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# Yeast

## A Newsletter for Persons Interested in Yeast

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# Editorials

## ICY 11 - Rio de Janeiro

Congratulations to Leda Mendonça-Hagler for the great success of the 11th International Yeast Congress in her magnificent city. An enriching scientific program in beautiful surroundings was complemented by fine Brazilian and international culinary delights.

## Electronic format

Our survey has indicated that many readers would enjoy receiving the Yeast Newsletter in electronic format. This will be possible starting with the June issue. Readers will have the choice of receiving a printed copy at cost, a free PDF copy, or both. Please read the accompanying instructions carefully. As announced in the June issue (2004), a uniform subscription rate of USD$12.00 now applies to all mailings outside Canada or the USA. Readers who have paid in advance will continue to receive mailings at the old rate until their current credit runs out.

## Yeast Newsletter / International Commission on Yeasts - Website

As suggested by members of the International Commission on Yeasts during the 11th ICY, I have begun to construct a Website to promote the activities of the Commission. At this time, the site is centered around the Yeast Newsletter and contains various links to items that may be of interest to our readers. In time, I hope to incorporate more archival materials about the Commission. A page that will allow readers to send their YNL communications directly by pasting text into their browser is being developed. I hope also to post some "hot news items" immediately upon receipt, so that a six month wait will not longer be necessary to find out about recent or forthcoming events of importance to yeast researchers. News items can be e-mailed at any time <lachance@uwo.ca>. Please feel free also to communicate links (URLs) to Websites associated with your laboratory or research program, for addition to the site.

All this is *work in progress*. I thank in advance users of the Website for their patience and any suggestions they might have. The following URL and associated server were put at my disposition by the University of Western Ontario Information Technology Services, for which I am grateful.

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

## Yeast Newsletter - Back Issues

Many thanks to Jack Fell, who kindly donated his back issues of the Yeast Newsletter to our archives. We are still missing issues published prior to November 1958 and would welcome these.

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

M. A. Lachance
Editor

# Essay

## Anomalous death/viability patterns of two yeasts cultured in Yeast Nitrogen Base

**Byron F. Johnson,[a] Monique Bassan,[a], and Gode B. Calleja[b]**
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*Schizosaccharomyces pombe*

In 1967,[1] lysis at the septum was observed in about 50% of fission yeast cells as they entered the stationary phase of growth, having been cultured in Yeast Nitrogen Base - amino acids (YNB; Difco). The loss of viability was subjectively tabulated by phase-contrast microscopy of dividing cells. Addition of $PO_4$ (5 mg $KH_2PO_4$/ml, 0.0015 **M**) or L-asparagine (0.2 mg/ml, 0.037 **M**) or both supplements seemed to remedy the problem. In 1990,[2] Bligh & Kelly repeated and extended those observations. In 2004,[3] objective assessment (counting CFU) of the YNB-fission yeast problem coupled with fluorescence microscopy of CFW-stained cells indicated that 99.9% of the fission yeast cells cultured in YNB were dying, but many had not lysed. Thus lysis at the septum was not the only parameter for loss of viability at the end of the log phase as was originally scored.

*Schwanniomyces occidentalis*

In the early to mid-1980's, Calleja and colleagues[4] needed a defined medium for cultures of *Schw. occidentalis*. Aware of the fission yeast problem, they experimented with $PO_4$ supplementation. O.2 **M** $PO_4$ appeared to be the ideal supplement to YNB. However, Dowhanick et al.[5] reverted to non-supplemented YNB. And thus were the first to show a serious viability problem in *Schw. occidentalis:* a 24 h-lag phase in cultures of organisms usually exhibiting a doubling time of ca. 1.5 h (their Figs. 1B, 1D, 1F). Subjective assessment[6,7] by phase contrast microscopy suggested (6, their Table 3) that ca. 97% of cells in 0.2 **M** $PO_4$-supplemented YNB were viable, ca. 93% viable in EMM2, but only ca. 36% viable in unsupplemented YNB. Note that at 36% viability, Dowhanick et al.[5] should not have had so long a lag. This is difficult to reconcile, but not really our problem. There are probably three factors here, one, a high variability of true viability (see ref. 6, their Table 2); two, the uncertainties intrinsic to phase contrast evaluation of viability of yeasts; and three, the role of pH control. (Some of these uncertainties are discussed[8].)

Accordingly, an objective evaluation was performed (ref. 8, and unpublished). In 10 paired comparisons of CFU, YNB viability as a percent of viability in YNB + 0.2 **M** $PO_4$ ranged from 26.4% to 79.4%; the median was 43%; the mean was 47.3% (s = 15.8). The percentile range for each median was a factor of 2.3, with no overlap between media.

A few general conclusions are apparent. The medium YNB - amino acids, is not adequate for either *Schizosaccharomyces pombe* or *Schwanniomyces occidentalis* without supplementation. From these observations, it seems reasonable to suggest that anyone who wishes to use a defined medium for any yeast should check it out. With budding yeasts (e.g., *Schw. occidentalis)* it is more difficult to classify modes of death, but for *Sch. pombe*, there clearly are at least two modes. A "nutritional" study of *Sch. pombe* could be very instructive.

It also clear that the use of phase contrast microscopy in this way can lead to a variety of problems -- lysis at the septum so dominated the image that the other dying cells were not perceived. On the other hand, the highly variable objective results with *Schw. occidentalis* obviously strained the capacity to determine the "real" loss of viability.

[1]B.F. Johnson. (1967) J. Bacteriol. 94: 192-195.

[2]H.F.J. Bligh and S.L. Kelly. (1990) FEMS Microbiol. Letters 68: 69-72.

[3]M. Bassan, 2004, Honours Thesis, Carleton University.

[4]G.B. Calleja, unpublished.

[5]T.M. Dowhanick, S.W. Scherer, G. Willick, I. Russell, G.G. Stewart, and V.L. Seligy. (1988) Can. J. Microbiol. 34: 262-270.

[6]B.F. Johnson, I. Curran, and T. Walker. (1994) Ant. v. Leeuwenhoek 68:107-109.

[7]B.F. Johnson, G.B. Calleja, and T. Walker. (2003)In: Wolf, Breunig & Barth, eds. Non-Conventional Yeasts in Genetics, Biochemistry and Biotechnology, Springer-Verlag, Berlin. pp. 267-269.

[8]B.F. Johnson, D. Wahalawatta, S. Mukherjee, C. Ficker, S. Boroumandi, O. Clarkin, B. Ramsingh, F. Siddiqi, M. Vessal, J. Escorcia, E. Chock, M. Lopez, S. Khulbe, H. Privora, R. Booth, and G.B. Calleja. (2003) In: Wolf, Breunig & Barth, eds. Non-Conventional Yeasts in Genetics, Biochemistry and Biotechnology, Springer-Verlag, Berlin. pp. 263 - 266.

## I. Russian Collection of Microorganisms (VKM), Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino 142292, Russia. Communicated by W.I. Golubev <wig@ibpm.pushchino.ru>.

Recent publications.

1. Golubev, W.I., Sampaio, J.P., Gadanho, M., and Golubeva, E.W. 2004. *Cryptococcus paraflavus* sp. nov. (Tremellales), isolated from steppe plants in Russia. J. Gen. Appl. Microbiol. 50, N 2, 65-70.

Three strains related to *Cryptococcus flavus* were isolated from plants in the Prioksko-terrasny biosphere reserve (Moscow region). Physiological characterization, mycocinotyping, sequencing of the D1/D2 domain of the 26S rDNA and the ITS region revealed their separate taxonomic position. The name *Cryptococcus paraflavus* is proposed to accommodate these isolates (type strain VKM Y-2923).

2. Pfeiffer, I., Farkas, Z., and Golubev, W.I. 2004. dsRNA viruses in *Nadsonia fulvescens*. J. Gen. Appl. Microbiol. 50, N 2, 97-100.

Isometric virus particles were revealed in *Nadsonia fulvescens* var. fulvescens VKM Y-2618 and the type strain of *N. fulvescens* var. *elongata*. Strong homology was detected between viral dsRNAs of these varieties but not between them and dsRNA of mycocinogenic strain (K1) of *Saccharomyces cerevisiae*. No mycocinogenic activity was detected in dsRNA virus infected strains of *Nadsonia*. It was noted that dsRNA viruses are present in all dominant species (*Trichosporon pullulans*, *Xanthophyllomyces dendrorhous* and *Nadsonia fulvescens*) of the yeast community in spring tree exudates.

3. Golubev W.I., Kulakovskaya T.V., Kulakovskaya E.V., Golubev N.W. 2004. The fungicidal activity of an extracellular glycolipid from *Sympodiomycopsis paphiopedili* Sugiyama et al. Mikrobiologiya (Moscow) 73 (6, in press).

The yeast *Sympodiomycopsis paphiopedili* (Ustilaginomycetes) produces an extracellular glycolipid which possesses the maximum antifungal activity at the pH of the medium equal to 4.0 - 4.5. Among the approximately 300 tested species of yeast-like and mycelial fungi, more than 80% (including pathogenic for plants, animals and humans) were found to be sensitive to this glycolipid.

4. Kulakovskaya T.V., Shashkov A.S., Kulakovskaya E.V., Golubev W.I. 2004. Characterization of an antifungal glycolipd secreted by the yeast *Sympodiomycopsis paphiopedili*. FRMS Yeast Res. 5, N 3, 247-252.

An antifungal glycolipid was purified from the culture liquid of the ustilaginomycetous yeast *Sympodiomycopsis paphiopedili* by column and thin-layer chromatography. According to nuclear resonance and mass-spectroscopy experiments it was a cellobioside containing 2,15,16-trihydroxypalmitic acid as an aglycon. The minimal effective concentrations leading to ATP leakage and growth inhibition were 45 and 160 µg/ml for *Cryptococcus terreus* and *Candida albicans*, respectively.

## II. Dipartimento di Scienze e Tecnologie Agro-Forestali e Ambientali, Università di Reggio Calabria, Piazza San Francesco 7, I-89061 Gallina (RC), Italia. Communicated by A. Caridi <acaridi@unirc.it>.

Recent publication.

1. Caridi A., Cufari A., Galvano F., Geria M., Postorino S., Tafuri A., Ritieni A. – New microbiological approach to reduce ochratoxin levels in alcoholic beverages. 19th International ICFMH Symposium Food Micro 2004, P11-13, p.264, Portorož (Slovenia), September 12-16, 2004.

Ochratoxin A (OTA) is a potently nephrotoxic and carcinogenic mycotoxin, that frequently contaminates various foods and beverages. Among others, one of the most effective strategy for controlling mycotoxins hazards is the use of specific materials that adsorb mycotoxins, thus avoiding or limiting their bioavailability. Increasing interest has been recently generated by the possibility of using microbiological-binding agents to remove mycotoxins. In alcoholic beverages OTA is formed prior to the alcoholic fermentation, during which the mycotoxin is partially removed or degraded. It is interesting to note that the decrease is strain-dependent. In spite of these reports, at present no study has been performed on yeasts selected for alcoholic fermentation regarding their ability to remove OTA by adsorption of the mycotoxin on yeast cell wall. So we decided to investigate the biodiversity of 20 strains of *Saccharomyces cerevisiae*, selected for winemaking, concerning their capacity to remove OTA in vitro. The strains were chosen for their different aptitude to adsorb phenolics using a simple method for screening yeasts in Petri dishes. A weighed quantity (40-60 mg) of fresh biomass of each strain was put in triplicate in test tubes containing 10 ml of physiological sterile saline and 1,100 ppt of OTA. The toxin was assayed in the supernatants after 15 days. The amount of removed toxin was calculated by subtracting the amount of toxin in experimental tubes from the amount found in control tubes. The removing capacity of the yeasts varies from 0 to 1,100 ppt of OTA and appears to be somewhat correlated with the strain aptitude to adsorb phenolics. These preliminary results confirm the hypothesis that it is consistent to screen starter yeasts regarding their aptitude to adsorb phenolics in view to select strains useful to remove OTA during alcoholic fermentation. The majority of the 20 tested strains showed a similar behaviour both for phenolics adsorption and for OTA removing. Moreover, it has been demonstrated that different strains of *S. cerevisiae* possess a strong aptitude to remove OTA in vitro. As regards the mechanism of OTA removing the hypotheses are fundamentally two: 1) cellular uptake followed by enzymatic degradation of the toxin; 2) cell wall adsorption by mannoproteins-binding capacity. Regarding the second hypothesis it is useful to remember that the

cell wall of *S. cerevisiae* consists of two major components, glucan and mannoproteins, and one minor component, chitin. Mannoproteins located in the outermost layer of yeast cell wall determine the wall's porosity and thereby regulate leakage of proteins from the periplasmic space and entrance of macromolecules from the environment. Although interesting, this is not the main part of our work, which aimed to establish the affinity of different strains of *S. cerevisiae* concerning OTA using a simple screening in vitro. Further researches will be carried out to confirm the ability of the most efficacious strains of S. cerevisiae to remove OTA also from naturally or artificially contaminated grape must and wort.

2. Ventrice D., Rizzo M., Procopio S., Caridi A., Cufari A., Geria M. – Determination of polyphenols adsorbed on yeasts by HPLC. PBA2004 - 15th International Symposium on Pharmaceutical and Biomedical Analysis, p.412, Firenze (Italy), May 2-6, 2004.

Many epidemiological researches support the observation that the high consumption of phenolics naturally present in food and beverages is associated with low incidence of cardiovascular diseases in humans. Phenolic compounds are among the major constituents of red wines. Type and quantity of phenolics largely influence different red wine properties, such as colour, astringency, mouth-feel characteristics, and health-giving features. Recently it has also been shown that several phenolics possess a wide range of antioxidant and pharmacological effects. During winemaking, wine yeasts show different aptitude to adsorb, on the external part of the cell-wall, phenolic compounds; this behaviour significantly affects wine composition. Recently, a simple screening method was proposed to differentiate yeasts with low, medium and high aptitude to adsorb phenolics, considering the colour modification of the microbial biomass. To our knowledge, at present there is not a specific analytical method able to detect phenolics adsorbed on microbial cell-walls. Therefore we tried to develop a new method of extraction and liquid chromatographic determination of phenolics, in detail monomeric anthocyans, non anthocyan flavonoids and phenol acids. An analytical RP-HPLC method was formulated to determine monomeric anthocyans, non anthocyan flavonoids, and phenol acids adsorbed on the cell-wall of 22 *Saccharomyces cerevisiae* strains. The yeasts were cultivated for 7 days at 25 °C on two media containing different grapes' phenolics. The microbial biomass was purified by liquid-liquid extraction of phenolics, followed by chromatographic separation on a Hypersil ODS 5 (250 mm x 4.6 mm; 5 µm) at 30 °C. The eluent was a mixture of acetic acid, methanol and water (5/45/50, v/v/v); the flow rate was 1 ml/min. The detection was performed at 220 and 260 nm with a diode-array detector. The validation of the method was carried out according to internationally accepted criteria for biological samples. The proposed method is highly responsive for the determination of different phenolics, and seems to be useful to evaluate the adsorption profile of phenolics on yeasts.

## III. Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <wout.middelhoven@wur.nl>.

The following papers appeared since December 2003.

1. K. Lopandic, T. Sugita, W.J. Middelhoven, M. Herzberg, J.W. Fell, S. Zelger and H.-J. Prillinger. *Trichosporon caseorum* sp. nov. and *Trichosporon lactis* sp. nov., two basidiomycetous yeasts isolated from cheeses. Frontiers in Basidiomycete Mycology 2004, pp. 99-116.

2. W.J. Middelhoven, G. Scorzetti and J.W. Fell. Systematics of the anamorphic basidiomycetous yeast genus *Trichosporon* Behrend with the description of five novel species: *Trichosporon vadense, T. smithiae, T. dehoogii, T. scarabaeorum* and *T. gamsii*. Int J Syst Evol Microbiol 54:975-986.

Phylogenetic trees of the genus *Trichosporon*, based on molecular sequence analysis of the ITS region and of the D1D2 region of the large subunit of ribosomal (26S) DNA are presented. This study includes three new species from soil. viz. *T. vadense* sp. nov. CBS 8901$^T$, *T. smithiae* CBS 8370$^T$ and *T. gamsii* CBS 8245$^T$, one new species from an insect, viz. *T. scarabaeorum* CBS 5601$^T$ and one species of unknown origin, viz. *T. dehoogii* CBS 8686$^T$. The phylogenetic positions and physiological characteristics that distinguish the new taxa from related species are discussed.

3. W.J. Middelhoven. Assimilation of polysaccharides and other non-conventional substrates, a diagnostic tool and an ecological marker. Abstracts 11th International Congress on Yeasts, Rio de Janeiro, 15-20th August 2004, p. 75.

4. W.J. Middelhoven. Identification of pathogenic and foodborne *Trichosporon* species. Abstracts 19th International ICFMH Symposium, FoodMicro2004, Portoroz, Slovenia, 12-16th September 2004, p. 360.

This paper is an extension of an earlier study published in Mycoses 46(2003)7-11, dealing with clinically relevant species only. It contains an identification key based on growth tests with carbon sources not traditionally used in yeast taxonomy, viz. uric acid, ethylamine, L-4-hydroxyproline, tyramine and L-phenylalanine as sources of carbon and nitrogen, and polygalacturonate, quinate at pH 5.5, 4-ethylphenol, 2,3-dihydroxybenzoate and orcinol as sole carbon sources. Of the standard growth tests, assimilation of L-rhamnose and the maximum growth temperature proved to be useful.

Not yet in print but already available on Internet.

5. W.J. Middelhoven. *Cryptococcus allantoinivorans* sp. nov., an anamorphic basidiomycetous yeast (Tremellales) physiologically resembling other species of the *Cryptococcus laurentii* complex that degrade polysaccharides and C2 compounds. Antonie van Leeuwenhoek 2005.

A novel *Cryptococcus* species is proposed to accommodate a yeast strain (CBS 9604$^T$) isolated from a decaying mushroom and able to assimilate allantoin as sole carbon source, a characteristic very uncommon among yeasts. By traditional methods, the strain could not be distinguished from *C. laurentii*, but nucleotide sequences of the D1D2 region of the 26S subunit and of the ITS region of ribosomal DNA showed relationship to the *Bulleromyces* clade of the genus *Cryptococcus* (order Tremellales). Data on the assimilation of some C2 compounds and polysaccharides are provided and compared with those of other type strains of novel species of the *C. laurentii* complex. Each showed a characteristic pattern.

6. W.J. Middelhoven. *Trichosporon wieringae* sp. nov., an anamorphic basidiomycetous yeast from soil, and assimilation of some phenolic compounds, polysaccharides and other non-conventional carbon sources by saprophytic *Trichosporon* species. Antonie van Leeuwenhoek 2005.

A novel species is proposed to accommodate strain CBS 8903$^T$ isolated from soil supplied with birch wood xylan. Sequencing of D1D2 and ITS regions of ribosomal DNA revealed close relationship to the Porosum clade of the genus.The ability of saprotrophic *Trichosporon* species to assimilate uric acid, ethylamine, L-4-hydroxyproline, some aromatic compounds including orcinol, phloroglucinol, 4-ethylphenol, 2,3-dihydroxybenzoate and L-phenylalanine, and some polysaccharides including polygalacturonate, xylan, galactomannan and tannic acid was studied. An identification key based on these characters is provided.

## IV. Department of Food Science and Technology, Dalhousie University, 1360 Barrington Street, D401, Halifax, Nova Scotia, Canada B3J 2X4. Communicated A. Speers <Alex.Speers@Dal.Ca>.

Recent publication.

1. Wan, Y.-Q. Speers, R.A. and Jin , Y-L. 2004. Effects of Fermentation Parameters and Cell Wall Properties on Yeast Flocculation. Proceedings of the Tsingtao International Symposium on Brewing Technology 2004 (TISBT'2004), Qingdao, China August 15-17, 2004 (Written presentation in Mandarin, Oral presentation English & Mandarin).

Industrial wort was fermented with a NewFlo phenotype ale yeast in lab-scale cylindrical fermenters. The effects of various fermentation parameters and yeast cell wall properties on yeast flocculation were studied during 120-h fermentation. The evaluation of the cell volume during the fermentation revealed a non-normal distribution ($p<0.05$) at most fermentation times. Overall yeast cell size initially decreased in the first 24 h of fermentation then increased during 24-60 h. Cell size then declined until the end of fermentation. These changes may reflect initial budding followed by individual cell growth and then settling of larger flocculent cells once fermenter shear forces declined. While yeast flocculation began after 24 h, most flocs remained in suspension until 60 h when the average turbulent shear rate caused by $CO_2$ evolution declined to below 8 $s^{-1}$. Both Helm's flocculence and cell surface hydrophobicity rapidly increased to high and stable values from 24 h onward. Although a significant correlation ($p<0.05$) was observed between zymolectin densities and cell surface area, the total zymolectin level on yeast cell walls did not change significantly with fermentation time ($p>0.05$). Interestingly, no significant difference existed in Helm's flocculation values of suspended and settled yeast cells ($p>0.05$). Changes in orthokinetic capture coefficient ($a_0$) value with fermentation time, measured in fermenting worts, indicated a significant increase ($p<0.001$) after 24 h of fermentation. Values of $a_0$ in sodium acetate buffers were significantly higher ($p<0.001$) than that measured in fermenting worts. Results suggested that fermentable sugar level and shear forces exert major influences on yeast flocculation in beer fermentation.

## V. Culture Collection of Yeasts, Institute of Chemistry, Dúbravská cesta 9, 842 38 Bratislava, Slovakia - *www.chem.sk/yeast*. Communicated by E. Breierová <chememi@savba.sk>.

The following are abstracts of articles that were published recently and are in press.

1. Kalebina T.S., Farkaš V., Laurinavichiute D.K., Gorlovoy P.M., Fominov G.V., Bartek P., Kulaev I.S. 2003. Detection of BGL2 results in an increased chitin level in cell wall of *Saccharomyces cerevisiae*. Antonie van Leeuwenhoek 84:179-184.

It is shown that the detection of BGL chemical composition differentiating fungal cells gene leads to increase in chitin content in cell wall of *Saccharomyces cerevisiae.* A part of the additional chitin can be removed from the *bgl*2? cell wall by alkali or trypsin treatment. Chitin syntase 1 (Ch1) activity was increased by 60 % in *bgl*2? mutant. No increase in chitin synthesis 3 (Chs3) activity in *bgl*2? cells was observed, while they become more sensitive to Nikkomycin Z. The chitin level in the cell wall of a strain lacking both *BGL2* and *CHS3 g*enes was higher than that in *bgl3*? and lower than that in *bgl*2? strain. Together these data indicate that the detection of BGL2 results in the accumulation and abnormal incorporation of chitin into the cell wall *S.cerevisiae*, and both and Chs3 take part in response to BGL2 detection in *S. cerevisiae* cells.

2. B. Košíková, E. Sláviková, V. Sasinková, F. Kaèík. 2004. The occurrence of yeasts in grass-grown soils. Selected Processes at the Wood Processing (Ed. V.Ve¾ková, A.Geffert, F. Koèík) Bobrovník, Slovakia ISBN 80-228-1328-1, 201-205.

The effect of various yeast microorganisms on pine wood sawdust was examined. We have tested the ability of ten different yeast species in order to remove extractives from wood before pulping. The growth of biomass was ranged from 0.72 to 4.56 g/L. The used strains remove the extractives in various extent. The removal of extractives was followed by FTIR analysis of acetone extracts from native pine wood and those obtained by extraction of wood samples after cultivation with yeast microorganisms. The obtained results indicate partial degradation of fatty and resin acids as well as sterols during biological treatment. Moreover, the acetone extracts were examined by HPLC analysis.

3. B. Košíková and E.Sláviková. 2004. Biotransformation of lignin polymers derived from beech wood pulping by *Sporobolomyces roseus* isolated from leafy material. Biotechnol. Lett. 26: 517-519.

The ability of the yeast, *Sporobolomyces roseus,* isolated from leafy material, to modify lignin derived from beech wood pulping was examined by FTIR and $^{13}C$ NMR spectroscopy, which revealed oxidative cleavage of the $C_\alpha$ - $C_\beta$ linkages between lignin units. Using veratryl alcohol as a model substrate confirmed that *Sp. roseus* could oxidize veratryl alcohol into veratric acid. This yeast might be suitable for the pretreatment of lignocellulosic materials and/or for biotransformation of technical lignins.

4. Sláviková E. and Vadkertiová R. 2003. Effects of Pesticides on Yeasts Isolated from Agricultural Soil - Zeitschrift fur Naturforschung 58c:855-859.

The effect of six various pesticides on the growth of yeasts isolated from agricultural soil was investigated. Two herbicides (with the effective substances lactofen and metazachlor), two fungicides (with the effective substances fluquinconazole and prochloraz), and two insecticides (with the effective substances cypermethrin +chlorpyrifos and triazamate) were tested. It is evident that there are considerable differences in inhibition effects of studied pesticides. The fungicide with the effective substance prochloraz inhibited the growth of majority of yeast strains. Insecticide triazamate at concentration 0.6 mM restricted or inhibited growth of all tested strains. The strains of the genus *Cryptococcus* were the most sensitive to pesticides, while the strains of the species *Cystofilobasidium capitatum*, *Debaryomyces occidentalis* var. *occidentalis*, and *Trichosporon cutaneum* were the most resistant.

5. Márová I., Breierová E., Koèí R., Friedl Z., Slovak B., Pokorná J. 2004. Influence of exogenous stress factors on production of carotenoids by some strains of carotenogenic yeasts. Ann. Microbiol. 54: 73-85.

The aim of this study was to compare composition and content of carotenoids produced by some yeasts strains in optimal growth conditions and in the presence of exogenous stress factors. Nine strains of carotenogenic yeasts were grown aerobically on glucose medium. As the stress factors 10 mmol/l $H_2O_2$ and 5-10 % NaCl were used, which were added into media i) at the beginning of growth and ii) to the exponentially growing cells. Changes of growth parameters as well as carotenoid production (lycopene, α-carotene and β-carotene) were followed. Ergosterol production was followed as additional parameter of biomass quality. Analyzed strains partially differed in the spectrum of produced carotenoids; the highest content of β-carotene was detected in *S. salmonicolor* CCY 19-4-10. Stress factors added to yeast cultures resulted in different responses. As good producers of enriched biomass could serve above all strains *R. glutinis* and *S. salmonicolor* grown under salt stress. Carotenoids act as lipid-soluble membrane antioxidants whose production is considered as an adaptive mechanism against adverse stress effects. Ability of red yeasts to adapt by means of overproduction of industrially significant metabolites could be of increasing interest for potential biotechnological applications.

6. Breierová E., Gregor T., Juršíková P., Stratilová E., Fišera M. 2004. The role of pullulan and pectin in the uptake of $Cd^{2+}$ and $Ni^{2+}$ ions by *Aureobasidium pullulans.* Annals of Microbiology 54, 247-255.

Three yeast-like strains of *Aureobasidium pullulans* that efficiently remove heavy metal ions from aqueous solution were studied. The production of the pullulan played an important role in the heavy metal accumulation. For better protection of cells against metals, this polysaccharide was added (0.3% w/v) into the cultivation medium and the result was compared the effect of pectin (0.3% w/v). Pectin due to its acidic character bound the heavy metals more effectively, while pullulan was better as a protective substance inhibiting penetration of heavy metals into the cells.

7. S. Bystricky, E. Paulovicova, E. Machova. 2004. Synthesis and immunogenicity of polysaccharide-protein conjugate composed of galactoglucoxylomannan of *Cryptococcus laurentii*. FEMS Microbiology Letters, 235, 311-314.

Galactoglucoxylomannan (GalGXMan) antigen of *Cryptococcus laurentii* was conjugated to protein carrier by a simple one step reaction. Prepared conjugate was immunogenic in rabbits and reinjection elicited booster response with significant increase of serum IgG( H+L) level. Induction of this Ig-isotype was confirmed in experiments with Protein A. Effectiveness of immune serum to inhibit the growth of *Cryptococcus laurentii* was demonstrated. The results indicate that the prepared conjugate could be considered as effective immunogen with potential for incorporation in the vaccine.

8. Miadoková E., Svidová S., Šubjanská I., Kogan G. 2003. Detection of antimutagenic potential of glucomannan in unicellular green algae and bacteria. Biologia (Bratislava) 58:627-631.

The potential bioprotective/antimutagenic effect of glucomannan (GM) isolated from *Candida utilis* was evaluated on two genetic model systems : bacteria *Salmonella tyohimurium* and unicellular green alga *Chlamydomonas reinhardtii*. Because GM exhibited antimutagenic effect an two repair-deficient strains of S. typhimurium (TA97 and TA 100), we decided to two repair-deficient strains of *C. reinhardtii* for the GM potential bioprotective effect elucidation. On the basis of the inhibition

index I evaluation at the highest concentration used, the results of DNA-repair assay on algae indicated that methyl methanesulphonate (MMS) had a strong genotoxic effect on recombination-repair-deficient strain (*uvs*10; I=0.16) and excision-repair-deficient strain (*uvs*12; I=0.05), and it was considerably less genotoxic for mismatch-repair-deficient strain (*uvs*14; I=0.77). A bioprotective effect of GM was observed in repair-deficient algal strains *uvs*10 and *uvs*14, but it unexpectedly increased toxicity of MMS applied on the wild type strain. It was revealed that bioprotectivity/ antimutagenicity of GM was dependent on the method of application, both in bacteria and algae.

9. Usova T. A., Zhanaeva S. Ya., Kogan G., Shandula I., Korolenko T.A. 2003 Mouse lymphosarcomas sensitive and resistant to cyclophosphamide therapy: activity of cathepsins B, L, and D during various schemes of treatment with cyclophophamide and SE-glycan. Bulletin of Experimental Biology and Medicine: 136: 451-454.

We measured activities of cysteine (cathepsins B and L) and aspartyl proteinases (cathepsin D) in tumor of mice with sensitive and resistant lymphosarcomas. In cyclophosphamide-resistant lymphosarcome tissue activities of cathepsins B, L, and D were lower than in cyclophosphamide-sensitive lymphosarcoma. After treatment with cyclophosphamide in high doses enzyme activities in mice with cyclophosphamide-resistant lympho-sarcoma increased more significantly than in animals with cyclophosphamide-sensitive lympho-sarcoma. Sulfoethylated α-1,3-D-glycan potentiated the effect of cyclophosphamide in mice with both forms of lymphosarcoma. This drug in lowest dose (10 mg/kg) was most effective.

10. Škrobáková A., Kogan G., Altomare C., Moretti A. 2003. The possibility of maize rot control by *Trichoderma* and fungal cell wall polysaccharide. Acta fytotechnica et zootechnica 2:50-52.

*Fusarium* fungi are common contaminants of important agricultural crops, known to produce several mycotoxins. This work reports on promising results of the tests carried out with a microbiological strain of antagonist *Trichoderma* and a fungal polysaccharide derivative sulfoethyl glucan (SEG) isolated from the cell walls of baker´s yeast *Saccharomyces cerevisiae* as the tools for disease control in field conditions. Besides observation of a lower degree of infestation with *F. graminearum* and *F. verticillioides,* we also analyzed the presence of the related toxins which are known as factors of pathogenesis or as being toxic to human and animals (the most toxic one being fumonisin). Both type of the used agents revealed high biological activity; they decreased or depressed toxin (FB1) production. In each sample the toxin FB2 was identified.

11. Kogan G., Šrobárová A., Tamas L. 2004. Effect of externally applied fungal polysaccharides on fusariosis in tomato plants. Chem. Pap.: 58, 139-144.

The chitin-glucan complex isolated from the waste mycelia of filamentous fungi *Aspergilus niger* that are left behind upon the industrial production of citric acid, β-D-glucan from bakers yeast (*Sacchraomyces cerevisiae*), and mannan from *Candida albicans* has been investigated. Five different water-soluble polysaccharide derivatives were obtained and used in the assay of the antifungal activity against plant pathogen *Fusarium oxysporum f.* sp. *lycopersici* in tomato (*Lycopersicum esculenta* L.). In the experiments, application of the polysaccharides led to the diminished infestation as well as to slightly increased productivity of fresh mass of the plants. The results demonstrated that the external application of the polysaccharides led to changes in production of cell-wall, as well as of some outer- and integral-membrane-bound proteins. Although the nature of the observed proteins has not been yet established, it can be speculated that they represent certain enzymes involved in the infective or anti-infective mechanisms in plants.

## VII. Department of Applied Microbiology, Lund University, PO Box 124, 221 00 Lund, Sweden. Communicated by M.F. Gorwa-Grauslund <marie-francoise.gorwa@tmb.lth.se>.

The department of Applied Microbiology pursues the following topics of research on yeast.

**A. Yeast as biocatalyst for stereoselective reductions.** The baker's yeast *Saccharomyces cerevisiae* is being genetically engineered to generate efficient biocatalysts for the reduction of dicarbonyl compounds of pharmaceutical or chemical interest. Work is focused on (i) the isolation and expression of reductase gene from various sources and (ii) the engineering of pathways that provide NADPH (that is the co-factor needed for the bioreduction).

Recent publications

1. M. Katz, B. Hahn-Hägerdal and M.F. Gorwa-Grauslund. 2003. Screening of two complementary collections of *Saccharomyces cerevisiae* to identify enzymes involved in stereo-selective reductions of specific carbonyl compounds: an alternative to protein purification. Enz. Microb. Technol. 33:163-172.
2. M. Katz, T. Frejd, B. Hahn-Hägerdal and M-F. Gorwa-Grauslund 2003. Efficient anaerobic whole-cell stereoselective bioreduction with recombinant *Saccharomyces cerevisiae.* Biotechnol. Bioeng. 84: 573-582.
3. M. Katz, T. Johanson and M-F. Gorwa-Grauslund. 2004. Treatment of *Candida tropicalis* with a mild detergent reveals an NADPH dependent reductase in the crude membrane fraction, which enables the production of pure bicyclic exo alcohol. Yeast. In press.

Recent thesis

4. Mikael Katz 2004. Bioreduction of carbonyl compounds to chiral alcohols by whole yeast cells: Process optimisation, strain design and non-conventional yeast screening. PhD thesis. Lund University.

**B. Design of pentose-utilising yeast.** Recombinant xylose-utilising *Saccharomyces cerevisiae* strains are designed for ethanol production from lignocellulosic hydrolysates using both metabolic engineering and inverse metabolic engineering strategies.

Recent publications.

5. Gárdonyi M, Österberg M, Rodrigues C, Spencer-Martins I, Hahn-Hägerdal B 2003. High capacity xylose transport in *Candida intermedia* PYCC 4715. FEMS Yeast Res 3:45-52 Gárdonyi M, Hahn-Hägerdal B 2003 The *Streptomyces rubiginosus* xylose isomerase is misfolded when expressed in *Saccharomyces cerevisiae*. Enzyme Microbiol Technol 32:252-259.
6. Wahlbom CF, Cordero Otero RR, van Zyl WH, Hahn-Hägerdal B, Jönsson LJ 2003 Molecular analysis of a *Saccharomyces cerevisiae* mutant with improved ability to utilize xylose shows enhanced expression of proteins involved in transport, initial xylose metabolism and the pentose phosphate pathway. Appl Environ Microbiol 69:740-746.
7. M. Jeppsson, K. Träff, B. Johansson, B. Hahn-Hägerdal and M.F. Gorwa-Grauslund 2003. Xylose reductase activity controls both xylose consumption rate and glycerol formation in xylose-fermenting recombinant *Saccharomyces cerevisiae. FEMS Yeast Research*. 3:167-175.
8. M. Gárdonyi, M. Jeppsson, G. Lidén, M.F. Gorwa-Grauslund and B. Hahn-Hägerdal 2003. Control of xylose consumption by xylose transport in recombinant *Saccharomyces cerevisiae*. *Biotechnol. Bioeng*. 82(7):818-824.
9. M. Jeppsson, B. Johansson, P. Ruhdal Jensen, B. Hahn-Hägerdal and M.F. Gorwa-Grauslund. 2003 The level of glucose-6-phosphate dehydrogenase activity strongly influences xylose fermentation and inhibitor sensitivity in recombinant *Saccharomyces cerevisiae* strains. *Yeast.* 20: 1263-72.
10. Lönn A, Träff-Bjerre KL, Otero Cordero RR, van Zyl WH, Hahn-Hägerdal B 2003 Xylose isomerase activity influences xylose fermentation with recombinant *Saccharomyces cerevisiae* strains expressing mutated *xylA* from *Thermus thermophilus*. Enzyme Microb Technol 32:567-573.
11. Wahlbom CF, van Zyl WH, Jönsson LJ, Hahn-Hägerdal B, Cordero Otero RR 2003 Generation of the improved recombinant xylose-utilizing *Saccharomyces cerevisiae* TMB 3400 by random mutagenesis and physiological comparison with *Pichia stipitis* CBS 6054. FEMS Yeast Res 3:319-326.
12. K. Träff, M. Jeppsson, B. Hahn-Hägerdal and M-F. Gorwa-Grauslund. 2004. Endogenous NADPH-dependent aldose reductase activity influences product formation during xylose consumption in recombinant *Saccharomyces cerevisiae*. Yeast 21:141-150.
13. M. Sonderegger, M. Jeppsson, C. Larsson, M.F. Gorwa-Grauslund, L. Olsson, I. Spencer-Martins, B. Hahn-Hägerdal and U. Sauer 2004. Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. Biotechnol. Bioeng. 87:90-98.
14. Sonderegger M, Jeppsson M, Hahn-Hägerdal B, Sauer U 2004 Molecular basis for anaerobic growth of *Saccharomyces cerevisiae* on xylose, investigated by global gene expression and metabolic flux analysis. Appl Environ Microbiol 70:2307-2317.

Recent thesis

15. Marie Jeppsson. 2004. Metabolic engineering of xylose-utilising *Saccharomyces cerevisiae* strains. A closer look at recombinant strains based on the xylose reductase-xylitol dehydrogenase pathway. PhD thesis. Lund University.

**C. Heterologous expression in yeast.** Both *S. cerevisiae* and *P. stipitis* are being studied for the expression of heterologous genes of interest.

Recent publications.

16. Passoth V, Cohn M, Schäfer B, Hahn-Hägerdal B, Klinner U 2003 Analysis of the hypoxia-induced *ADH2* promoter of the respiratory yeast *Pichia stipitis* reveals a new mechanism for sensing of oxygen limitation in yeast. Yeast 20:39-51

17. Görgens JF, Planas J, van Zyl WH, Knoetze JH, Hahn-Hägerdal B 2004. Comparison of three expression systems for heterologous xylanase production by *Saccharomyces cerevisiae* in defined medium. Yeast. 21:1205-1217.

18. Görgens JF, van Zyl WH, Knoetze JH, Hahn-Hägerdal B 2004 Amino acid supplementation improves heterologous protein production by *Saccharomyces cerevisiae* in defined medium. Appl Microbiol Biotechnol. Accepted for publication.

## VIII. Yeast Molecular Genetics Laboratory, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. Communicated by G. Miloshev <miloshev@obzor.bio21.bas.bg>.

Our new Yeast Molecular Genetics Laboratory was created earlier this year. Listed below are summaries of our recent work and publications from our laboratory.

1. E. Peycheva and G. Miloshev. Yeast Comet Assay (YCA) could precisely detect small amounts of DNA damaging chemicals (in press).

During last decade Single Cell Gel Electrophoresis (SCGE) also known as Comet assay has become an inevitable tool for detecting damages of DNA. The alkali version of the method could reveal double strand breaks, nicks, base modifications and other changes in the DNA molecule caused by different chemical or physical agents. Initially Comet assay has been used on mammalian and plant cells. We were first who applied Comet assay on yeast and called it Yeast Comet Assay (YCA). According to our data yeast DNA appears to be at least 100 times more sensitive than mammalian cells to the action of DNA damaging chemicals. Here we present our attempt to improve the YCA method by applying it on *S. cerevisiae* cell wall mutant strain. Although, preliminary, the results could be used to develop a test system. Such test system, based on *S. cerevisiae* mutant could be up to 1000 times more sensitive to DNA damaging agents presented in the environment.

2. E. Peicheva, N. Dodoff and G. Miloshev. Search for the molecular mechanisms of action of potentially antitumor compounds – methanesulfonylhydrazine and its hydrazones (in press).

Sulfonamide derivatives attract much attention as enzyme inhibitors and anticancer drugs. Sulfonylhydrazones, combining in the same molecule two pharmacophoric groups – sulfonamide and hydrazone – are of particular interest as cytostatic agents. Recently we synthesized and characterized new Schiff bases derived from methanesulfonylhydrazine (MSH) and found that they, along with the parent compound, exhibit antibacterial and antineoplastic effect. In the present work we demonstrate the cytostatic activity of MSH and its hydrazones on *Saccharomyces cerevisiae*. In an attempt to highlight their molecular mechanisms of action, the Comet assay and FACS analysis were applied. The results show that the tested compounds do not damage directly DNA and do not block the cell cycle.

3. M. Kirilova and G. Miloshev. Single Cell Gel Electrophoresis (SCGE) as a tool for yeast chromatin studies (submitted).

The genomic sequence of many organisms is known now but how those genomes are folded in the nucleus is remaining unsolved issue. Models for such folding involve loops (50-150 kb) attached to the peripheral lamina or internal nuclear structures such as matrix. It is thought that all important nuclear processes take place at these attachments. A method for detection damages in DNA called *Single Cell Gel Electrophoresis* or *Comet assay* reveals the loops of DNA attached to the nuclear structures . We were the first who applied the method on yeast developing in this way (YCA). On the other hand the endonucleases as micrococcal nuclease (MNase) and deoxyribonuclease I (DNase I) are traditionally used as tools in chromatin research. In this research we combined the nuclease digestion with Comet assay to expand the method and learn more about the organization of chromatin and nuclear matrix in yeast. Of special interest for the studies of chromatin structure is histone H1. It is common believe that the main role of H1 histones is involvement in the organizing of higher-ordered chromatin structures. Our results with YCA for the first time represent evidence that yeast H1 is engaged in the organization of higher-order chromatin structures in the nucleus of *Saccharomyces cerevisiae*. These results confirm also, that the method YCA could be used in chromatin research providing new data for more profound analysis of the nuclear organization.

## XIX. Department of Molecular Ecology, Institute of Cryobiology and Food Technology, 53A Cherni vrah Blvd, 1407 Sofia, Bulgaria. Communicated by P. Venkov <p_venkov@biofac.uni-sofia.bg>.

The following are summaries of articles from our Department, which have been published or accepted for publication.

1. D. Staneva, D. Uccelletti, F. Farina, P. Venkov & C. Palleschi. 2004. *KlSEC53* is an essential *Kluyveromyces lactis* gene and is homologous with the *SEC53* gene of *Saccharomyces cerevisiae*. Yeast 21: 41-51.

Phosphomannomutase (PMM) is a key enzyme, which catalyses one of the first steps in the glycosylation pathway, the conversion of D-mannose-6-phosphate to D-mannose-1-phosphate. The latter is the substrate for the synthesis of GDP-mannose, which serves as the mannosyl donor for the glycosylation reactions in eukaryotic cells. In the yeast *Saccharomyces cerevisiae* PMM is encoded by the gene *SEC53* (*ScSEC53*) and the deficiency of PMM activity leads to severe defects in both protein glycosylation and secretion. We report here on the isolation of the *Kluyveromyces lactis SEC53* (*KlSEC53*) gene from a genomic library by virtue of its ability to complement a *Saccharomyces cerevisiae sec53* mutation. The sequenced DNA fragment contained an open reading frame of 765 bp, coding for a predicted polypeptide, *Kl*Sec53p, of 254 amino acids. The *Kl*Sec53p displays a high degree of homology with phosphomannomutases from other yeast species,

protozoans, plants and humans. Our results have demonstrated that *KlSEC53* is the functional homologue of the *ScSEC53* gene. Like *ScSEC53*, the *KlSEC53* gene is essential for *K. lactis* cell viability. Phenotypic analysis of a *K. lactis* strain overexpressing the *KlSEC53* gene revealed defects expected for impaired cell wall integrity. The sequence of the *KlSEC53* has been deposited in the EMBL database under Accession No. AJ428418.

2. D. Staneva, D. Uccelletti, **N.** Petrova, P.Venkov, C. Palleschi. Isolation and nucleotide sequence of the *PSA1* gene from the yeast *Kluyveromyces lactis*. FEMS Yeast research (in press).

GDP-mannose is the mannosyl donor for protein glycosylation reactions in eukaryotic cell. GDP-mannose pyrophosphorylase, a key enzyme of the glycosylation pathway, catalyzes the synthesis of GDP-mannose from D-mannose-1-phosphate and GTP. In the yeast *S. cerevisiae*, this enzyme is encoded by *PSA1/VIG9/SRB1*, an essential, cell cycle regulated gene. The isolation of the *Kluyveromyces lactis PSA1* gene was attempted using three different approaches, the successful one being *in vivo* complementation of the hypo-osmotic lethality of *Saccharomyces cerevisiae srb1* mutant. Sequencing of the isolated genomic fragment revealed that it contains an ORF of 1083 bp, with 84% homology to *ScPSA1* gene, coding for a predicted polypeptide, *Kl*Psa1p, of 361 amino acids. *Kl*Psa1p showed high homology to yeasts' GDP-mannose pyrophosphorylases.

3. I. Alexandar, P. San Segundo, P. Venkov, F. del Rey, C.R. Vázquez de Aldana. 2004. Characterization of a *Saccharomyces cerevisiae* thermosensitive lytic mutant leads to the identification of a new allele of the NUD 1 gene. Int. J. Biochem. Cell Biol. 36:2196- 2213.

To improve our understanding of the factors involved in the osmotic stability of yeast cells, a search for novel conditional *Saccharomyces cerevisiae* cell lysis mutants was performed. Ten temperature-sensitive (ts) mutant strains of *S. cerevisiae* were isolated that lyse at the restrictive temperature on hypotonic, but not on osmotically supported medium. The ten mutants fell into four complementation groups: *ts1* to *ts4*. To clone the wild-type gene corresponding to the *ts4* mutation, a strategy aimed at complementing the thermosensitive phenotype-using low-copy and high-copy DNA libraries-was followed, but only two extragenic suppressors were identified. Another approach, in which classic genetic methods were combined with the use of yeast artificial chromosomes and traditional cloning procedures, allowed the identification of the *NUD1* gene - which codes for a component of the spindle-pole body - as the wild-type gene corresponding to the *ts4* mutation. Cloning and sequencing of the defective allele from the chromosome of the mutant cells resulted in the identification of a point mutation that produces a single amino acid change in the protein: a Gly-to-Glu change at position 585 (the *nud1-G585E* allele). Further analysis revealed that cells carrying this allele show a thermosensitive growth defect. At the restrictive temperature, the cells arrest with large buds, elongated spindles, and duplicated nuclei. In addition, with longer incubation times they are unable to maintain cellular integrity and lyse. Our results have allowed the identification of the first single amino acid mutation in *NUD1*, and suggest a link between cell cycle progression and cellular integrity.

M. Pesheva, O. Krastanova, L. Staleva, V. Dentcheva, M. Hadzhitodorov, P. Venkov. 2004. The Ty1 Transposition Assay: A New Short-Term Test for Detection of Carcinogens. J. Microbiol. Meth. (in press).

An assay based on induction by carcinogens of Ty1 transposition in *Saccharomyces cerevisiae* is proposed. A tester strain was developed that contains a marked Ty1 element, which allows following the transposition in the genome as a whole and a mutation, which increases cellular permeability. Hypersensitivity to chemical agents, higher cell wall porosity and transformability with plasmid DNA evidenced an enhanced cellular permeability of the tester cells. The increased permeability resulted in higher sensitivity to carcinogens. The treatment with different laboratory carcinogens induced Ty1 transposition rates in the tester strain by a factor of 10 to 20, compared to the controls. The induction is not stress-generated by the cytotoxicity of carcinogens, since treatment with $NaN_3$ at concentrations killing 50% of the cells did not increase the transposition rate. The increase of Ty1 transposition in tester cells is specific for active carcinogens and a positive response with pro-carcinogens was obtained only in presence of S9 mix. The Ty1 transposition test responded positively to a number of Ames-test or DEL-test negative carcinogens. The positive response of Ty1 test was statistically significant and verified in kinetics and concentration-dependent experiments. It is concluded, that the Ty1 transposition test can be used, in addition to the Ames assay, as a short-term test for detection of carcinogens.

## X. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nuβdorfer Läende 11, A-1190 Vienna, Austria. Communicated by H. Prillinger <hansjoerg.prillinger@boku.ac.at>.

The following are abstracts of our recent work.

1. O. Molnar, G. Schatzmayr, E. Fuchs and H. Prillinger. *Trichosporon mycotoxinivorans* sp. nov., a new yeast species useful in biological detoxification of various mycotoxins. Syst. Appl. Microbiol.

A yeast strain isolated from the hindgut of the lower termite *Mastotermes darwiniensis* (Mastotermitidae) was found to represent a new member of the genus *Trichosporon*. *Trichosporon mycotoxinivorans* is closely related to *T. loubieri* on the basis of the phylogenetic trees based on the D1/D2 region of 26S rDNA, an approx. 600 bp fragment of the 18S rDNA and both ITS regions. However, the two species differ at nine positions in the D1/D2 region of 26S rDNA. The IGS1 region of *T. mycotoxinivorans* is 401 bp long. *T. mycotoxinivorans* is distinguished from *T. loubieri* by its ability to assimilate inulin and galactitol, and its inability to grow at 40°C. The name of this newly isolated strain refers to an important characteristics of *T. mycotoxinivorans* to detoxify mycotoxins such as ochratoxin A and zearalenone. Therefore this strain can be used for the deactivation of the respective mycotoxins in animal feeds.

2. K. Lopandic, O. Molnár, H. Prillinger. Application of ITS sequence analysis, RAPD and AFLP fingerprinting in characterising the yeast genus *Fellomyces*. Microbiol. Res. (in press).

Three molecular techniques, ITS sequence analysis, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) were used to study phylogenetic and genotypic relationships among strains of the genus *Fellomyces*. In the analyses were included strains isolated predominantly from epiphytic lichens collected in Indonesia, China and Mexico. The polyphasic approach indicated that the *Fellomyces* isolates are genotypically heterogeneous and that lichens represent a specific environment for selection of large number of the sterigmatoconidia producing species. The phylogenetic and genotypic analysis confirmed the existence of 11 currently accepted *Fellomyces* species and indicated that several species may be the new representatives of the genus. The RAPD and AFLP analyses demonstrated a higher potential in distinguishing the *Fellomyces* strains than the ITS regions. Since the sequence analysis showed low or no divergence among several strains, both RAPD and AFLP fingerprinting indicated that the strains may be discriminated at the species level.

3. K. Lopandic, O. Molnár, H. Prillinger. *Fellomyces mexicanus* sp. nov., a new member of the yeast genus *Fellomyces* isolated from lichen *Crypthothecia rubrocincta* collected in Mexico. Microbiol. Res. (in press).

Two strains isolated from the lichen *Cryptotheca rubrocincta* collected in Mexico are described as new members of the sterigmatoconidia producing genus *Fellomyces*. Based on the identical sequences of the D1/D2 regions of 26S rDNA as well as the results of the cluster analysis of the AFLP fingerprints, the strains have been shown to be conspecific. The 26S rDNA-based phylogeny has indicated that *F. mexicanus* cluster within a clade including species such as four other *Fellomyces* isolates from lichens: *F. chinensis*, *F. lichenicola*, *F. sichuanensis*, *F. thailandicus*. *F. mexicanus* is characterised by the presence of xylose in the cell walls and CoQ10 in the mitochondrial membranes. The type culture is strain HB25 = CBS8279.

## XI. VTT Biotechnology, P.O.Box 1500, FIN-02044 VTT, Finland. Communicated by J. Londesborough <john.londesborough@vtt.fi>.

Publications since our last communication include the following.

1. Pitkänen, J.-P., Rintala, E., Aristidou, A., Ruohonen, L. and Penttilä, M. 2004. Xylose chemostat isolates of *Saccharomyces cerevisiae* show altered metabolite and enzyme levels compared with xylose, glucose and ethanol metabolism of the original strain. Appl. Microbiol. Biotechnol, in press.
2. Toikkanen, J.H., Sundqvist, L. and Keranen, S. 2004. *Kluyveromyces lactis* SSO1 and SEB1 genes are functional in *Saccharomyces cerevisiae* and enhance production of secreted proteins when overexpressed. Yeast. 21:1045-55.
3. Toivari, M.H., Salusjärvi, L., Ruohonen, L. and Penttilä, M. 2004. Endogenous xylose pathway in Saccharomyces cerevisiae Appl. Environ Microbiol. 70:3681-3686.
4. Verho, R., Putkonen, M., Londesborough, J., Penttilä, M. & Richard, P. 2004. A novel NADH-linked L-xylulose reductase in the L-arabinose catabolic pathway of yeast J. Biol. Chem. 279:14746-14751.
5. Vidgren, V., Virkajärvi, I., Ruohonen, L., Salusjärvi, L. and Londesborough, J. 2003. The free and carrier-bound yeast populations from a two-stage immobilised yeast reactor are in different physiological conditions. Proc. 29th EBC Congr. Dublin, 17-22 May 2003. Nürnberg: Fachverlag Hans Carl. ISBN 90-70143-22-4 [CD-ROM], 609-617.

The following theses have been successfully presented.

6. Anu Saloheimo 2004. Yeast *Saccharomyces cerevisiae* as a tool in cloning and analysis of fungal genes. Applications for biomass hydrolysis and utilisation. PhD Thesis, Department of Biological and Environmental Sciences, University of Helsinki, Finland.
7. Titta Manninen 2004. Effects of regulatory and permease mutations in the uptake and metabolism of xylose by the xylose-utilising *Saccharomyces cerevisiae* yeast (in Finnish). M.Sc. Thesis, Department of Applied Chemistry and Microbiology, University of Helsinki, Finland.

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

Current publications.

1. Barnett, J.A. 2004. A history of research on yeasts 7: enzymic adaptation and regulation. Yeast 21:703-746.
2. Barnett, J.A. 2004. A history of research on yeasts 8: taxonomy. Yeast 21:1141-1193.

3. Barnett, J.A. 2005. Glucose catabolism in yeast and muscle. Comprehensive Biochemistry 44: in the press.

4. Barnett, J.A. & Entian, K.D. 2005. A history of research on yeasts 9: regulation of sugar metabolism. Yeast - in preparation.

5. Barnett, J.A. & Eddy A.A. 2005. A history of research on yeasts 10: metabolite transport. Yeast - in preparation.

## XIII. Centraalbureau voor Schimmelcultures, Yeast Division, Uppsalalaan 8, P.O.Box 85167 AD Utrecht, The Netherlands. Communicated by M.Th. Smith <smith@cbs.knaw.nl>.

Publications:

2003

1. Bossler, A.D., Richter, S.S., Chavez, A.J., Vogelgesang, S.A., Sutton, D.A., Grooters, A.M., Rinaldi, M.G., Hoog, G.S. de & Pfaller, M.A. 2003. *Exophiala oligosperma* causing olecranon bursitis. J. Clin. Microbiol. 41: 4779-4782.

2. De Leo, F., Urzi, C. & Hoog, G.S. de, 2003. A new meristematic fungus, *Pseudotaeniolina globosa*. Antonie van Leeuwenhoek 83: 351-360.

3. Gunde-Cimerman, N., Zalar, P., Petrovic, U., Turk, M., Kogej, T., Hoog, G.S. de & Plemenitas, A. 2003. Fungi in the salterns. In Ventosa, A. (ed.) Halophilic Microorganisms, 6: 103-113. Springer Verlag.

4. Göttlich, E., Hoog, G.S. de, Genilloud, O., Jones, B.E., & Marinelli, F. 2003. MICROMAT: Culturable fungal diversity in microbial mats of Antarctic lakes. In: Huiskes, A.H.L., Gieskes, W.W.C., Rozema, J., Schorno, R.M.L., Vies, S.M. van der & Wolff, W.J. (eds): Antarctic biology in a global context. Proc. VIII Scar Int. Biol. Symp., Amsterdam. Backhuys, Leiden, pp. 251-254.

5. Hoog, G.S. de, Vicente, V., Caligiorne, R.B., Kantargliocu, S., Tintelnot, K., Gerrits van den Ende, A.H.G. & Haase, G. 2003. Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. J. Clin. Microbiol. 41: 4767-4778.

6. Horré, R., Schröteler, A., Marklein, G., Breuer, G., Siekmeier, R., Sterzik, B., Hoog, G.S. de, Schnitzler, N. & Schaal, K.P. 2003. Vorkommen von *Exophiala dermatitidis* bei Patienten mit zystischer Fibrose in Bonn. Atemw.-Lungenkrkh. 29: 373-379.

7. Kurzai, O., Keith, P., Hopp, H., Hoog, G.S. de, Abele-Horn, M. & Frosch, M. 2003. Post mortem isolation of *Pseudotaeniolina globosa* from a patient with aortic aneurysm. Mycoses 46: 141-144.

8. Matos, T., Haase, G. & Hoog, G.S. de. 2003. Molecular diversity of oligotrophic and neurotropic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. Antonie van Leeuwenhoek 83: 293-303.

9. Naumov, G.I., Naumova, E.S., Smith, M.Th & Hoog, G.S. de. 2003. Ribosomal DNA sequencing and reinstatement of the genus *Arthroascus* von Arx. J. Gen. Appl. Microbiol. 49: 267-270.

10. Onofri, S., Cockell, C., Edwards, H., Friedmann, E.I., Billi, D., Zucconi, L., Selbmann, L., Hoog, G.S. de, Sterflinger, K. & Grady, M. 2003. Lithobionts - Ecological niches for life in lithic habitats: models for searching past life on Mars. Proc. III Eur. Workshop Exo-Astrobiol. Mars: The Search for Life, pp. 253-254

11. Peltroche-Llacsahuanga, H., Schnitzler, N., Jentsch, S., Platz, A., Hoog, G.S. de, Schweizer, K. & Haase, G. 2003. Analysis of phagocytosis, evoked oxidative burst, and killing of black yeasts by human neutrophils: a tool for estimating their pathogenicity? Med. Mycol. 41: 7-14.

12. Vitale, R.G., Hoog, G.S. de, Rijs, T.G. & Verweij, P.E. 2003. In vitro activity of amphotericin B and itraconazole in combination with fluorocytosine, sulphadiazine and quinolones against *Exophiala spinifera*. J. Antimicrob. Chemotherap. 51: 1297-1300.

13. Ball, L.M., Bes, M.A., Theelen, B., Boekhout, T., Egeler, R.M., Kuijper, E.J. 2003. Significance of amplified fragment length polymorphism in the identification and epidemiology of *Candida* species colonization in children undergoing allogeneic stem cell transplantation. J. Clin. Microbiol. 42: 1673-1679.

2004

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    b. 15th Congress of the International Society for Human and Animal Mycology (ISHAM), May 25-29 2003, San Antonio, TX, USA.

    c. 1st Trends in Medical Mycology, joint meeting of the 9th Congress of the European Confederation of Medical Mycology and the 7th Trends in Invasive Fungal Infections, September 28- October 1, Amsterdam, The Netherlands.

    d. 47 th Annual meeting of the Japanese Society for Medical Mycology, Oct. 16-17, Tokyo, Japan. FEMS Yeast Res. 4: 667.

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17. D'Souza, C.A., Hagen, F., Boekhout, T., Cox, G.M. & Heitman, J. 2004. Investigation of the basis of virulence in serotype A strains of *Cryptococcus neoformans* from apparently immunocompetent individuals. Curr. Genet. 47: 92-102.

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23. Kantarcioğlu, A.S. & de Hoog, G.S. 2004. Infections of the central nervous system by melanized fungi: a review of cases presented between 1999 and 2004. Mycoses 47: 5-13.

24. Kerrigan J, Smith MTh, Rogers JD & Poot GA. 2004. *Botryozyma mucatilis* sp. nov., an anamorphic ascomycetous yeast associated with nematodes in poplar slime flux. FEMS Yeast Res.

25. Marinelli, F., Brunati, M., Sponga, F., Ciciliato, I., Losi, D., Van Trappen, S., Mergaert, J., Swings, J., Göttlich, E., Hoog, G.S. de, Rojas, J.L. & Genilloud, O. 2004. Biotechnological exploitation of heterotrophic bacteria and filamentous fungi isolated from benthic mats of Antarctic lakes. In Kurtböke, I. & Swings, J. (Eds): Microbial genetic resources and discovery. WFCC, Egham, pp. 163-184.

26. Nakagawa Y, Robert V, Kawarazaki J, Epping W, Smith MTh, Poot GA, Mizuguchi I, Kanbe T, Doi M 2004. Recurrent emergence of a less common yeast *Candida pararugosa* from a sarcoma patient. Medical Mycology 42:267-271.

27. Sztejnberg, A., Paz, Z., Boekhout, T., Gafni, A. and Gerson, U. 2004. *Meira geulakonigii*, an unique fungus able to reduce both phytophagous mites and fungal plant pathogens. Crop Science 23: 1125-1129.

28. Taj-Aldeen, S.J., Al-Ansari, H.I., Boekhout, T. & Theelen, B. 2004. Co-isolation of *Trichosporon inkin* and *Candida parapsilosis* from a scalp white piedra case. Med. Mycol. 42: 87-92.

In press.

29. Bonifaz, A., McGinnis, M.R., Hoog, G.S. de, Mercadillo, P., Rodríguez-Cortés, O., Araiza, J. & Saúl, A. Cladophialophora carrionii: a new etiologic agent of eumycetoma. Med. Mycol.

30. Caron, S., Avis, T.J., Boekhout, T., Hamelin, R.C. and Bélanger, R.R. 2004. Fingerprinting techniques as tools towards a molecular quality control of *P. flocculosa.* Mycol. Res.

31. Hölker, U., Bend, J., Pracht, R., Müller, T., Tetsch, L. & de Hoog. *Hortaea acidophila*, a new acidophilic black yeast from lignite. Antonie van Leeuwenhoek.

32. Hofmann, H., Choi, S.-M., Wilsmann-Theis, D., Horré, R., Bieber, Th. & de Hoog, G.S. *Phialophora verrucosa* causing invasive chromoblastomycosis and sinusitis in a child from northern Africa.

33. Hoog, G.S. de & Guého, E. Agents of white piedra, black piedra and tinea nigra. In Hay, R. (ed.): Topley & Wilson's Microbiol. Microb. Infect., ed. 10.

34. Hoog, G.S. de, Matos, T., Sudhadham, M., Luijsterburg, K.F. & Haase, G: Intestinal prevalence of the neurotropic black yeast *Exophiala dermatitidis* in healthy and impaired individuals. Mycoses.

35. Hoog, G.S. de & Smith, M.Th.: Ribosomal gene phylogeny and species delimitation in *Geotrichum* and its teleomorphs. Stud. Mycol.

36. Kidd, S., Hagen, F., Tscharke, R., Huynh, M., Bartlett, K., Fyfe, M., MacDougall, L., Boekhout, T., Kwon-Chung, K.J. & Meyer, W. (2004) A rare genotype of *Cryptococcus gattii* caused the *Cryptococcosis* outbreak on Vancouver Island (British Columbia, Canada). Proc. Natl. Acad. Sc. USA.

37. Onofri, S., Selbmann, L., Zucconi, L., Tosi, S. & Hoog, G.S. de: The Mycota of continental Antarctica. Terra Antarct. Repts.

38. Porteous, N.B., Redding S.W., Thompson, E.H., Grooters, A.M., Hoog, G.S. de & Sutton D.A.: Isolation of an unusual fungus in treated dental unit waterlines. J. Amer. Dental Assoc.

39. Porteous, N.B., Grooters, A.M., Redding, S.W., Thompson, E.H., Rinaldi, M.G., Hoog, G.S. de & Sutton, D.A. *Exophiala mesophila* in dental unit waterlines. J. Clin. Microbiol.

40. Selbmann, L., Hoog, G.S. de, Mazzaglia, A., Friedmann, E.I. & Onofri, S. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic deserts. Stud. Mycol.

41. Selbmann, L., Hoog, G.S. de, Mazzaglia, A. & Onofri, S. Caratterizzazione biologica e molecolare di microfunghi isolati da comunità criptoendolitiche in Antartide. Informatore Bot. Ital.

42. Taj-Aldeen, S.J., El Shafie, S., Alsoub, H., Eldeeb, Y & de Hoog, G.S. Isolation of *Exophiala dermatitidis* from endotracheal aspirate of a cancer patient. Mycoses (in press).

## XIV. Research Institute for Viticulture and Enology, Matúškova 25, 831 01 Bratislava, Slovakia Communicated by E. Minárik.

Recent publications

1. Minárik E 2002 Vine yeast ability to adsorb wine thiol volatiles. Vinohrad 40:19 (in Slovak).

The ability to adsorb many unpleasant volatiles in wine by yeasts was investigated. The O.I.V. code in Paris for fining wine by fresh wine lees had permitted this procedure. Mercaptans in wine cause disagreeable odour and taste. By this procedure ethyl- and methylmercaptan may be efficiently eliminated. The method "sur lies" may be thus evidently confirmed.

2. Minárik E 2004 Causes and elimination of sluggish alcoholic fermentation in grape must. Vinohrad 43:6-7 (in Slovak).

In case of problems with prolonged or sluggish alcoholic grape must fermentation aeration of yeasts may be recommended. Addition of assimilable nitrogen , yeast ghosts or a combination of both agents might be useful too. In case that fermentation has stopped refermentation is necessary using alcohol resistant yeast strain combined with aeration.

3. Minárik E 2004. Influence of sulphur dioxide in the transformation of anthocyanins and proanthocyanidins in wine. Vinić a víno 4: suppl. 9.

The presence of $SO_2$ according to its concentration decreases the degradation of proanthocyanidin B1 in model red wine. $SO_2$ has no influence on malvidin-7-glucoside or colour degradation in red wine. Acetaldehyde, on the other hand, may increase the degradation. In the presence of sulphur dioxide proanthocyanidin disappears in the course of time.

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

We are grateful to the Organising Committee of the ICY2004 (Rio de Janeiro) for the invitation to have oral communications and for financial support to participate in the symposium. Many thanks to I. Masneuf, M. Aigle, D. Dubourdieu (Bordeaux) for the opportunity to visit their labs in October 2004.

The following are publications for 2004 or in press.

1. Naumov G.I. 2004. Domestication of dairy yeasts *Kluyveromyces lactis*: transfer of cluster of genes of beta-galactosidase (*LAC*4) and lactose permease (*LAC*12)? Dokl. Biol. Sci. (in press).

2. Naumov G.I. 2004. Why the yeast *Kluyveromyces wickerhamii* assimilates but cannot ferment lactose? Dokl. Biol. Sci. (submitted).

3. Naumova E.S., Sukhotina N.N., Naumov G.I. 2004. Molecular genetic differentiation of the dairy yeast *Kluyveromyces lactis* and its closest wild relatives. FEMS Yeast Research (in press).

4. Naumova E.S., Sukhotina N.N., Naumov G.I. 2004. Molecular markers differentiating the dairy yeast *Kluyveromyces lactis* var. *lactis* and its wild European ancestor. Microbiology (Moscow) (submitted).

We have conducted a comparative molecular-genetic study of 36 *Kluyveromyces* strains of different origin. On the basis of restriction analysis of intergenic spacer 2 (IGS2) rDNA, the dairy yeast *Kl. lactis* var. *lactis* can be distinguished from genetically very closely related wild lactose-negative strains of European population 'krassilnikovii'. Molecular markers for differentiation of physiologically similar yeasts *Kl. lactis* var. *lactis* and *Kluyveromyces marxianus* are described. Origin of clinical *Kl. lactis* isolates is discussed.

5. Naumova E.S., Zholudeva M.V., Martynenko N.N., Naumov G.I. 2004. Molecular genetic differentiation of cultured *Saccharomyces* yeasts. Microbiology (Moscow) (in press).

We conducted a comparative molecular genetic study of cultured *Saccharomyces* yeasts, isolated from different berries and various fermentation processes. In addition to the yeast *S. cerevisiae*, hybrid strains *S. cerevisiae* x *S. bayanus* var. *uvarum* were documented for the first time among baker's yeasts and strains isolated from berries of black currant. Molecular karyotyping revealed a polyploidy of distiller's, baker's and brewing strains. Restriction analysis of non-coding rDNA regions (5.8S-ITS and IGS2) was shown to be useful both for differentiating *Saccharomyces* species and revealing interspecific hybrids. Microsatellite primer (GTG)5 is recommended for studying populations of cultured *S. cerevisiae* yeasts.

6. Naumov G.I., Sharga B.M., Nikolaitchuk V.I. 2004. Advances and prospects in genetical and selection studies of wine yeasts from Transcarpathia. Biotechnologia (Moscow) (in press).

Short review on the studies of Transcarpathian wine yeasts is presented. We describe natural polymorphism of the *Saccharomyces* yeasts on a number of properties, which are of importance for breeding and genetics, viz. fermentation of maltose and galactose, competitive ability determined by plasmids of toxin formation, homo- and heterothallism. Using Transcarpathian strains the cultured biological species *S. cerevisiae* was established. The prospects of further studies of the gene pool of Transcarpathian wine yeasts are discussed.

7. Naumov G.I. Genetic approaches to yeast classification and evolution and evolution: species, varieties, genera and families. In: Yeast in science and technology the quest for sustainable development, 11th International Congress on Yeasts. Rio de Janeiro, Brazil, 15-20th August 2004, P. 66.

A new field in zymology, evolutionary and taxonomic genetics of yeasts is investigated in our laboratory. Genetic fundamentals of species, variety, genus and family classification of yeasts have been elaborated. Genetic genus is characterized by a common mating type system of their member species, probably providing the horizontal transfer of some genes, for example telomeric ones, and cytoplasmic determinants. The genetically defined genus of yeasts reflects an evolutionary relationship between species and is not only theoretical, but also operational concepts. Truly genetic genera are *Saccharomyces* sensu stricto,

*Williopsis* s. str., *Zygofabospora* s. str. *Arthroascus, Metschnikowia* s. str., *Metschnikowia* sensu lato and *Galactomyces* s. str. In addition to the *Saccharomyces* s. str., biological species as genetically isolated populations have been found in several other taxa: *Zygofabospora, Hansenula polymorpha, Williopsis, Arthroascus,* and *Metschnikowia.* Species within each of this complex can be easily crossed but their hybrids remain sterile having non-viable ascospores. Geographical populations are species in statu nascendi. Genetic and allozyme analyses revealed the existence of several semi-isolated geographical populations of wild yeast *S. paradoxus*: European, Far East Asian, North American and Hawaiian. Within *S. bayanus* species there are two varieties: *bayanus* and *uvarum*, having partial genetic isolation. Using genetic analysis and different molecular markers we differentiated eight wild populations as varieties of *Z. lactis*: five North-American (including the known varieties *Z. lactis* var. *drosophilarum* and *Z. lactis* var. *phaseolospora*), one European (*Z. lactis* var. *krassilnikovii*), one South-African (*Z. lactis* var. *vanudenii*), and one Far East Asian. There is a promising test for differentiation of taxa at a higher than genus hierarchical level: pheromone reaction between genetically related genera – members of the same family. Pheromone interaction is documented for species of the *Z. lactis* (*Zygofabospora* s. str.) and *Z. aestuarii* (*Zygofabospoa* s. lato), as well as for *S. cerevisiae* and *S. kluyveri, S. cerevisiae* and several *Saccharomyces* s. lato species. We consider that the family Saccharomycetaceae is heterogeneous.

8. Naumova E.S. Molecular markers in classification and identification of ascomycetous yeasts. In: Yeast in science and technology the quest for sustainable development, 11th International Congress on Yeasts. Rio de Janeiro, Brazil, 15-20th August 2004, P. 76.

Molecular and genetic phylogeny of organisms is a rapidly developing area of biology. Particularly, this approach is widely used in yeast studies. Many yeast genera are heterogeneous, and, within them, there are groups of closely related species corresponding to a genetic genus. The genetic genus form a well-separated cluster in phylogenetic trees based on the comparison of the nucleotide sequences of the ribosomal genes. Its member species have the same system of mating types responsible for their crossing. During the past decade, our laboratory has been developing the molecular and genetic basis of the classification and evolution of different ascomycetous yeasts: *Saccharomyces* sensu stricto, *Artroascus, Zygofabospora/Kluyveromyces lactis* complex, *Williopsis* sensu stricto, *Zygowilliopsis* and *Pichia anomala*. Depending on the yeasts studied, various molecular markers show different capacities to discriminate strains at species or variety level. RAPD and microsatellite fingerprinting were shown to be very useful for preliminary screening of new taxa in ecological and geographical populations of yeasts. Using four primers, M13, (GTG)5, OPA-09 and OPA-11, we found strains with peculiar PCR profiles among *Arthroascus* and *Williopsis* yeasts. Sequencing of rDNA ITS region and D1/D2 domain of the 26S rDNA, DNA-DNA reassociation and genetic hybridization analysis confirmed their belonging to new taxa and determined species or variety status of the strains. PCR restriction fragment length polymorphism of the ribosomal internal transcribed spacers and intergenic sequences (IGS2) are suitable to study molecular and genetic heterogeneity of *Zygofabospora /Kluyveromyces lactis* and *Saccharomyces* sensu stricto yeasts, as well as interspecific hybrid strains of the latter complex. Different molecular approaches not always give consistent results. It is commonly believed that the sequences of the D1/D2 region of the 26S rDNA of strains having the same species belonging are usually identical or differ only in one to three nucleotide positions (Kurtzman and Robnett, 1998). However, the type cultures of *Zygowilliopsis californica* and its synonym *Hansenula dimennae*, having 96% DNA-DNA reassociation, showed six nucleotide substitutions in the D1/D2 domain and significant differences in the ITS region. In contrast, *Saccharomyces* sensu stricto species having very similar rDNA sequences displayed a great divergence of DNA-DNA reassociation values. Possible contradiction between the data obtained by rDNA sequencing and molecular karyotyping is also discussed.

## XVI. CREM – Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Communicated by J. P. Sampaio <jss@fct.unl.pt>.

The following papers have been recently published (abstracts were included in the last issue).

1. Sampaio, J.P. 2004. Diversity, phylogeny and classification of basidiomycetous yeasts. In *Frontiers in Basidiomycote Mycology.* Agerer, R., Blanz, P., and Piepenbring, M. (eds). Eching: IHW – Verlag, pp. 49 - 80.

2. Sampaio, J.P., Golubev, W.I., Fell, J.W., Gadanho, M. and Golubev, N.W. 2004. *Curvibasidium cygneicollum* gen. nov., sp. nov. and *Curvibasidium pallidicorallinum* sp. nov., novel taxa in the Microbotryomycetidae (Urediniomycetes), and their relationship with *Rhodotorula fujisanensis* and *Rhodotorula nothofagi*. International Journal of Systematic and Evolutionary Microbiology **54**: 1401-1407.

3. Golubev, W.I., Sampaio, J.P., Gadanho, M. and Golubev, E.W. 2004. *Cryptococcus paraflavus* sp. nov. (Tremellales), isolated from steppe plants in Russia. Journal of General and Applied Microbiology **50**: 65-69.

The following papers have been accepted for publication.

4. Gadanho, M. and Sampaio, J.P. 2004. Application of temperature gradient gel electrophoresis to the study of yeast diversity in the estuary of the Tagus river, Portugal. FEMS Yeast Research.

Temperature Gradient Gel Electrophoresis (TGGE) was employed for the assessment of yeast diversity in the estuary of the Tagus river (Portugal). The molecular detection of yeasts was carried out directly from water samples and, in parallel, a cultivation approach by means of a enrichment step was employed. A nested PCR was employed to obtain a fungal amplicon containing the D2 domain of the 26S rRNA gene. For identification the TGGE bands were extracted, re-amplified, and sequenced. Fourteen fungal taxa were detected and all except one were yeasts. Most yeast sequences corresponded to members of the Ascomycota and only three belonged to the Basidiomycota. Five yeasts (four ascomycetes and one basidiomycete) could not be identified to the species level due to the uniqueness of their sequences. The number of species detected after enrichment was higher than the number of taxa found using the direct detection method. This suggests that some yeast populations are present in densities that are bellow the detection threshold of the method. With respect to the analysis of the yeast community structure, our results indicate that the dominant populations belong to *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, *Cryptococcus longus* and to an uncultured basidiomycetous yeast phylogenetically close to *Cr. longus*. The combined analysis of direct detection and cultivation approaches indicates a somewhat similar community structure in the two sampled sites since nine species were present at both localities.

2. Libkind, D., Gadanho, M., van Broock, M. and Sampaio, J.P. 2004. *Sporidiobolus longiusculus* sp. nov. and *Sporobolomyces patagonicus* sp. nov., two novel yeasts of the Sporidiobolales isolated from aquatic environments in Patagonia, Argentina. Int J Syst Evol Microbiol.

During a survey of carotenogenic yeasts carried out in northwestern Patagonia (Argentina), several ballistoconidia-producing strains belonging to the order Sporidiobolales were isolated from aquatic environments. Five strains were found to represent two novel species, for which the names *Sporidiobolus longiusculus* (type strain CBS $9654^T$ = PYCC $5818^T$ = CRUB $1044^T$) and *Sporobolomyces patagonicus* (type strain CBS $9657^T$ = PYCC $5817^T$ = CRUB $1038^T$) are proposed. The particular micromorphological feature of *Sporidiobolus longiusculus* are the elongated basidia, which are 5 to 6 times longer that those of the remaining species of the genus *Sporidiobolus*. Based on the sequences of the D1/D2 domains of the 26S rDNA, the most closely related species to *Sporidiobolus longiusculus* is *Sporobolomyces bannaensis*, whereas *Sporobolomyces marcillae* is the closest relative of *Sporobolomyces patagonicus.* Complete internal transcribed spacer (ITS) sequence analysis confirmed the separated position of *Sporidiobolus longiusculus* whereas for *Sporobolomyces patagonicus* no nucleotide differences were found towards *Sporidiobolus pararoseus* CBS $491^T$. Negative mating experiments between strains of *Sporobolomyces patagonicus* and strains of *Sporidiobolus pararoseus* and low DNA-DNA reassociation values for the type strains of the two species validated the proposal of *Sporobolomyces patagonicus* as a distinct species. Information on additional Patagonian *Sporobolomyces* isolates is also included in this report.

3. Kolozsvári-Nagy, J., Süle, S. and Sampaio, J.P. 2004. Apple tissue culture contamination by *Rhodotorula* spp.: identification and prevention. In Vitro Cellular Development Biology – Plant

Tissue cultures of apple cv. JTH-E and Pinova were contaminated with a faint pink pigmented yeast. Yeast isolates were identified as *Rhodotorula slooffiae* with standard physiological and molecular methods. The isolated yeasts were tested against different fungicides. The following fungicides inhibited the growth of the yeast isolates, and were not phytotoxic to apple plantlets: Proclin (3 mg $l^{-1}$), Dithane M45 (10–100 mg $l^{-1}$), Saprol (100 mg $l^{-1}$), Systane 12E (250 mg $l^{-1}$), thiabendazole (40 mg $l^{-1}$), potassium sorbate (100 mg $l^{-1}$), Electis (50 mg $l^{-1}$), Amistar (100 mg $l^{-1}$), and silver nitrate (50–100 mg $l^{-1}$). Some fungicides, inhibited the growth of yeasts but were phytotoxic in the used concentrations: miconazole (20 mg $l^{-1}$), PPM™ (2000 - 5000 mg $l^{-1}$), copper sulfate (200 mg $l^{-1}$), and Cycloheximide (400 mg $l^{-1}$). Fundazol (benomyl) was not phytotoxic but was active only in high doses (750-1000 mg $l^{-1}$). Contaminated shoots were freed from yeasts by the combination of two treatments: the plantlets were soaked in a half strength MS liquid medium with silver nitrate (50 mg $l^{-1}$) and Silvet 77 (0.025%) overnight, and then were inserted in solidified complete MS medium with Dithane M45 (15 mg $l^{-1}$).

## XVII. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze.

Recent publications.

1. E. Böer, T. Wartmann, K. Dlubatz, G. Gellissen, and G. Kunze. 2004. Characterization of the *Arxula adeninivorans AHOGI* gene and the encoded mitogen-activated protein kinase. Curr. Genet (in press).

*Arxula adeninivorans* is an osmo-resistant yeast species that can tolerate high levels of osmolytes like NaCI, PEG400 and ethylene glycol. As in other yeast species this tolerance is elicited by components of the high osmolarity glycerol response (HOG) pathway. In the present study we isolated and characterized as a key component of this pathway the *Arxula adeninivorans-AHOG1* gene encoding the mitogen-activated protein kinase Ahoglp, an enzyme of 45.9 kDa. The gene includes a coding sequence of 1203 bp disrupted by a 57 bp intron. The identity of the gene was confirmed by complementation of a *hog1* mutation in a *S. cerevisiae* mutant strain and the high degree of homology of the derived amino acid sequence with that of mitogen-activated protein kinases from other yeasts and fungi. Under stress-free conditions the inactive Ahog1p is present in low levels. When exposed to osmotic stress, Ahoglp is rendered active by phosphorylation. In addition *AHOGl* expression is increased. Assessment of the *AHOG1*-promoter activity with a *lacZ* reporter gene confirmed its inducibility by osmolytes, a characteristic not observed in homologous *HOG1* genes of other yeast species. This specific property could account for the fast adaptation and high osmoresistance encountered in this species.

2. E. Böer, T. Wartmann, S. Schmidt, R. Bode, G. Gellissen, and G. Kunze. 2004. Characterization of the *AXDH* gene and the encoded xylitol dehydrogenase from the dimorphic yeast *Arxula adeninivorans*. Antonie van Leeuwenhoek (in press).

The xylitol dehydrogenase-encoding *Arxula adeninivorans AXDH* gene was isolated and characterized. The gene includes a coding sequence of 1107 bp encoding a putative 368 amino acid protein of 40.3 kDa. The identity of the gene was confirmed by a high degree of homology of the derived amino acid sequence to that of xylitol dehydrogenases from different sources. The gene activity is regulated by carbon source. In media supplemented with xylitol, D-sorbitol and D-xylose induction of the *AXDH* gene and intracellular accumulation of the encoded xylitol dehydrogenase is observed. This activation pattern was confirmed by analysis of *AXDH* promoter - *GFP* gene fusions. The enzyme characteristics are analysed from isolates of native strains as well as from those of recombinant strains expressing the *AXDH* gene under control of the strong *A. adeninivorans*-derived *TEF1* promoter. For both proteins a molecular mass of ca. 80 kDa was determined corresponding to a dimeric structure, a pH optimum at pH 7.5 and a temperature optimum at 35°C. The enzyme oxidizes polyols like xystol and D-sorbitol whereas the reduction reaction is preferred when providing D-xylulose, D-ribulose and L-sorbose as substrates. Enzyme activity exclusively depends on $NAD^{-}$ or NADH as coenzymes.

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

The following lecture was presented recently.

1. Lachance MA 2004 Recent developments in the systematics and evolution of yeasts in the large-spored *Metschnikowia* clade. 11th International Congress on Yeasts, Rio de Janeiro.

The following papers have now appeared in print.

2. Herzberg, M. and M.A. Lachance. 2004. *Candida bombiphila* sp. nov., a new asexual yeast species in the *Wickerhamiella* clade. Int. J. Syst. Evol. Microbiol. 54:1857-1859.
3. Carreiro, S.C., F.C. Pagnocca, M. Bacci Jr, M.A. Lachance, O.C. Bueno, M.J.A. Hebling, C.C.C. Ruivo, and C.A. Rosa. 2004. *Sympodiomyces attinorum* sp. nov., a novel yeast species associated with nests of the leaf-cutting ant *Atta sexdens*. Int. J. Syst. Evol. Microbiol. 54:1891-1894.
4. Morais, P.B., L.C.R.S. Teixeira, J.M. Bowles, M.A. Lachance, and C.A. Rosa. *Ogataea falcaomoraisii* sp. nov., a sporogenous methylotrophic yeast from tree exudates. FEMS Yeast Res. 5:81-85.
5. Ruivo, C.C.C., M.A. Lachance, M. Bacci Jr, S.C. Carreiro, C.A. Rosa & F.C. Pagnocca. *Candida leandrae* sp. nov., an asexual ascomycetous yeast species isolated from tropical plants. Int. J. Syst. Evol. Microbiol. 54: 2405-2408.

Papers in press.

6. Pimenta, R.S. P.D.D. Alves, A. Corrêa Jr., M.A. Lachance, G.S. Prasad, R. Rajaram, B.R.R.P. Sinha, and C.A. Rosa. *Geotrichum silvicola*, a novel asexual arthroconidial yeast species related to genus *Galactomyces*. Int. J. Syst. Evol. Microbiol.
7. Anderson, T.M., M.A. Lachance, and W.T. Starmer. The relationship of phylogeny to community structure: the cactus-yeast community. Amer. Naturalist.
8. Lachance MA & Bowles JM *Metschnikowia similis* sp. nov. and *Metschnikowia colocasiae* sp. nov., two ascomycetous yeasts isolated from *Conotelus* spp. (Coleoptera: Nitidulidae) in Costa Rica. Studies in Mycology 50.

# International Commission on Yeasts

## Meeting of Commissioners, 17 August 2004
## Eleventh International Congress on Yeasts Rio de Janeiro, Brazil

Minutes of Meeting

**Present:** Lex Scheffers (Chair); Graham Fleet (Vice-Chair), Peter Biely, Monique Bolotin-Fukuhara, Tibor Déak, Hans van Dijken, James du Preez, Lucia Figueroa, Barbel Hahn-Hagerdal, Mike Ingledew, Mogens Jakobsen, Byron Johnson, Eric Johnson, Lodi Kock, Matti Korhola, Clete Kurtzman, André Lachance, Patricia Lappe Oliveras, Cecilia Leão, Anna Maraz, Sandro Martini, Leda Mendonça Hagler, Sally Meyer, Gernadi Naumov, Isak Pretorius, Hans Prillinger, Bernard Prior, Amparo Querol, Peter Raspor, Doris Rauhut, Patrizia Romano, Anna Clara Schenberg, Andrei Sibirny, Isabel Spencer-Martins, John Thevelein, Ricardo Vazquez.

**Apologies:** A. Panek, J. Peinado, M. Penttila, R. Sentandreu, A. Toh-E.

**Report of Chair**

Lex Scheffers welcomed the 36 delegates to the meeting and gave apologies for those who could not

attend. He commented on the large number of delegates at the meeting and expressed appreciation for their support. He presented the agenda, requested any additional items and asked Graham Fleet to record the minutes.

**Minutes of the Previous Meeting**

Lex Scheffers referred the delegates to the Minutes of the previous meeting of Commissioners which was held in Budapest on 27 August 2003. The Minutes were published in the December 2003 issue of the *Yeast Newsletter*.

**ICY, Rio de Janeiro (2004)**

Lex Scheffers expressed his personal thanks to Leda Christina Mendonça for her excellent commitment to hosting the eleventh ICY in Brazil. There were some 200 delegates at the Congress, representing 34 countries. The Commissioners proposed a toast to Leda, thanking her and her organising committee for their great work and support.

**ISSY24, Spain (2005)**

Lex reported correspondences with Rafael Sentandreu who is organising the symposium. In Professor Sentandreu's absence, Amparo Querol commented on the arrangements for this symposium. **Symposium theme**: Yeast cell surface; plasma membrane; cell wall; dimorphism. **Location and date**: Oropesa del Mar (near Valencia), Spain, 28 September – 2 October 2005.

**IUMS 11th International Congress of Mycology, San Francisco 24-29 July (2005)**

As Vice Chair of the Mycology Division of IUMS and International Chair of the organising committee for the Mycology Congress, Graham Fleet commented on arrangements to date. The congress is being hosted by the American Society for Microbiology who have appointed a national (US) organising committee chaired by John Taylor (U C Berkeley). This committee has put its own stamp on the structure of the scientific program but Graham Fleet has worked to see that there is good balance between topics on yeasts and filamentous fungi and there is good international representation of speakers. The program is organised under topics of ecology, systematics, evolution, development and molecular biology, medical and industrial. While there is no ICY sponsored/organised session, there is excellent representation of yeasts throughout the entire program. Members of ICY will find much interest, and are encouraged to attend. The program can be found at *www.iums2005.org*.

Graham is encouraging ASM to publicise the Congress more broadly.

**ISSY25 Finland (2006)**

Merja Penttila is organising this symposium, but in her absence Matti Korhola noted that the theme of the symposia will be "**Metabolic Engineering and Systems Biology**" and that it will be held at Espoo/Ontaniems in June 2006.

**ISSY26 Italy (2007)**

Patrizia Romano is organising this symposium. As it is some years away, the theme and location are not yet finalised but tentatively, the location is Ravello, some time in June 2007.

**ICY12 (2008)**

During their last meeting (Budapest 2003), Commissioners were hoping to organise the next ICY12 in the Asian/S E Asian region in order to encourage stronger participation of scientists from that region in ICY activities. Unfortunately, attempts to host ICY12 in Japan or Thailand were not successful for a range of logistical reasons. Andrei Sibirny proposed that ICY12 be hosted by Ukraine at Kiev, and offered to organise this congress. The Commissioners considered his suggestions and proposal and unanimously agreed that ICY12 (2008) would be hosted by Ukraine through Andrei Sibirny. The Commissioners warmly thanked Andrei for his dedication and effort and noted the success of ISSY21, which he had organised in Lviv, 2001.

**ISSY27 (2009)**

There were suggestions from Monique Bolotin-Fukuhara and Peter Raspor for organising ISSY27 in either France or Thailand. The Commissioners welcomed submissions from delegates of these two countries in due course.

**Yeast Newsletter**

André Lachance commented that interest in the **Yeast Newsletter** remained strong and that it served a most useful function in communication between yeast researchers. He proposed to communicate it in electronic format in the future. This new direction was supported by a good majority of the Commissioners. However, hard copies will still be distributed to those who prefer that format. André acknowledged the support of the co-editors of the Newsletter. On behalf of ICY, Lex Scheffers thanked André for his excellent contributions in managing the **Yeast Newsletter**.

**New Chair and Vice Chair, ICY**

Lex Scheffers announced that his tenure as Chair of ICY had come to an end and that Graham Fleet's tenure as Vice Chair had also expired. Lex would become the next Vice Chair in accordance with ICY statutes. The Commissioners expressed their appreciation of the contributions of Lex and Graham by applause. The Commissioners unanimously elected Leda Christina Mendonça-Hagler as the next Chair (2004-2008) of ICY, with much acclaim.

**List of Commissioners**

Lex commented that commissioners of ICY should be active in ICY affairs and that dormant commissioners should be replaced by active members. While there had been success in addressing this issue over recent years there was still some dormancy and that

this affected the participation of some countries in ICY activities. It was proposed that dormant commissioners should be removed from ICY. After discussion, it was unanimously agreed that, if commissioners had not attended any of the previous four ICY/ISSY symposia, they would be automatically excluded from the Commission, and new delegates would be recruited to represent their country.

The following changes to Commissioners were received.

**Retirements:** Byron Johnson (Canada), Sally Meyer (USA), Sandro Martini (Italy). The meeting expressed a deep sense of appreciation to these long-serving and committed Commissioners, noting that Sandro and Sally were former Chairs of ICY.

**New Commissioners:** Doris Rauhut (Germany), Monique Bolotin-Fukuhara (France), Anne Vaughn-Martini (Italy), Kyria Boundy-Mills (USA), Mogens Jakobsen (Denmark), Sakkie Pretorius (Australia).

These new Commissioners were warmly welcomed by the meeting.

**Other Business**

Peter Raspor expressed some concern about the relationship of ICY as a COMCOF within IUMS, and Graham Fleet indicated that he would raise the issue of COMCOFs and relationship with IUMS at the next IUMS Executive meeting. Peter also expressed the need for an ICY website to promote the visibility and activities of ICY. André Lachance suggested he may be able to assist on this initiative.

**Meeting close**

Lex Scheffers closed the meeting expressing, on behalf of ICY, appreciation to Leda Mendonça-Hagler, for arranging the excellent food and wine for the meeting.

Graham H Fleet

## Archive of ICY Symposium Books

This summer in Rio de Janeiro, Johan Thevelein proposed to establish a collection, including all the ICY Symposium Books, right from the beginning in 1964. This appealing and important idea would require a suitable depository. I am grateful that meanwhile an opportunity has been offered by the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. In order to save as many of these books from oblivion, I herewith invite all who are prepared to place one or more of such Abstract Books and Proceedings at disposal. Those who are able and prepared to contribute, are invited to just send me an e-mail, indicating the details (year, venue, shape) of the book(s) in question. In order to avoid unnecessary duplication, it is advised not yet to forward the book(s) but to wait until I will have an overview of the supply. Eventually, I then will send a request for the particular offers.

I hope that this initiative will meet with approval and that many will contribute one or more of their ISSY and ISY books for this historical collection. For your convenience, below follows the complete list of ICY events (published earlier in Yeast Newsletter by André Lachance). I look forward to your cooperation.

Lex Scheffers <lex.scheffers@tnw.tudelft.nl>

**International Symposium on Yeasts:**

1 Smolenice (Czechoslovakia) 1964
2 Bratislava (Czechoslovakia) 1966
3 The Hague/Delft (The Netherlands) 1969
4 Vienna (Austria) 1974
5 London,ON (Canada) 1980
6 Montpellier (France) 1984
7 Perugia (Italy) 1988
8 Atlanta, GA (USA) 1992
9 Sydney (Australia) 1996
10 Papendal (The Netherlands) 2000.
11 Rio de Janeiro (Brazil) 2004

**International Specialized Symposium on Yeasts:**

1 Smolenice (Czechoslovakia) 1971
2 Kyoto(Japan)1972
3 Otaniemi (Finland) 1973
4 Berlin West (Germany) 1976
5 Keszthely (Hungary)1977
6 Montpellier (France)1978
7 Valencia (Spain) 1981
8 Bombay (India) 1983
9 Smolenice (Czechoslovakia) 1983
10 Plovdiv (Bulgaria) 1985
11 Lisbon (Portugal) 1986
12 Weimar (German DR) 1987
13 Leuven (Belgium) 1989
14 Smolenice (Czechoslovakia) 1990
15 Riga (Latvia) 1991
16 Papendal (The Netherlands) 1993
17 Edinburgh (UK) 1995
18 Bled (Slovenia) 1997
19 Braga (Portugal) 1998
20 Smolenice (Slovakia) 1999
21 Lviv (Ukraine) 2000
22 Pilanesberg (South Africa) 2002
23 Budapest (Hungary) 2003

# Recent Meeting

## 32nd Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts Smolenice, Slovakia, May 12.-14. 2004

The 32nd Annual Conference on Yeasts organized by the Czech and Slovak Commission for Yeasts and the Institute of Chemistry, Slovak Academy of Sciences, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, during May 12-134 by three guests from other countries. The oral program consisted of 21 plenary lectures in three sessions: biochemistry and cell biology, biotechnology, and molecular biology and genetics. The lectures were complemented by 53 posters. A real refreshment of the program was the sensorial evaluation of Slovak spirits which was moderated by I. Vajcziková. The titles of all contributions are listed below.

**Lectures in the session Biochemistry and cell biology**

1. Opekarová M., Malinská K., Malinský J., Tanner W.: Lipid rafts in *Saccharomyces cerevisiae.*
2. Hronská L., Schneiter R., Hapala I.: Sterol trafficking - yeast *Saccharomyces cerevisiae* as a model for studying mechanisms of sterol trafficking.
3. Marešová L., Sychrová H.: Localization and function of the putative $K^+/H^+$ antiporter *KHA1*.
4. Novotná D., Flegelová H., Janderová B.: Interaction of K1 and K2 killer toxins with the cell surface of *S. cerevisiae* deletants with different resistance to toxins.
5. Šesták S., Tanner W., Strahl S.: Scw4p and Scw10p are new cell wall glucanases important for cell wall stability in *Saccharomyces cerevisiae*.

**Lectures in the session Biotechnology**

6. Čertík, M., Breierová, E., Márová, I.: Response of pigment producing yeasts to exogenous stress: An introduction.
7. Breierová, E., Čertík, M., Márová, I., Gregor, T., Omelková J.: Response of yeasts to exogenous stress: Morphology, exopolymer formation and heavy metal accumulation.
8. Čertík, M., Breierová, E., Márová, I. : Response of yeasts to exogenous stress: Membrane lipids.
9. Márová, I., Breierová, E., Čertík, M., Kočí, R., Drábková, M., Pokorná, J.: Response of yeasts to exogenous stress: Production of carotenoid pigments.
10. Rapta, P., Čertík, M., Breierová, E: Scavenging and antioxidant properties of pigments generating by yeasts grown under exogenous stress.
11. Přibylová, L., de Montigny, J., Potier, S., Sychrová, H. : Physiological properties of the osmotolerant yeast *Zygosaccharomyces rouxii.*
12. Biely, P., Kremnický, Ľ., Vršanská, M. : Utilization of plant polysaccharides by *Aureobasidium pullulans*: Enzymology and regulation.
13. Fiala, J., Novák, J., Basařová, G.: Flow cytometric analysis of yeasts.

**Lectures in thesession Molecular biology and genetics**

14. Tomáška Ľ., Valent I., and Nosek : Rhythm'n'yeasts: A short guide through yeast oscillatory behavior.
15. Gregan J., Rabitsch K. P., Schleiffer A., Javerzat J.P., Eisenhaber F. , Nasmyth K.: Two fission yeast homologs of *Drosophila* Mei-S332 are required for chromosome segregation during meiosis I and II.
16. Hikkel I., Lucau-Danila A., Deveaux F., Delaveau T., Marc P., Bouchoux C., Potier M.-C., Jacq C. : Using artificial transcription factors to identify the genome- wide properties of yeast zinc finger ($Zn_2Cys_6$) transcription factors.
17. Špryngar M., Janatová I., Hašek J. : EIF3A/RPG1P affects reassembly of microtubules in *Saccharomyces cerevisiae*.
18. Kozovská Z., Lucau-Danila A., Jacq C. : Global kinetic response of *Saccharomyces cerevisiae* strains to the antimitotic drug benomyl.
19. Poliaková D., Šabová Ľ.: Search for primary targets of ROS induced by expression of pro-apoptotic Bax protein in *Kluyveromyces lactis*.
20. Mentel M., Piškur J., Kolarov J. : Mitochondrial ADP/ATP carrier in yeast: Identification and characterization of three isogenes encoding ADP/ATP carrier in the dimorphic yeast *Yarrowia lipolytica.*

**List of Posters**

21. Klobučníková V., Strhanová K., Hapala I.: Isolation and characterization of yeast mutants selectively resistant to nystatin and amphothercin B.
22. Flegelová H., Sychrová H.: Yeast as a model system for characterization of mammalian $Na^+/H^+$ antiporters.
23. David M., Gabriel M., Kopecká M. : Microtubules and actin structures in the basidiomycetous yeast, *Cryptococcus laurentii.*
24. Gášková D., Maláč J., Urbánková E.: Monitoring the performance of yeast membrane ABC transporters by fluorescence assay: Effect of cultivation conditions.
25. Havelková M., Unger E.: The role of calmodulin in karyokinesi and in cytokinesis.
26. Vališ K., Mašek T., Novotná D., Pospíšek M., Janderová B.: Suicidal phenotype: Does exist in K1killer toxin producing cells?
27. Kinclová-Zimmermannová O., Sychrová H.: *Saccharomyces cerevisiae* $Na^+/H^+$-antiporter Nha1p participates in the regulation of intracellular alkali- metal-cation homeostasis and in cell response to sudden changes of external osmolarity.
28. Dudíková J., Kolarova N.: Galactosyltransferase activity in the acapsular mutant *Cryptococcus laurentii.*
29. Koláčná L., Sychrová H.: Yeast as a model for mammalian potassium channel expression.
30. Mrózová Z., Czabany Z., Čertík M., Hapala I.: Utilisation of external fatty acids for storage lipid synthesis in the yeast *Saccharomyces cerevisiae.*
31. Pevala V., Kolarov J.: Mitochondrial oxidative phosphorylation is essential for Bcl-$x_L$ protection against cell death.
32. Kovářová J., Šťavíková J., Kofroňová O., Pichová A. : Monitoring of yeast aging.

33. Sekavová B., Melzoch K., Maternová J.: Fluorescent probes as a tool for characterisation of yeast physiological state.
34. Sigler K., Hendrych T., Chládková K., Gášková D.: Biocides and *S. cerevisiae* cells: from cell depolarization to impairment of membrane integrity and cell killing.
35. Machová E., Paulovičová E., Bystrický S., Masárová J., Mislovičová D.: Preparation and immunogenicity of *Saccharomyces cerevisiae* mannan conjugate.
36. Čertík M., Breierová E., Bronišová Ž, Oláhová M., Hanusová V.: Changes in membrane lipids of yeasts stressed by heavy metals and hydrogen peroxide.
37. Kolouchová I., Melzoch K., Macková M.: Impact of resveratrol on *Saccharomyces cerevisiae.*
38. Masrnová S., Čertík M., Spurná O., Sitkey V., Minárik M.: Biotechnological production of asthaxanthin by *Phaffia rhodozyma*.
39. Omelková J., Breierová E., Stratilová E.: The influence of the method of storage on survival of chosen yeast strains.
40. Palian Z., Breierová E., Šandula J.: Hyaluronidase activity in different yeast species.
41. Pavlíček J., Ohkusu M., Takeo K., Raclavský V.: Image analysis and flow cytometry in cell cycle analysis of *Cryptococcus neoformans*.
42. Drábková M., Pokorná J., Kočí R., Márová I.: Changes of carotenoid production in red yeast cells stressed by salt, hydrogen peroxide and heavy metals.
43. Kočí R., Drábková M., Pokorná J., Márová I.: Influence of UV-irradiation and ethanol on the production of carotenoids by industrial red yeasts.
44. Raclavský V., Trtková J., Pavlíček J., Ohkusu M., Takeo K.: Hypoxia response in the basidiomycetous yeast *Cryptococcus neoformans*.
45. Plachý R., Hamal P., Raclavský V.: New approach to Candida yeast identification based on melting curve analysis of RAPD-products (McRAPD).
46. Šajbidor J., Čertík M., Breierová E, Garajová S.:The influence of ethanol on growth and lipid composition of *Saccharomyces cerevisiae.*
47. Sláviková E., Košíková B., Sasinková V.: The use of various yeast strains for pretreatment of pine wood.
48. Mikeš J., Siglová M., Masák J., Čejková A., Jirků V.: Determination of the heavy metals toxicity in yeasts by flow cytometry.
49. Mikeš J., Dostálek P., Matějka P.: Chemical composition of the yeast cell wall as an important factor in the biosorption of heavy metals.
50. Rapta P., Čertík M., Breierová E., Polovka M., Zalibera M.: Physico-chemical studies of protective compounds formed by yeasts grown under environmental stress.
51. Stratilová E., Čigašová H., Breierová E., Omelková J. : Polygalacturonases of *Aureobasidium pullulans* and *Aspergillus niger* and their possible influence on the pathogenic or saprophytic character of microorganisms.
52. Tomšíková A.: New information of the genus *Cryptococcus*.
53. Vadkertiová R., Sláviková E.: Biodiversity of yeasts in terrestrial ecosystems.
54. Breierová E., Gregor T., Bronišová Ž.: Role of exopolymers in protective properties of yeasts stressed by heavy metals.
55. Dorosh A., Hašek J., Janatová I.: Isolation and characterization of various homologous and heterologous promoters in *Schwanniomyces (Debaryomyces) occidentalis.*
56. Fekete V., Lencz P., Hapala I., Sulo P.: Strain collection with the mutations affecting mitochondria in W303 genetic background.
57. Poláková S., Slamka T., Sulo P.: Simple PCR taxonomic criterion a tool for the construction of hybrids and new species from natural and industrial *Saccharomyces sensu stricto* strains.
58. Frýdlová I., Trachtulcová P., Janatová I., Hašek J.: The pheromone signaling pathway is responsible for the mating-type specific invasive growth of the *Saccharomyces cerevisiae isw2* deletants.
59. Imrichová D., Takáčová M., Gbelská Y., Šubík J.: Molecular characterization of the *Kluyveromyces lactis* gene conferring resistence to 4-nitroquiline-N-oxide and azole antifungals.
60. Holič R., Griač P.: Role of phosphatidylcholine (PC) turnover products in transcriptional regulation of phospholipid biosynthetic genes.
61. Sidorová M., Hikkel I., Šubík J.: Screening system to identify the loss of function mutations in transcription factor Pdr3p in the yeast *Saccharomyces cerevisiae.*
62. Strádalová V., Janatová I, Hašek J.: Searching for a nuclear transport in of the chromatin-remodeling factor Isw1p in *Saccharomyces cerevisiae.*
63. Černická J., Hnátová M., Šubíková V., Šubík J.: Strobilurin resistance in baker´s yeast and pathogenic *Candida albicans.*
64. Polákova S., Slamka T., Minárik G., Sulo P.: Variability in *Saccharomyces cerevisiae* natural isolates - the first step towards speciation or the consequence of interspecies mating?
65. Obernauerová M., Tichá E., Tyčiaková S., Šubík J.: Regulation of phosphatidylglycerphosphate synthase expression in *Kluyveromyces lactis*
66. Tyčiaková S., Obernauerová M., Sulo P., Šubík J.: Essentiality of the PEL1/PGS1 gene in *Kluyveromyces lactis*.
67. Vašicová P., Dorosh A., Hašek J., Janatová I.: Expression of human thyroid peroxidase and CD5 antigens in *Pichia pastoris.*
68. Velková K., Přibylová L., Sychrová H.: $Na^+/H^+$ antiporters in different yeast species – structure and function comparison.
69. Vlčková V., Dúhová V., Vráblová I., Závodná K., Moráňová Z., Kogan G., Miadoková E.: Potential antimutagenic effect of sulfoethylglucan on model test systems.
70. Vlčková V., Hermanská K., Marková E., Chovanec M., Brozmanová J.: Response of the Saccharomyces cerevisiae *rad52* and *yku70* mutants to oxidative stress.
71. Yu V. P.C.C., Reed S. I.: Role of CKS1 in transcription regulation.
72. Novák J., Basařová G., Fiala J.:Analysis of brewery yeast changes during main fermentation by optical methods usage.
73. Poljšak B., Pesti M., Gazadag Z., Plesničar S., Belágyi J., Dergecz T., Farkas N., Rapor P.: The importnace of Trolox in Cr(VI) mediated intracellular damage prevention.

Abstracts of all contributions published in English (ISSN 1336-4839) are available on the web-site www.chem.sk/yeast. On the meeting of the Czech and Slovak Commission for Yeasts it was agreed that the 33rd Annual Conference on Yeasts will be held in Smolenice during May11.-13. 2005. The main lines of the program will be biochemistry, cell biology and genetics, biotechnology and yeasts in human and animal medicine. Abstracts are required to be submitted in English, however the conference language will be *ad libitum* for more senior scientists from Czech Republic and Slovakia. Presentations in English are recommended for young scientists and students. Consequently, English as the conference language is changing the traditional conference of the two nations speaking similar languages to a more open meeting to foreign scientists, who are cordially invited to attend the annual conferences in future.

Peter Biely, former Chair of the Czech and Slovak Commission for Yeasts

# Forthcoming Meeting

## ISSY 2006
## Systems Biology and Metabolic Engineering of Yeasts
## June 18-22 2006, Hanasaari, Finland

The ISSY 2006 meeting on Systems biology and metabolic engineering of yeasts will be held June 18-22, 2006, at Hanasaari, an island on the edge of Helsinki.

For further information, contact

Dr. M. Penttilä <merja.penttila@vtt.fi>

# Brief News Items

## Change of Address: Dr. Heide-Marie Daniel

I have recently accepted a position at the Mycothèque de l'Université Catholique de Louvain, where I shall be pursuing studies in the taxonomy and diversity of *Candida* and other yeasts. My address will be the following.

Heide-Marie Daniel
BCCM/MUCL, Mycothèque de l'Université Catholique de Louvain
Croix du Sud 3
B-1348 Louvain-la-Neuve
Belgium <daniel@mbla.ucl.ac.be>

## Change of address - Ing. Vaclav Svejcar

I have recently retired, but shall continue to be active in yeast research. Also, the following diploma thesis was defended recently.

Pavelkova, I. 2004 Influence of yeast on the quality of wines. Mendel University of Agriculture and Forestry Brno, Faculty of Horticulture in Lednice na Morave. The thesis deals with the most frequent yeasts of South Moravia and their influence on the quality of wines. Extra care is given to a application of pure cultures of the yeast, especially in their dry form. The information about application of *Schizosaccharomyces* spp. will also be of interest (in Czech).

Ing. Vaclav Svejcar, CSc
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696 11 Mutenice
Czech Republic <Svejcar.Vaclav@seznam.cz>

## Alternative supplier for Vitamin-free Yeast Base and related media

In a recent issue of the Yeast Newsletter, I reported the existence of a British supplier for Vitamin-free Yeast Base (FORMEDIUM). Fernando Pagnocca of São Paulo State University, Rio Claro, Brazil, brought to my attention the existence of an American supplier with distributors in many countries world-wide. The company, called USBIOLOGICAL of Swampscott, Massachusetts, provides a number of synthetic and complex yeast media. I have recently tried the Vitamin-free formulation, side by side with the DIFCO product, in the characterization of nearly 200 isolates from Hawaiian floricolous beetles. The results were essentially identical with the two media. Dr. Pagnocca also reports good success with the new product. For more information on USBIOLOGICAL, please consult their website.

http://www.usbio.net/.

M.A. Lachance

The above information is provided for the benefit of our readers and does not constitute an official endorsement by the Yeast Newsletter or the International Commission on Yeasts.

# Yeast on the Web

(yeest) - Yeast is alive! It is a microscopic, single-cell organism that, as it grows and ferments, produces alcohol and carbon dioxide. The carbon dioxide bubbles get trapped in the gluten strands of bread, causing it to rise. **The most commonly available form is active dry yeast**; the tiny organisms are dehydrated, and therefore dormant due to the lack of moisture. Yeast should be "proofed" (or "activated") in water heated to approximately 110 degrees F.
www.geocities.com/NapaValley/4079/Glossary/U-Y.htm

A microscopic, single-cell organism that converts its food into alcohol and carbon dioxide through a process known as fermentation.
www.sunset.com/sunset/Reference/FoodRef/FoodGlossary.html

Yeast is a fungus, **a member of the plant family**. Yeast exists on plants, in the air, in soil, and in and on humans and animals. Yeast metabolize simple sugars and produce alcohol and carbon dioxide through the process of fermentation. Different strains of yeast are used for different processes, such as brewing and dough-rising.
www.angelfire.com/ab/bethsbread/sdDefinitions.html

Kind of fungi or microbe. Yeast are used in bread-, wine- and beer-making to produce fermentation products.
ucbiotech.org/glossary/

The magical ingredient of beer. A microscopic fungi that is able to convert sugar into alcohol and carbon dioxide in a process know as fermentation.
www.leebrewery.com/glossary.htm

The enzyme-producing one-celled fungi of the genus *Saccharomyces* that is added to wort before the fermenting process for the purpose of turning fermentable sugar into alcohol and carbon dioxide.
www.vendomecopper.com/obgloss.htm

Living substance responsible for the production of the enzymes that permit fermentation, the conversion of sugar into alcohol, with heat and carbon dioxide as by products. **Yeast occurs naturally on grape skins**, but many winemakers today use specially selected cultured yeasts to allow better control of the fermentation. Yeasty - Tasting term used to describe the distinctive smell of yeast (as in unbaked bread dough). In wine this usually indicates a fault, though the smell of freshly-baked bread can be desirable in Champagne and lees aged whites.
www.marquis-wines.com/101/soundsavvy.htm

The one-celled micro-organism that turns sugar into alcohol and carbon dioxide
www.geocities.com/mipeman/glossary.html

Any of various single-cell fungi capable of fermenting carbohydrates.
www.ott.doe.gov/biofuels/student_glossary.html

Unicellular micro-organism (fungus) **naturally present on the skin of grapes**. It provokes alcoholic fermentation and is indispensable in the elaboration of wine.
www.terroir-france.com/wine/glossary.htm

Micro-organisms that produce the enzymes which convert sugar to alcohol. Necessary for the fermentation of grape juice into wine.
128.200.136.180/ea/wine/tastings/glossary.html

Micro-organism responsible for the conversion of sugar to alcohol. They are **endemic in many vineyards**, but if not they may be cultured in the laboratory and introduced to commence the fermentation.
www.thewinedoctor.com/wineglossary.htm

Converts the sugar into alcohol and carbon dioxide. Two main types of yeast are used, suited to different conditions and producing different styles of beer *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*.
www.badgerbrewery.com/talk/glossary.htm

A single-celled fungus capable of fermentation.
www.alabev.com/glossary.htm

A single-celled organism that breaks its food down into alcohol and carbon dioxide in a process known as "fermentation." Brewers capitalize on the alcohol. Carbon dioxide gives beer and champagne effervescence and causes bread to rise.
www.nutribase.com/cookingi.shtml

A unicellular fungus that belongs to the phylum Ascomycetes, has a single nucleus ,and reproduces either asexually by budding or fission, or sexually through spore formation.
www.hardydiagnostics.com/Glossary-Y.html

Yeast are fungus cells that eat and drink the sugars, proteins and water found in wort. The waste of yeast help to produce the various smells and flavours found in beer, as well as creating carbon dioxide and ethanol production.
www.briansbelly.com/glossary2.shtml

A group of unicellular cellular fungi of the class Hemiascomycetae and phylum Ascomycota. They occur as single cells or as groups or chains of cells. They reproduce both sexually and asexually. Yeasts of the genus *Saccharomyces* ferment sugars and are hence used in baking and brewing. Mans ability to and long history of culturing yeasts has made them useful tools in genetic engineering.
www.genewatch.org/GeneSrch/Glossary.htm

A fungi that produces enzymes converting sugar to alcohol and carbon dioxide. Used in skin conditioners. No known toxicity.
www.makingcosmetics.com/makingcosmetics/glossary/25_glossary_y.html

A living organism used in the production of bread and beer. Yeast, in the environment of sugar, produces carbon dioxide and alcohol. This process is called fermentation. Bread yeast comes in dry granulated and fresh cakes. A new form of yeast, called instant yeast, has been developed which allows the user to mix the yeast directly into the flour without dissolving it first in water.
www.recipegoldmine.com/glossary/glossaryY.html

Any of various unicellular fungi reproducing by budding and from ascospores and capable of fermenting carbohydrates.
home.earthlink.net/~tmlphx/Dictionary/terms_and_definitions.htm

Microscopic fungi. Some species catalyze useful fermentation in bread and winemaking.
www.stavin.com/glossary.htm

Used to convert natural sugars into equal parts of alcohol and carbon dioxide in wine and beermaking.
www.arborwine.com/def.html

Yeast is a living microorganism that causes fermentation when it comes into contact with liquid sugar (sugar and water), and heat. Yeast dies when there is too much alcohol in its environment (&plusmn16% alc./vol.)
www.happyhour.ca/dictionary.html

**A group of enzymes** which promote fermentation of grape juice. **A natural bloom or fungus found on grapes** whose metabolism of grape sugars causes sugars to break down into alcohol and Carbon dioxide. **The 'dust' on a grape, known as the 'bloom' is wild yeast**. Most wine makers prefer to use their own yeast strains.
www.lynnwoodinn.com/content/wineterm.htm

A commercial leavening agent containing yeast cells; used to raise the dough in making bread and for fermenting beer or whiskey
www.cogsci.princeton.edu/cgi-bin/webwn

any of various single-celled fungi that reproduce asexually by budding or division
www.cogsci.princeton.edu/cgi-bin/webwn

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