequence of G-S-X-S-G in their protein architecture. These structural features contribute towards the unique characteristics of lipases to modulate their specific functions.

Lipases may be obtained from plants, animals and microorganisms. Microbial sources are more potent sources in terms of unlimited availability, ease of extraction, feasibility of genetic manipulations, simpler production and downstream processing. In this context, microbial lipases have received a great deal of attention as biocatalysts due to their stability, regiospecificity, chiral selectivity and broad substrate specificity with significant potential for commercial exploitation. The interest in microbial lipase production has increased in the past decades in view of its applications in food, dairy, leather, detergent, pharmaceutical, medical and oleochemical industries thereby constituting an important group of biocatalysts for biotechnological applications. Although many commercial lipases are available, only a few meet the optimal requirements. Hence, there is an increasing demand to identify and characterize new lipases with emphasis on their applications. Microbial lipases may be obtained from bacteria, fungi and yeasts. Innumerous bacterial and fungal lipases have been identified and well characterized. The search for novel lipases is still in the forefront since the sources and the characteristics acquired from various habitats are multifold offering thermostability, solvent tolerance and unique specificities which manifest a wide range of applications.

Yeast lipases are ideal and promising candidates for biotechnological innovations such as in the production of biopolymers, biodiesel, enantiopure pharmaceuticals, agrochemicals and flavour compounds. Yeast lipases exhibit chemo-, regio- and enantiospecificities for pharmaceutical applications and the most studied yeast lipase is from *Candida rugosa*, which has been in commercial production for decades with versatile industrial uses. Yeasts also confer desirable characteristics attributed to their cytoskeletal structures with a rigid cell wall composition and their physiological and metabolic features. Hence, yeast sp. are targets of significant contemplation for lipase production. Many yeast sp. display lipase activities including *Yarrowia lipolytica*, *Trichosporon fermentans*, *Cryptococcus* sp., *Rhodotorula* sp. etc. *Pichia pastoris* is highly suitable as an expression host system for production of recombinant lipases. We describe here the lipase production potentials of some major yeast producers which have been investigated recently for commercial exploitations as well.

Candida rugosa lipase has been studied by solid state fermentation (SSF) in early 1990s and is known as a potent lipase producer. The significant parameters in the production of *Candida rugosa* lipase using rice bran as solid substrate have been optimized by response

surface technique. The optimum values found were: 0•25% urea, 4•5% maltose and 15% oil (w/w dry bran) for biomass production and 0•5% urea, 1•5% maltose and 7•5% oil for lipase production. The optimum C/N ratio for lipase and biomass production were found to be 6–6•5 and 9–9•5 respectively. Studies in a tray fermenter indicated 98% humidity and 1 litre/min aeration rate as additional parameters for lipase production.

Yarrowia lipolytica is a non-conventional yeast which possesses 16 paralogs of genes coding for lipase with three isoenzymes Lip2p, Lip7p and Lip8p characterized so far. The synthesis of Lip2p is greatly modulated by the environmental conditions and the nature of the carbon and nitrogen sources used for growth. In the presence of carbon sources, a lipase activity of 174.4 U/ml/h/A_{600} was obtained which raised to 500 U in the presence of tryptone due to the action of bioactive peptides.

A new yeast lipase from *Trichosporon asahii* has been reported in 2008 with 30-fold enhancement in lipase production by medium optimization with 104 U/ml using one variable-at-a-time and a statistical approach. Glucose and calcium chloride were identified as insignificant variables. Requirement of malt extract and yeast extract varied with the type of inducer used. Corn oil favoured lipase production in malt extract, while a high concentration of 3% yeast extract with Tween 80 served as the best components for maximum lipase production.

Yeast strains isolated from the phylloplane of *Hibiscus rosasinensis* were found to be potent lipase producers with 3.2-fold lipase production in a bioreactor than with flask submerged culture. The strains that were identified as *Pseudozyma hubeiensis*, *Debaryomyces occidentalis* and *Cryptococcus* sp. were the highest lipase producers among the microbial flora. Also, the production process was significantly reduced from 48 h in flasks to 18 h in the bioreactor with good cost/benefit rate for enzyme attainment.

Olive mill wastewater has been shown to be a promising substrate for microbial lipase production and the lipase from *Candida rugosa* was the highest producer of lipase activity on all the typologies of olive mill wasters; and, was markedly affected by the type of nitrogen source and was induced by the addition of olive oil. *Pichia pastoris* is a methylotrophic yeast which is a well established host system for heterologous protein production. Use of a multicarbon substrate in addition to methanol is an essential requirement to increase cell density, process productivity and to reduce the induction time. It has therefore been observed that sorbitol co-feeding allowed for sustained cell growth and lipase production thereby reducing the metabolic burden for lipase overexpression. In the case of *Yarrowia lipolytica* lipase production was induced in the presence of methanol. Lipase BTL2 from *B. thermocatanulatus* overexpressed in *E.coli* under the control of native promoter and a strong temperature inducible promoter exhibited 600 U/g wet cells and 54,000 U/g wet cells lipase productivities respectively and was intracellular requiring further purification procedures. However, BTL2 lipase overexpressed in *Pichia pastoris*, produced 309,000 U/L of extracellular lipase, indicating the potency of the yeast host for high level lipase production.

Mutagenesis of yeast strains also offer enhanced lipase productivities by the mutants. It has been observed that batch culture of *Yarrowia lip*olytica mutant exhibited high yields of lipase with 158246 U/ml after 80 h cultivation in a media with feeding of olive oil and tryptone at the end of the exponential growth phase. In another study, *Yarrowia lipolytica* mutant obtained by UV mutagenesis produced 356 U/ml lipase activity after 24 h, which was about 10.5-fold higher activity than the wild type strain.

The production of lipase plays a meaningful role when it is applicable for industrial uses such as in biosurfactants, biodiesel production, as laundry formulations etc. Hence, it is also important to develop novel enzymes with novel characteristics for commercialization, and yeast lipases are interesting candidates with tremendous potentials. Recently, methanol-tolerant lipase producing yeast has been employed as a whole cell biocatalyst for green biodiesel industrialization in terms of enzyme stability and cost effectiveness. Thereby, innovative approaches have been demonstrated for effective selection of lipase-producing yeasts in biodiesel production.

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D'Annibale A, Sermanni GG, Federici F & Petruccioli M 2006 Olive-mill waste waters: a promising substrate for microbial lipase production. Bioresour Technol, 97: 1828-1833.

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Fickers P, Marty A & Nicaud JM 2011 The lipases from *Yarrowia lipolytica*: genetics, production, regulation, biochemical characterization and biotechnological applications. Biotechnol Adv, in press.

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Ramon R, Ferrer P & Valero F 2007 Sorbitol co-feeding reduces metabolic burden caused by the overexpression of a *Rhizopus oryzae* lipase in *Pichia pastoris*. J Biotechnol, 130: 39-46.

Song HT, Jiang ZB & Ma LX 2006 Expression and purification of two lipases from *Yarrowia lipolytica* AS 2.1216. Protein Expr Purif, 47: 393-397.

Srimhan P, Kongnum K, Taweerodjanakarn S & Hongpattarakere T 2011 Selection of lipase producing yeasts for methanol-tolerant biocatalyst as whole cell application for palm oil transesterification. Enz Microb Technol, 48: 293-298.

Recent Meeting

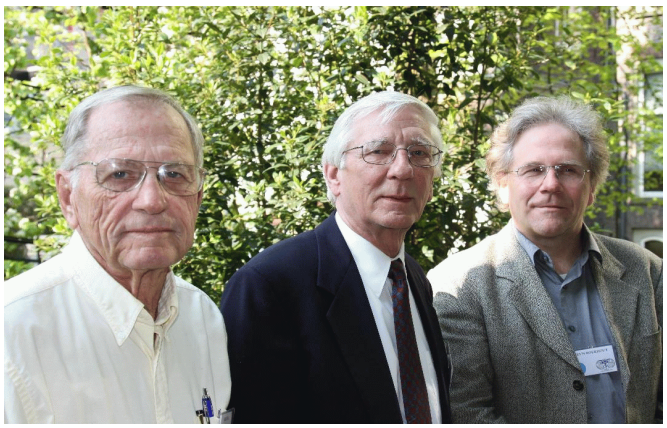
One Fungus One Name (1F=1N) CBS Symposium
Amsterdam, April 20, 2011

Johanna Westerdijk Award presented to Jack Fell by Pedro Crous, Director of the CBS Fungal Biodiversity Centre

The 'One Fungus = One Name' Symposium was held in Amsterdam, April 20, 2011, at Trippenhuis, the headquarters of the Royal Netherlands Academy of Arts and Sciences. The program of the symposium can be seen at:
http://www.cbs.knaw.nl/News/NewsDetails.aspx?Rec=53

During the meeting, the CBS 'Johanna Westerdijk Award' was presented to Jack Fell for his extensive studies on the diversity, ecology of basidiomycetous yeasts, including the genetics of their mating cycles. His involvement in the development of molecular diagnostics for yeast pathogens is seen as significant. Furthermore, it was particularly appreciated that Jack deposited many strains originating from his research in the CBS collection thus allowing them to be studied by future generations of scientists.

Jack is of course one of the editors of the newly published treatise "The Yeasts, a Taxonomic Study", along with Teun Boekhout and Clete Kurtzman.



Jack Fell, Clete Kurtzman, and Teun Boekhout, editors of "The Yeasts, a Taxonomic Study", 5th Edition (2011)

Clete Kurtzman presents Prof. Dr. Theo Mulder, of the Royal Netherlands Academy of Arts and Sciences, with a copy of the recently published treatise, "The Yeasts, a Taxonomic Study"

Forthcoming Meetings

25th International Conference on Yeast Genetics and Molecular Biology
July 11th - 16th 2011, Olsztyn-Kortowo, Poland

Early registration to 25th International Conference on Yeast Genetics and Molecular Biology that will be held July11-16, 2011 in Olsztyn, Poland is finished, but you can still register with higher yet still reasonable registration fees: 500 EURO regular rate and 270 EURO student rate. If you register and submit your abstract by April 15th it will be included in the supplementary issue of the journal Yeast, which is also the abstract book of our Conference.

Please visit **www.yeast-2011.org** to see detailed instructions of the registration and abstract submission, as well as all the facts regarding the Conference Program and venue. If you have any questions or need additional information, please do not hesitate to contact the organizers at **yeast2011@ibb.waw.pl**.

ISSY 29 - 29th International Specialized Symposium on Yeasts
August 29 - September 2 2011
Guadalajara, Jalisco, Mexico

On behalf of the International Commission on Yeasts (ICY), the "Universidad Nacional Autónoma de México", the "Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco", the "Universidad Iberoamericana" and the Organizing Committee, we have the pleasure to invite all colleagues in yeast research to attend the 29th Specialized Symposium on Yeasts (ISSY 29) that will be held from August 29 to September 2 in the colonial and marvelous city of Guadalajara, State of Jalisco, Mexico.

Until 2009 most of the symposia were held in Europe. With the aim to increase the participation of researchers of countries all over the world, the ICY decided to organize several meetings in other continents. During the XI International Congress held in Kiev in 2008 the ICY gave the Mexican Commissioners the honor to organize the 29th ISSY to enhance the participation of researchers from America and the Caribbean, being the first time that this symposium is hold in Latin-America.

The theme of the symposium The Relevance of Yeasts and Microbial Consortia in Traditional and Industrial Fermentations was chosen with the aim to emphasize the central role of yeast and microbial consortia in the production of important commodities for human consumption and activities. Certainly this topic will be of interest for all delegates attending the meeting and should be relevant to the developing world in the region this symposium is held.

The program of the ISSY29 will comprise plenary lectures, keynote lectures, oral presentations, poster sessions and round table discussions to overview up-to-date knowledge, to contribute to the dissemination of recent research achievements and to stimulate discussion and exchange of information among yeast researchers.

Welcome to Mexico and discover a colorful country with rich culture, history, traditions and gastronomy!

Patricia Lappe-Oliveras • Anne Gschaedler Mathis • Rubén Moreno-Terrazas
Chairs of the Organizing Committee

Please visit the website regularly for updates: **http://issy29.com/index.html**

The 23rd Annual Meeting of the Thai Society for Biotechnology
Systems Biotechnology: Quality & Success
27-28 October 2011, The Imperial Queen's Park Hotel, Bangkok, Thailand

For additional information, consult: **http://www.tsb2011.com/index.html**

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ICY 13 - 13th International Congress on Yeasts
Yeasts for a Sustainable Future
August 26-30 2012, Madison, Wisconsin, USA

This is the first announcement for the forthcoming 13th International Congress on Yeasts, to be held at the Monoma Terrace Community and Convention Center, in Madison, Wisconsin, USA, August 26-30, 2012. For further information on the conference or the beautiful venue, please consult: **http://conferencing.uwex.edu/conferences/icy2012/**

The conference organization is headed by Professor Thomas Jeffries of the Forest Products Laboratory in Madison.

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

For information on this meeting, please consult **www.ncy2011.org**

For up-to-date announcements on other forthcoming meetings, please see the YNL website
http://publish.uwo.ca/~lachance/Future%20meetings.html

Fifty Years Ago in "YEAST: A News Letter for Persons Interested in Yeast"
(Volume X Number 1, May 1961)

Editor: Herman J. Phaff, University of California, Davis, California; Associate Editors: Leslie R. Hedrick, Illinois Institute of Technology, Urbana, Illinois, and Cecil G. Dunn, Massachusetts Institute of Technology, Cambridge, Massachussetts.

'A contribution of $0.50 from those who have not contributed for some time would be appreciated to finance future editions of tne News Letter.'

Dr. Moshe Shifrine reports: 'A paper "Classifying yeasts on punch cards" has been accepted for publication by Antonie van Leeuwenhoek. The advantages of such a method are: great speed in both filling and retrieving information; ease of recording data; cards of yeasts with common characteristics can easily [be] retrieved. Translation of information from punch card onto IBM cards (the method of the future) is simple.'