

Yeast

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Editorial

ISSY-23 Budapest, August 26-29, 2003

Dr. Tibor Deak informs us that preparations are well underway for the 23rd International Specialized Symposium on Yeasts, to be held in Budapest in August 2003. The preliminary program includes sessions on ecology, taxonomy and phylogeny, biodiversity, food & yeasts, wine yeasts, clinical yeasts, and yeast methodology. A wide array of researchers from across the international community have agreed to present lectures or coordinate sessions on the general topic of interactions between yeasts and other organisms. A second circular is in preparation. Look for an update in the December issue of the Yeast Newsletter.

M.A. Lachance
Editor

Letters to the editor

Herman Jan Phaff remembered in Russia

Russian zymologists of Moscow State University express their great regret on learning of the death of Herman Jan Phaff - our great yeast leader. For many years he was the most respected teacher in yeast science. His "Life of Yeasts", translated into Russian by V.I. Golubev and I.P. Babjeva, is considered to be the most interesting book about these tiny friends of mankind till now. Many years ago he organized the "Yeast

Newsletter", an essential publication for zymologists of all over the world. H.J. Phaff was a world leader in all aspects of yeast life. His activity in yeast science continued during many years after his retirement. Russian zymologists consider H.J. Phaff to be the greatest teacher in yeast science and will always remember him with gratitude and love.

I.P. Babjeva, I.Yu. Chernov, I.A. Maximova

Request for information on selenium in fungi

For the last couple of years I have temporarily neglected the yeasts. Considerable interest currently attends the working hypothesis that selenium dietary supplements may lower the incidence of prostatic cancer. A large trial involving 32,400 men to explore the potential benefits of selenium, vitamin E, or both, is underway. While much information has accrued on selenium concentrations in serum with regard to nutrition and cancer there are no published data on selenium status in the target organ. I recently analyzed samples from prostatectomy specimens and found a range of 1.24 - 1.42 microgram Se per

gram dry weight. This is the first measurement of selenium in the human prostate and the values refer to healthy tissue within a cancerous prostate. They indicate that the gland is reasonably well endowed with selenium and indeed comparable in concentration to the human kidney. The assembly and operation of atomic fluorescence equipment has been expensive and time consuming. However, I would like to do some measurements (en passant) on yeast and yeastlike organisms, industrial and pathogenic. Have any of the readers of the yeast newsletter had experience with selenium in the fungal world?

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The following papers appeared recently. Reprints are available.

1. J. Middelhoven. 2001. Identification of pathogenic *Trichosporon* species. Abstracts (Resumos) XXI Congresso Brasileiro de Microbiologia, p. 308, Foz do Iguaçu, Brazil, October 2001.
2. W.J. Middelhoven. 2002. History, habitat, variability, nomenclature and phylogenetic position of *Hansenula polymorpha*. In: G. Gellissen (Ed.) *Hansenula polymorpha*, Biology and Applications, Wiley-VCH, Weinheim, Germany, April 2002, p 1-7.

A review is given of the history of this thermotolerant, methanol-assimilating yeast, currently known as *Pichia angusta*, a name that is used by yeast taxonomists, but did not get

popularity among other investigators of this intriguing yeast species.

3. W.J. Middelhoven and C.P. Kurtzman. 2002. Relation between phylogeny and physiology in the *Stephanoascus/Blastobotrys* clade of ascomycetous yeasts. Abstracts 22nd International Specialized Symposium on Yeasts, Pilanesberg National Park, South Africa, March 2002, p. 28.

II. Russian Collection of Microorganisms, Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino 142292, Russia. Communicated by W.I. Golubev <WIG@ibpm.serpukhov.su>.

Recent publications.

1. Golubev, W.I., Ikeda, R. and Shinoda, T., 2000. Anti-*Cryptococcus neoformans* activity of saprotrophic tremelloid yeasts. *Problems in Med. Mycol.* **2(3)**:39-43.

Cryptococcus neoformans (66) strains were examined for sensitivity to 12 killer toxins secreted by 9 saprotrophic species belonging to genera *Bullera*, *Cryptococcus*, *Cystofilobasidium* and *Filobasidium*; 90-100% of them were sensitive to toxins of *Cr. humicola*, *Cys. bisporidii* and

F. capsuligenum. All strains of *Cr. neoformans* var. *gattii* were also sensitive to mycocins of *B. alba*, *B. hanna*e, *B. sinensis*, *Cr. podzolicus* and *Cr. laurentii* type strain. It seems likely that sensitivity of this variety to many killer toxins is the cause of its restricted geographic distribution.

2. Golubev, W.I. and Churina, L.G., 2001. Taxonomic specificity of sensitivity to the mycocin of *Tilletiopsis flava* VKM Y-2823. *Mikrobiologiya* **70(1)**:51-54.

The mycocinogenic strain of *Tilletiopsis flava* that has fungicidal activity (at pH 3.5-4.5) was characterized. It retained this activity after treatment with agents that eliminate extrachromosomal genetic elements. Its killer toxin with molecular mass above 10kDa is thermolabile and sensitive to proteolysis. This mycocin is most active against species of the

genus *Tilletiopsis*. Another representatives (with rare exceptions) of the Tilletiales and all other basidiomycetous (including ballistosporeous organisms) yeasts belonging to the Sporidiales, Tremellales and ascomycetous yeasts are resistant to *T. flava* mycocin.

3. Puchkov, O.E., Zahringer, U., Lindner, B., Kulakovskaya, T.V., Seydel, U. and Wiese, A., 2001. Mycocidal, membrane-active complex of *Cryptococcus humicola*, is a new type of cellobiose lipid with detergent features. *Biochim. Biophys. Acta (Biomembranes)* **1558(2)**:161-170.

The chemical composition of the mycocidal complex (formerly known as microcin) secreted by *Cr. humicola* was investigated by chemical, mass spectrometric and nuclear magnetic resonance methods. The results indicate that the mycocidal complex is composed of glycolipids with a highly acetylated (up to five acetyl groups) cellobiose backbone linked to the hydroxyl group of dihydroxy palmitate with an unsubstituted carboxyl group. The acyl chain forming aglycone can be replaced by hydroxy C18:0 fatty acids. The complex has a comparatively high surface activity; 0.5 mg/ml of it reduced the surface tension of 0.1 M sodium bicarbonate from 71 mN/m to 37 mN/m and interfacial tension against n-hexadecane from 39 mN/m to 10 mN/m. The critical micelle concentration of the complex at pH 4.0, determined by the fluorometric method with

N-phenyl-1-naphthylamine as fluorescent probe and by the De Nouy ring method, was 2×10^{-6} M (taking the average molecular mass of the complex to be 750); it did not depend on the presence of 100 mM KCl and was an order of magnitude higher at pH 7.0. By fluorescence resonance energy transfer spectroscopy with N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine as energy donor and N-(rhodamine B sulfonfyl)-phosphatidylethanolamine as energy acceptor the complex was shown to intercalate into the liposomal lipid matrix. Primary lesions caused by the complex in planar lipid bilayers were revealed as short-living current fluctuations of a broad spectrum of amplitudes. The mycocidal effect of the complex is suggested to be associated with its detergent-like properties.

III. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

We are grateful to the Organising Committee of the ISSY2002 (Pilanesberg National Park, South Africa) for the invitation to have an oral communication and for financial support to participate in the symposium. Two three-years (2001-2004)

Ph.D. projects have been started in our laboratory. They are: I.V. Korshunova - "Genetics of melebiose fermentation in *Saccharomyces* yeasts." and E.S. Kazaryan - "Evolutionary genetics of the *Saccharomyces sensu stricto* species."

The following are publications for 2001-2002 or in press.

1. Naumova E.S., Smith M.Th., Boekhout T., Hoog G.S., Naumov G.I. 2001. Molecular differentiation of sibling species in the *Galactomyces geotrichum* complex. *Antonie van Leeuwenhoek* **80**:263-273.
2. Naumov G.I., Naumova E.S., Korshunova I.V. 2001. Genetic identification of cultured *Saccharomyces* yeasts from Asia. *J. Gen. Appl. Microbiol.* **47**(6):335-338.
3. Naumov G.I., Naumova E.S. 2002. Five new combinations in the yeast genus *Zygofabospora* Kudriavzev emend. G. Naumov (pro parte *Kluyveromyces*) based on genetic data. *FEMS Yeast Research* (in press).

The rDNA sequencing data obtained during last five years in several labs clearly demonstrate that within the heterogeneous genus *Kluyveromyces* there is a group of highly related species, which we refer to as the genus *Zygofabospora* Kudriavzev 1960 emend. G. Naumov 2002. This genus includes four hybridizing species *Zf. marxiana*, *Zf. dozhzhanskii*, *Zf. lactis*, *Zf. wickerhamii* (*Zygofabospora* sensu stricto) and two taxonomically related species *Zf. aestuarii*, *Zf. nonfermentans* (Nagahama et al.) G. Naumov comb. nov. (*Zygofabospora* sensu lato). We studied the relationships of the yeasts composing the *Zf. lactis* complex. Genetic hybridization analysis and molecular karyotyping revealed partial genetic isolation varieties *Zf. lactis* var. *drosophilorum* (Shehata et al.) G. Naumov comb. nov. and

Zf. lactis var. *phaseolospora* (Shehata et al.) G. Naumov comb. nov. from North America, and *Zf. lactis* var. *krassilnikovii* (Kudriavzev) G. Naumov comb. nov. from Europe. Dairy yeast *Zf. lactis* var. *lactis* G. Naumov comb. nov. yields highly fertile hybrids with its wild ancestor *Zf. lactis* var. *krassilnikovii* and semi-sterile hybrids with North American taxa. Besides, *Zf. lactis* var. *lactis* and *Zf. lactis* var. *krassilnikovii* formed fertile hybrids with South African yeast *Zf. lactis* var. *vanudenii* (van der Walt et Nel) G. Naumov comb. nov. The reinstatement of the latter yeast at the variety level has been done taking into account the results of the present study and the literature data on its geographic isolation, high divergence of the karyotype and mitochondrial DNA.

4. Naumova E.S., Bulat S.A., Mironenko N.V., Naumov G.I. 2002. Differentiation of six sibling species in the *Saccharomyces* sensu stricto complex by multilocus enzyme electrophoresis and UP-PCR analysis. *Antonie van Leeuwenhoek* (in press).

UP-PCR analysis and multilocus enzyme electrophoresis were used to characterize 37 strains of the sibling species *Saccharomyces cerevisiae*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii*, *S. mikatae* and *S. paradoxus*. The results demonstrate that both molecular approaches are useful for

discriminating these phenotypically indistinguishable *Saccharomyces* species. The data obtained are in excellent agreement with previously reported genetic analyses, sequencing of the 18S rRNA and ITS regions, and DNA-DNA reassociation data.

5. Naumov G.I., Naumova E.S., Korshunova I.V., Jakobsen M. 2002. Comparative genetics of yeasts Communication XXIV. A new alpha-galactosidase gene *MEL15* in *Saccharomyces cerevisiae*. *Russian Journal of Genetics* (in press).

In addition to the earlier identified European family of highly homologous α -galactosidase genes *MEL1-MEL11* and African family of divergent genes *MEL12-MEL14*, a new gene *MEL15* of *Saccharomyces cerevisiae* strains isolated from fermented maize dough in Ghana has been found. Southern hybridization and restriction analyses showed that the *MEL15*

gene belongs to the African family of *MEL* genes. The new gene is located in doublet containing chromosomes XII/IV. Tetrad analysis indicated that the *MEL15* gene locates neither on left arm of chromosome IV or on right arm of chromosome XII where the genes *MEL5* or *MEL10* have been mapped earlier.

6. Naumova E.S., Korshunova I.V., Jespersen L., Naumov G.I. 2002. Molecular genetic identification of *Saccharomyces* strains isolated from African sorghum beer. 22nd Int. Spec. Symp. on Yeasts (ISSY 2002), 25-28 March 2002, Pilanesberg, South Africa, p. 26.

Naumov G.I., Naumova E.S., Naumoff D.G., Korshunova I.V., Jakobsen M. 2002. Divergent alpha-galactosidase genes *MEL12-MEL15* in African indigenous strains of *Saccharomyces cerevisiae*. 22nd Int. Spec. Symp. on Yeasts (ISSY 2002), 25-28 March 2002, Pilanesberg, South Africa, p. 135.

**IV. 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. & Robinow, C.F. 2002. A history of research on yeasts 4: cytology part I, 1890-1950. *Yeast* **19**:151-182.
2. Barnett, J.A. & Robinow, C.F. 2002. A history of research on yeasts 4: cytology part II, 1950-1990. *Yeast*, in the press.
3. Barnett, J.A. & Zimmermann, F.K. 2003. A history of research on yeasts 5. the fermentation pathway. *Yeast*, in preparation.

V. Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602, U.S.A. Communicated by A. Bakalinsky <Alan.Bakalinsky@orst.edu>.

Recent publication.

1. Zara, S., Farris, G.A., Budroni, M., and Bakalinsky, A.T. 2002. *HSP12* is essential for biofilm formation by a Sardinian sherry strain of *S. cerevisiae*. *Yeast* **19**:269-276.

Sardinian sherry strains of *S. cerevisiae* form a biofilm on the surface of wine at the end of the ethanolic fermentation, when grape sugar is depleted and when further growth becomes dependent on access to oxygen. A point mutation in *HSP12* or deletion of the entire gene resulted in inability to form this film. *HSP12* encodes a heat-shock protein previously found by others

to be active during stationary phase, in cells depleted for glucose, and in cells metabolizing ethanol and fatty acids, all conditions associated with sherry biofilms. The DNA sequence of the *HSP12* allele of strain Ar5-H12 has GenBank accession no. AY046957.

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

The following papers were recently published.

1. E. Minárik. 2002. Optimizing ageing conditions of wines on lees. *Vinič a víno* **2**:8 (in Slovak).

Wine ageing on lees is practised in the technology of white grape wines in some countries. This type of ageing has been also used in red wine production. The aim of winemaking technology is to obtain mannoproteins, amino acids and peptides improving thus sensory and aroma properties as well as stability

in wine. The enzyme Rapidase filtration intensifies ageing otherwise requiring usually 8-12 month. By Rapidase filtration after alcoholic fermentation the ageing process may be cut down to 1-2 months.
2. K. Furdíková, Z. Dömény, L. Krištofiková, E. Minárik. 2002. Yeasts of the Slovak Tokay wine-growing region: 2nd part. Technological qualities of the isolates. *Vinič a víno* **2**: Supplement 2-4 (in Slovak).

Technological properties of isolated strains were tested for the Tokay Aszu wine production. For the isolation of such strains samples of the Slovsk Tokay wine-growing region were used. Based on the results and comparison with the commercial

strain UVAFERM PM and spontaneous fermentation, the strain T5p showed most suitable properties for the fermentation of Tokay Aszu wines.
3. E. Minárik. 2002. Biochemical reasons of sluggish or stopped must fermentation. *Vinič a víno* **2**: Supplement 10-11 (in Slovak).

Winemakers are interested to be acquainted with factors responsible for sluggish or prematurely stopped alcoholic fermentation of grape must as it is connected with economic problems. Many factors are involved (insufficient vitamin B1, oxygen insufficiency, high alcohol content, toxic fatty acids/octoic and decanoic acids/ and high SO₂ level. Inhibition of

key enzymes, low pH, acetic acid and change of plasmatic yeast membrane are usually case of fermentation disturbances Low yeast biomass formation of viable cells may also influence fermentation rapidity. Interactions of those factors may also cause fermentation difficulties.

4. E. Minárik. 2002. Acetifying and ethylacetate formation in wine. *Vinohrad* **40**:(1) 18-19.

Basic requirements for wines displaying low volatile acids produced by wine yeasts were summarized: a) rapid grape processing, b) quick pressing/de-stemming, c) immediate inoculation after SO₂ addition, d) regulating malolactic

fermentation by *Oenococcus oeni*, e) maintaining the level of free sulphur dioxide (40-50 mg/L). The main yeast species causing volatile acid and ethylacetate formation in wine are elucidated.

5. E. Minárik. 2002. Ability of wine lees to absorb volatile thiols in wine. *Vinohrad* **40**(2):1 (in Slovak).

The ability of wine lees to adsorb some constituents causing unpleasant odour or flavour formation in wine cannot always be eliminated by routine fining. The International Code of the O.I.V. (Paris) recommends wine ageing on fresh wine lees

by which methyl- and ethylthiols may be successfully eliminated. This procedure is regarded as another beneficial procedure by the method "sur lies".

6. E. Minárik. 2002. Possibilities to increase aroma profiles of wine by non-*Saccharomyces* yeasts. *Vinohrad* **40**(2):22-23 (in Slovak).

In spite of classic conceptions selected apiculate yeasts might increase the aroma profile of wines according to Italian researchers. Wine microbiologists should direct their attention

to these and other non-*Saccharomyces* species and confirm or controvert possibilities to use such yeasts in enology.

VIII. Instituto de Investigaciones Biomédicas del CSIC, Arturo Duperier 4. E-28029 Madrid. Spain. Communicated by C. Gancedo <cgancedo@iib.uam.es>.

The following paper is in press.

1. O. Zaragoza, C. de Virgilio, J. Ponton and C. Gancedo. Disruption in *Candida albicans* of the *TPS2* gene encoding trehalose-6-phosphate phosphatase affects cell integrity and decreases infectivity. *Microbiology*.

The gene *CaTPS2* encoding trehalose-6-P phosphatase from *Candida albicans* has been cloned and disrupted in this organism. The *Catps2/Catps2* mutant did not accumulate trehalose and accumulated high levels of trehalose-6-P. Disruption of the two copies of the *CaTPS2* gene did not abolish growth even at 42°C but decreased the growth rate. In the stationary phase, the *Catps2/Catps2* mutant aggregated, more than 50% of its cells became permeable to propidium iodide and a high amount of proteins was found in the culture medium. Aggregation occurred only at pH values higher than 7 and was

avoided by osmoprotectants; it was never observed during the exponential phase of growth. The mutant formed colonies with a smooth border on Spider medium. Mice inoculated with 1.5 x 10⁶ c.f.u. of wild type cells died after 8 days while more than 80% of those inoculated with the same number of c.f.u. of the *Catps2/Catps2* mutant survived for at least one month. Reintroduction of the wild type *CaTPS2* gene in the *Catps2/Catps2* mutant abolished the phenotypes described. We hypothesize that the accumulation of trehalose-6-P interferes with the assembly of a normal cell wall.

XIX. Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Canada S7N 5A8. Communicated by W. M. (Mike) Ingledew <ingledew@sask.usask.ca>.

Dr. K. C. Thomas, an integral part of the research activity in this lab, has now retired.

The following papers have been published since our last report.

1. D. Bayrock, and W.M. Ingledew. 2001. Changes in steady state on introduction of a *Lactobacillus* contaminant to a continuous ethanol fermentation. *J. Indust. Microbiol. Biotechnol.* **27**:39-45.

Lactobacillus paracasei was introduced as a contaminant into a multistage continuous culture ethanol fermentation system at ratios of 1:100, 1:1, and 70:1 with *Saccharomyces cerevisiae*, but failed to overtake the yeast. None of the inoculation ratios allowed *L. paracasei* to affect *S. cerevisiae* in the first fermentor in the multistage system. *S. cerevisiae* remained constant at 23 x 10⁷ CFU/ml regardless of the bacterial inoculation level, and even at the 70:1 inoculation ratio, glucose, ethanol, and lactic acid concentrations did not

change from the steady-state concentrations seen before bacterial inoculation. However, *L. paracasei* decreased steadily from its initial inoculation level of 22.2 x 10⁹ CFU/ml and stabilized at 3.7 x 10⁵ CFU/ml after 10 days of steady-state operation. Both organisms then persisted in the multistage system at an approximate *L. paracasei*/*S. cerevisiae* ratio of 1:100 which confirms that, in continuous fuel ethanol production, it would be difficult to eliminate this bacterium. Only when the pH was controlled at 6.0 in fermentor 1 (F1) were changes seen which

would affect the multistage system. Ethanol concentration then decreased by 44% after 4 days of pH - controlled operation. This coincided with an increase in *L. paracasei* to $>10^{10}$ CFU/ml, and a 4 fold increase in lactic acid concentration to 20 g/l. When the clarified contents from other fermentors (F2–F5) in the

multistage system were used as growth media, *L. paracasei* was not able to grow in batch culture. This indicated that the first fermentor in the multistage system was the only fermentor capable of supporting the growth of *L. paracasei* under the described conditions.

2. N.V. Narendranath, K.C. Thomas, and W.M. Ingledew. 2001. Acetic acid and lactic acid inhibit growth of *Saccharomyces cerevisiae* by different mechanisms. *J. Amer. Soc. Brew. Chemists* **59**:187-194.

Two strains of *Saccharomyces cerevisiae* were grown in minimal medium with glucose as the carbon source and with added acetic acid or lactic acid. The intracellular pH (pHi) of yeast cells was not significantly affected by acetic acid up to a concentration of 0.25% w/v. The pHi was maintained at a more or less constant value by pumping out excess protons through the increased activity of plasma membrane H⁺-ATPase. With lactic acid at a concentration of 0.4% w/v or higher, the pHi dropped enough to affect yeast growth. This drop in pHi was the result of

reduced activity of membrane bound H⁺-ATPase. Cell membrane lipids of yeast cells grown in the presence of 0.5% w/v lactic acid contained considerably reduced levels of the unsaturated fatty acids, palmitoleic and oleic acids. Changes in the fatty acid composition of cells grown with acetic acid were relatively small. These differences in internal pH, plasma membrane H⁺-ATPase activities and membrane fatty acid composition all indicate that acetic acid and lactic acid inhibit the growth of *S. cerevisiae* by different mechanisms.

3. D.P. Bayrock, and W.M. Ingledew. 2001. Application of multistage continuous fermentation for production of fuel alcohol by very high gravity (VHG) fermentation technology. *J. Indust. Microbiol. Biotechnol.* **27**:87-93.

A fermentation system to test the merging of very-high-gravity (VHG) and multistage continuous culture fermentation (MCCF) technologies was constructed and evaluated for fuel ethanol production. Simulated mashes ranging from 15% to 32% w/v glucose were fermented by *Saccharomyces cerevisiae* and the dilution rates were adjusted for each glucose concentration to provide an effluent containing less than 0.3% w/v glucose (greater than 99% consumption of glucose). The MCCF can be operated with glucose concentrations up to 32% w/v, which indicates that the system can successfully operate under VHG conditions. With 32% w/v glucose in the medium reservoir, a maximum of 16.73% v/v ethanol was produced in the MCCF.

The introduction of VHG fermentation into continuous culture technology allows an improvement in ethanol productivity while producing ethanol continuously. In comparing the viability of yeast by methylene blue and plate count procedures, the results in this work indicate that the methylene blue procedure may overestimate the proportion of dead cells in the population. Ethanol productivity (Yps) increased from the first to the last fermentor in the sequence at all glucose concentrations used. This indicated that ethanol is more effectively produced in later fermentors in the MCCF, and that the notion of a constant Yps is not a valid assumption for use in mathematical modeling of MCCFs.

4. Y-H. Lin, D. Bayrock, and W. M. Ingledew. 2001. Metabolic flux variation of *Saccharomyces cerevisiae* cultivated in a multistage continuous stirred tank reactor fermentation environment. *Biotechnol. Progr.* **17**:1055-1060.

The technique of metabolic flux analysis was implemented to elucidate the flux balancing of *Saccharomyces cerevisiae* cultivated in a multistage continuous stirred tank reactor fermentation environment. The results showed that the majority of the substrate ($97.70 \pm 0.49\%$) was funneled into the glycolytic pathway, while the remainder was subdivided between the pentose phosphate pathway and pathways for polysaccharide synthesis. At the pyruvate node, $87.30 \pm 1.38\%$ of the flux was channeled through the reaction governed by pyruvate decarboxylase. Fluxes through the pyruvate dehydrogenase bypass were maintained at a constant level ($82.65 \pm 1.47\%$)

irrespective of the configuration of the fermentation setup. Activity through the TCA "cycle" was replenished by the reaction catalyzed by pyruvate carboxylase and by the transport of cytosolic oxaloacetate across the mitochondrial membrane. The CO₂ evolution rate varied as fermentation progressed; however, the yield coefficient of CO₂ remained at a constant value. Although a constant yield of ethanol (0.42 g of ethanol/g of glucose) was obtained, operations of the TCA cycle were gradually switched from partially reductive to partially oxidative pathways from the first fermentor to the fourth fermentor.

5. Y-H. Lin, D. Bayrock and W.M. Ingledew. 2002. Evaluation of *Saccharomyces cerevisiae* grown in a multistage chemostat environment under increasing levels of glucose. *Biotechnol. Lett.* **24**:449-453.

A method monitoring net consumption and/or production rates of fermentation metabolites has been proposed and applied to evaluate the performance of *Saccharomyces cerevisiae* grown in a multistage chemostat environment under

increasing glucose concentration (increased gravity). Results show that both rates are effected by high glucose concentrations (osmotic pressure) and that ethanol inhibition has a profound effect on the performance of yeast cells.

6. K.C. Thomas, S. H. Hynes and W.M. Ingledew. 2002. Influence of medium buffering capacity on growth inhibition of *Saccharomyces cerevisiae* by acetic and lactic acids. *Appl. Environ. Microbiol.* **64**:1616-1623.

Acetic acid (167 mM) and lactic acid (548 mM) completely inhibited growth of *Saccharomyces cerevisiae* both in minimal medium and in media which contained supplements, such as yeast extract, corn steep powder, or a mixture of amino acids. However, the yeast grew when the pH of the medium containing acetic acid or lactic acid was adjusted to 4.5, even though the medium still contained the undissociated form of either acid at a concentration of 102 mM. The results indicated that the buffer pair formed when the pH was adjusted to 4.5 stabilized the pH of the medium by sequestering protons and by lessening the negative impact of the pH drop on yeast growth, and it also decreased the difference between the extracellular and intracellular pH values (Δ pH), the driving force for the

intracellular accumulation of acid. Increasing the undissociated acetic acid concentration at pH 4.5 to 163 mM by raising the concentration of the total acid to 267 mM did not increase inhibition. It is suggested that this may be the direct result of decreased acidification of the cytosol because of the intracellular buffering by the buffer pair formed from the acid already accumulated. At a concentration of 102 mM undissociated acetic acid, the yeast grew to higher cell density at pH 3.0 than at pH 4.5, suggesting that it is the total concentration of acetic acid (104 mM at pH 3.0 and 167 mM at pH 4.5) that determines the extent of growth inhibition, not the concentration of undissociated acid alone.

**X. Department of Chemical Engineering, University of Melbourne, Victoria, Australia 3052.
Communicated by N. B. Pamment <nbp@unimelb.edu.au>.**

Recent publications on yeast.

1. A.R. Barber, M. Henningson & N.B. Pamment. In press. Acceleration of high gravity yeast fermentations by acetaldehyde addition. *Biotechnol. Lett.*

In high gravity *Saccharomyces cerevisiae* fermentations containing 300 g glucose/l, daily addition of acetaldehyde to a total of 93 mM shortened the time required to ferment the first 250 g glucose/l from 790 h to 585 h. Acetaldehyde feeding had no effect on the ethanol yield but increased by 135%, 78% and

77% the final concentrations of 2,3-butanediol, 2-methylpropanol and acetate, while decreasing that of glycerol by 14%. Controlled acetaldehyde feeding has potential as a technique for accelerating high gravity fuel or industrial ethanol fermentations and may be useful in preventing incomplete fermentations.

2. H. Golias, G.J. Dumsday, G.A. Stanley and N.B. Pamment. In press. Evaluation of a recombinant *Klebsiella oxytoca* strain for ethanol production from cellulose by simultaneous saccharification and fermentation: comparison with native cellobiose-utilising yeast strains and performance in co-culture with thermotolerant yeast and *Zymomonas mobilis*. *J. Biotechnol.*

In the simultaneous saccharification and fermentation to ethanol of 100 g l⁻¹ microcrystalline cellulose, the cellobiose-fermenting recombinant *Klebsiella oxytoca* P2 outperformed a range of cellobiose-fermenting yeasts used in earlier work, despite producing less ethanol than reported earlier for this organism under similar conditions. The time taken by *K. oxytoca* P2 to produce up to about 33 g l⁻¹ ethanol was much less than for any other organism investigated, including ethanol-tolerant strains of *Saccharomyces pastorianus*, *Kluyveromyces marxianus* and *Zymomonas mobilis*. Ultimately, it produced slightly less ethanol (maximum 36 g l⁻¹) than these organisms, reflecting its lower ethanol tolerance. Significant advantages were obtained by

co-culturing *K. oxytoca* P2 with *S. pastorianus*, *K. marxianus* or *Z. mobilis*, either isothermally, or in conjunction with temperature-profiling to raise the cellulase activity. Co-cultures produced significantly more ethanol, more rapidly, than either of the constituent strains in pure culture at the same inoculum density. *K. oxytoca* P2 dominated the early stages of the co-cultures, with ethanol production in the later stages due principally to the more ethanol tolerant strain. The usefulness of *K. oxytoca* P2 in cellulose simultaneous saccharification and fermentation should be improved by mutation of the strain to increase its ethanol tolerance.

3. A.R. Barber, F. Vriesekoop and N.B. Pamment. 2002. Effects of acetaldehyde on *Saccharomyces cerevisiae* exposed to a range of chemical and environmental stresses. *Enzym. Microbial Technol.* **30**:240-250.

The ability of small quantities of added acetaldehyde to stimulate growth in environmentally-stressed cultures of *Saccharomyces cerevisiae* was examined over a broad range of stress conditions. Acetaldehyde addition substantially reduced the lag phase of cultures suddenly inoculated into medium containing inhibitory quantities of low M.W. alcohols and higher

fatty acids. For ethanol-stressed cultures, acetaldehyde was effective whether added initially or during fermentation. The effects of acetaldehyde were not universal, only minor stimulation being observed for cultures exposed to heat shock, or experiencing changes in cultivation temperature or pH, despite the occurrence of long lag phases under these conditions.

Acetaldehyde strongly inhibited the growth of osmotically-shocked cultures, in contrast to the effects of small quantities of ethanol. The beneficial effects of acetaldehyde appear to be largely confined to cultures exposed to chemical stress, especially

by agents which disturb membrane structure or function. Acetaldehyde addition has potential practical application in overcoming inhibition in such fermentations.

XI. Center of Biological Research, Division of Experimental Biology, P.O. Box 128, La Paz 23000 B.C.S., México. Communicated by R. Vasquez Juárez <rvazquez@cibnor.mx>.

Current research.

1. D. Tovar, J. Zambonino, C. Cahu, F.J. Gatesoupe, R. Vázquez-Juárez, and R. Lésel. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass larvae.

Yeasts produce polyamines, and some strains have a strong adhesion potential to intestinal mucus, an important condition for probiotic efficiency. The aim of this study was to explore an *in-situ* production of polyamine by *Debaryomyces hansenii* HF1 (DH), a yeast strain isolated from fish gut, in comparison with *Saccharomyces cerevisiae* X2180 (SC) (Goteborg University Collection). The production of polyamines by DH was 3-times higher than that of SC. The main polyamines were spermine and spermidine, produced at a similar level. Both strains adhered to the gut of seabass larvae. When the yeasts were introduced into a compound diet, the colonization was

effective in the larvae (10^4 CFU g^{-1} on a body weight basis). The diet DH led to an increase in amylase secretion in 27-day-old larvae, in comparison with the control diet. The secretion of amylase and trypsin was lower with the SC diet, and some delay in trypsin secretion was still observed in this group at day 42. At day 27, the activity of brush border membrane enzymes was stimulated by DH diet, and delayed by SC diet, in comparison with the control diet. The survival of the larvae was also increased with diet DH, but the growth rate was lower than in the control group. That may be due to the introduction of live yeast into the diet, which need to be optimised.

XII. Department of Microbiology, Institute of Applied Molecular Biology, University of the Saarland, Im Stadtwald, Building 2, D-66041 Saarbrücken, Germany. Communicated by M.J. Schmitt <mjs@microbiol.uni-sb.de>.

The following are summaries of recent published papers of the group.

1. Breinig, F., Tipper, D.J. & M.J. Schmitt. 2002. Kre1p, the plasma membrane receptor for the yeast K1 viral toxin. *Cell* **108**:395-405.

Saccharomyces cerevisiae K1 killer strains are infected by the M1 double-stranded RNA virus encoding a secreted protein toxin that kills sensitive cells by disrupting cytoplasmic membrane function. Toxin binding to spheroplasts is mediated by Kre1p, a cell wall protein initially attached to the plasma membrane by its C-terminal GPI anchor. Kre1p binds toxin directly. Both cells and spheroplasts of $\Delta kre1$ mutants are completely toxin resistant; binding to cell walls and spheroplasts

is reduced to 10 and <0.5%, respectively. Expression of K28-Kre1p, an inactive C-terminal fragment of Kre1p retaining its toxin affinity and membrane anchor, fully restored toxin binding and sensitivity to spheroplasts while intact cells remained resistant. Kre1p is apparently the toxin membrane receptor required for subsequent lethal ion channel formation.

2. Breinig, F. & M.J. Schmitt. 2002. Spacer-elongated cell wall fusions improve cell surface expression in the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **58**:637-644.

Fusion proteins for cell surface expression in the yeast *Saccharomyces cerevisiae* were constructed that consisted of the N-terminal leader sequence of Kre1p, followed by the nine amino acid viral epitope hemagglutinin (HA), and the carboxyterminal anchoring domain of either Cwp2p or Flo1p. All fusions were constitutively expressed under transcriptional control of the phosphoglycerate kinase promoter and immunofluorescence analysis indicated that in each construct the HA peptide was correctly anchored to the outer yeast cell surface. Successful solubilisation of the cell wall fusions by laminarinase treatment indicated that the fusions are *in vivo* covalently linked to cell wall β -1,3-D-glucans. FACS analyses further demonstrated that 70%

of the yeast cell population expressed the corresponding cell wall fusion. Neither the number of positive cells within the population nor the distribution of the fusion at the single cell level were negatively affected by replacing the "heterologous" Kre1p leader by the "native" Cwp2p leader. Insertion of a 350 amino acid Ser/Thr-rich spacer sequence into the fusions led to a dramatic increase in HA peptide accessibility on the yeast cell surface. Our data show that FACS analyses represent a valuable means for investigating cell surface expression, and indicate that artificially spacer-elongated cell wall fusions might rise novel possibilities for cell surface expression of heterologous proteins in yeast.

3. Riffer, F., Einfeld, K., Breinig, F. & M.J. Schmitt. 2002. Mutational analysis of K28 pre-protoxin processing in the yeast *Saccharomyces cerevisiae*. *Microbiology* **148**:1317-1328.

K28 killer strains of *Saccharomyces cerevisiae* are permanently infected with a cytoplasmic persisting double-stranded (ds)RNA virus encoding a secreted α/β heterodimeric protein toxin that kills sensitive cells by cell cycle arrest and inhibition of DNA synthesis. *In vivo* processing of the 345 amino acid toxin precursor (preprotoxin; pptox) requires multiple internal and carboxyterminal cleavage events by the prohormone convertases Kex2p and Kex1p. By site-directed mutagenesis of the pptox gene and phenotypic analysis of its *in vivo* effects we now demonstrate that secretion of a biological active virus toxin requires signal peptidase cleavage after Gly³⁶ and Kex2p-mediated processing at the α N-terminus (after GluArg⁴⁹), the α C-terminus (after SerArg¹⁴⁹) and at the β N-terminus (after

LysArg²⁴⁵). The mature C-terminus of β is trimmed by Kex1p which removes the terminal Arg³⁴⁵ residue, thus uncovering the toxin's endoplasmic reticulum (ER) targeting signal (HDEL) which - in a sensitive target cell - is essential for retrograde toxin transport. Interestingly, both toxin subunits are covalently linked by a single disulfide bond between α -Cys⁵⁶ and β -Cys³⁴⁰ and expression of a mutant toxin in which β -Cys³⁴⁰ had been replaced by Ser³⁴⁰ resulted in the secretion of a non-toxic α/β heterodimer that is blocked in retrograde transport and incapable of entering the yeast cell cytosol, indicating that one important *in vivo* function of β -Cys³⁴⁰ might be to ensure accessibility of the toxin's β -C-terminus to the HDEL-receptor of the target cell.

4. Schmitt, M.J. & F. Breinig. 2002. The viral killer system in yeast: from molecular biology to application. *FEMS Microbiol. Rev.*, in press.

Since the initial discovery of the yeast killer system almost 40 years ago, intensive studies have substantially strengthened our knowledge in many areas of biology and provided deeper insights into basic aspects of eukaryotic cell biology as well as into virus-host cell interactions and general yeast virology. Analysis of killer toxin structure, synthesis and secretion has fostered understanding of essential cellular mechanisms such as posttranslational prepro-protein processing in the secretory pathway. Furthermore, investigation of the

receptor-mediated mode of toxin action proved to be an effective means for dissecting the molecular structure and *in vivo* assembly of yeast and fungal cell walls, providing important insights relevant to combatting infections by human pathogenic yeasts. Besides their general importance in understanding eukaryotic cell biology, killer yeasts, killer toxins and killer viruses are also becoming increasingly interesting with respect to possible applications in biomedicine and gene technology. This review will try to address both aspects.

5. Weiler, F. & M.J. Schmitt. Zygotin, a secreted antifungal toxin of the yeast *Zygosaccharomyces bailii* and its effect on sensitive cells, submitted.

Zygotin, a protein toxin produced and secreted by a killer virus-infected strain of the osmotolerant yeast *Zygosaccharomyces bailii*, kills a great variety of human and phytopathogenic yeast and filamentous fungi. Toxicity of the viral toxin is envisaged in a two-step receptor-mediated process in which the toxin interacts with cell surface receptors at the level of the cell wall and the plasma membrane. Isolated and partially purified Zygotin cell wall receptors were successfully used as biospecific ligand for efficient one-step purification of

the 10 kDa protein toxin by receptor-mediated affinity chromatography. Evidence is presented that Zygotin-treated yeast cells are rapidly killed by the toxin, and intensive propidium iodide staining of Zygotin-treated cells indicated that the toxin is affecting cytoplasmic membrane function, most probably by lethal ion channel formation. The presented findings suggest that Zygotin has the potential as a novel antimycotic in combatting yeast and fungal infections.

XIII. Department of Microbiology, Biochemistry & Food Science / UNESCO MIRCEN, University of the Free State, P.O. Box 339, 9300 Bloemfontein, South Africa. Communicated by James du Preez <dpreezjc@sci.uovs.ac.za> www.uovs.ac.za/faculties/nat/mkboc/.

Dear friends:

The alert among you may have noticed our name change; following the merger between the faculties of Natural and Agricultural Sciences, in January this year the Department of Microbiology & Biochemistry merged with the Dept. of Food Science (we are working on the rather unimaginative new name for our department). This also brought about changes in our e-mail addresses and website address. Also, the "Orange" was dropped from the name of our province and,

accordingly, from the name of the university (formerly U. of the Orange Free State).

To those of you who attended the 22nd International Specialised Symposium on Yeasts that took place in South Africa this year, on behalf of the symposium organising committee, I would like to thank you for participating and making ISSY 22 a great success. Our best wishes to Tibor Deák with his task of organising the 23rd ISSY in Budapest next year.

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

1. D.P. Smith, J.L.F. Kock, P.W.J. van Wyk, P. Venter, D.J. Coetzee, E. van Heerden, D. Linke and S. Nigam. 2000. The occurrence of 3-hydroxy oxylipins in the ascomycetous yeast family Lipomycetaceae. *S. Afr. J. of Sci.* **96**:247-249.
2. J.L.F. Kock, P. Venter, D.P. Smith, P.W.J. van Wyk, P.J. Botes, D.J. Coetzee, C.H. Pohl, A. Botha, K.H. Riedel & S. Nigam. 2000. A novel oxylipin-associated "ghosting" phenomenon in yeast flocculation. *Ant. v. Leeuwenhoek* **77**:401-406.
3. D.P. Smith, J.L.F. Kock, M.I. Motaung, P.W.J. van Wyk, P. Venter, D.J. Coetzee & S. Nigam. 2000. Ascospore aggregation and oxylipin distribution in the yeast *Dipodascopsis tóthii*. *Ant. v. Leeuwenhoek* **77**:389-392.
4. R. Deva, R. Coccoli, T. Schewe, J.L.F. Kock, & S. Nigam. 2000. Arachidonic acid stimulates cell growth and forms a novel oxygenated metabolite in *Candida albicans*. *Biochimica et Biophysica Acta* **1486**:299-311.
5. R. Deva, J.L.F. Kock, & S. Nigam. 2001. Involvement of aspirin-sensitive oxylipins in vulvovaginal candidiasis. *FEMS Lett.* **198**(1):37-43.
6. O. Sebolai, J.L.F. Kock, C.H. Pohl, D.P. Smith, P.J. Botes, E.E. Pretorius, P.W.J. Van Wyk & S. Nigam. 2001. Bioprospecting for novel hydroxyoxylipins in fungi: Presence of 3-hydroxy palmitic acid in *Saccharomyces malanga*. *Antonie v. Leeuwenhoek* **80**:311-315.
7. De Kock, S.H., J.C. du Preez & S.G. Kilian. 2001. The effect of growth factors on anoxic chemostat cultures of two *Saccharomyces cerevisiae* strains. *Biotechnology Letters* **23**:957-962.

In anoxic chemostat cultures of *Saccharomyces cerevisiae* ATCC 4126 and CBS 8066 grown in a medium containing yeast extract, a sharp increase in the steady-state residual glucose concentration occurred at relatively low dilution rates, contrary to the expected Monod kinetics. However,

supplementation with vitamins and amino acids facilitated efficient glucose uptake. This enhanced requirement for growth factors under anoxic conditions and at high growth rates could explain the exceptionally high apparent k_s values for *S. cerevisiae* reported in the literature.

8. Du Preez, J.C., Maré, J.E., Albertyn, J. & Kilian, S.G. 2001. Transcriptional repression of *ADH2*-regulated β -xylanase production by ethanol in recombinant strains of *Saccharomyces cerevisiae*. *FEMS Yeast Research* **1**:233-240.

The regulation of endo- β -(1,4)-xylanase production by two different strains of *Saccharomyces cerevisiae*, each transformed with the *XYN2* gene from *Trichoderma reesei* under control of the promoter of the alcohol dehydrogenase II (*ADH2*) gene of *S. cerevisiae*, was investigated. In batch culture, the rate of xylanase production was severely reduced by the pulse addition of 390 mmol ethanol l⁻¹. Pulses of 190 to 630 mmol

ethanol l⁻¹ into aerobic glucose-limited steady-state continuous cultures reduced the xylanase activity about five-fold and showed that ethanol repressed the *ADH2* promoter, as was evident from Northern blot analyses. Derepression of the *ADH2*-regulated xylanase gene occurred at ethanol concentrations below approximately 50 mmol l⁻¹.

9. G. Morakile, E.E. Pretorius, C.H. Pohl & J.L.F. Kock. 2002. Comparison of preservation methods for the maintenance of brewing yeast. *S. Afr. J. Sci.* **98** (Jan./ Feb.), 23-24.
10. G. Morakile, J.L.F. Kock & P.J. Botes. 2002. The use of fatty acid and sterol analyses as quality control methods in the brewing industry. *J. Inst. Brew.* (In Press).

Fatty acid and sterol analyses were evaluated as alternative quality control methods to conventional differentiation and characterisation systems in the brewing industry. The presence of linoleic acid (18:2) in brewing yeast could be used to distinguish these from closely related yeast species. Furthermore, the absence of lanosterol and stigmasterol enabled

differentiation of the brewing yeast from the rest of the closely related species tested. However, both fatty acid and sterol methods were not sensitive enough to detect mutants (variants) of brewing yeast. Conventional brewing identification tests proved sensitive enough to detect variants at low concentrations.

11. G. Morakile, J.L.F. Kock, W. van Zyl, C.H. Pohl, & B.C. Viljoen. 2002. Differentiation of brewing and related yeasts based on PCR amplification and restriction fragment length polymorphism of ribosomal DNA. *J. Inst. Brew.* (In Press).

A study to differentiate commercially applied brewing yeasts selected from the culture collection of the University of the Free State from related yeasts of the genus *Saccharomyces* using PCR amplification and RFLP of the internal transcribed spacers region was conducted. Differentiation was dependent on the restriction enzymes used to digest the amplified rDNA. Digestion with *Hae* III, *Cfo* I, *Sau* 3AI and *Msp* I divided representatives of the genus *Saccharomyces* into several unique

groups. With *Msp* I the DNA patterns for the two brewing strains were similar, but could be differentiated from *Sacch. cerevisiae* and other species tested. It was also possible to distinguish some members of the *Saccharomyces sensu stricto* group i.e. *Sacch. bayanus* and *Sacch. pastorianus* from *Sacch. cerevisiae* and *Sacch. paradoxus* using *Hae* III as well as *Sacch. paradoxus* from the other *sensu stricto* members using *Msp* I digestion.

XIV. VTT Biotechnology, P.O.Box 1501, FIN-02044 VTT, Finland. Communicated by John Londesborough <john.londesborough@vtt.fi>.

Publications since our last communication include the following.

1. Brummer, M.H., Kivinen, K.J., Jäntti, J., Toikkanen, J., Söderlund, H. and Keränen, S. 2001. Characterization of the *sec1-1* and *sec1-11* mutations. *Yeast* **18**:1525-1536.
2. Brummer, M.H., Richard, P., Sundqvist, L., Väänänen, R. and Keränen, S. 2001. The *GDII* genes from *Kluyveromyces lactis* and *Pichia pastoris*, cloning and functional expression in *Saccharomyces cerevisiae*. *Yeast* **18**:897-902.
3. Jäntti, J., Aalto, M.K., Öyen, M., Sundqvist, L., Keränen, S. and Ronne, H. 2002. Characterization of temperature sensitive mutations in the yeast syntaxin homologues *Sso1p* and *Sso2p*, and evidence for a distinct function for *Sso1p* in sporulation. *J. Cell Sci.* **115**:409-420.
4. Londesborough, J. 2001. Fermentation of maltotriose by brewer's and baker's yeasts. *Biotechnol. Lett.* **23**:1995-2000.
5. Maaheimo, H., Fiaux, J., Çakar, Z.P., Bailey, J.E., Sauer, U. & Szyperski, T. 2001. Central carbon metabolism of *Saccharomyces cerevisiae* explored by biosynthetic fractional ¹³C labeling of common amino acids. *Eur. J. Biochem.* **268**:2464-2479.
6. Nakari-Setälä, T. Azeredo, J., Henriques, M., Oliveira, R., Teixeira, J., Linder, M., and Penttilä, M. 2002. Expression of a fungal hydrophobin in the *Saccharomyces cerevisiae* cell wall: Effect on cell surface properties and immobilization, *Appl. Environ. Microbiol.* **68**:in press.
7. Poutanen, M., Salusjärvi, L., Ruohonen, L., Penttilä, M., Kalkkinen, N. 2001. Use of matrix-assisted laser desorption/ionization time-of-flight mass mapping and nanospray liquid chromatography/electrospray ionization tandem mass spectrometry sequence tag analysis for high sensitivity identification of yeast proteins separated by two-dimensional gel electrophoresis. *Rapid Commun. Mass Spectrom.* **15**:1685-1692.
8. Richard, P., Londesborough, J., Putkonen, M. and Penttilä, M. 2001. Cloning and expression of a fungal L-arabinitol 4-dehydrogenase gene. *J. Biol. Chem.* **276**:40631-40637.
9. Richard, P., Putkonen, M., Väänänen, R., Londesborough, J. and Penttilä, M. 2002. The missing link in the fungal L-arabinose catabolic pathway, identification of the L-xylulose reductase gene. *Biochemistry* **41**:6432-6437.
10. Toivari, M.H., Aristidou, A., Ruohonen, L. & Penttilä, M. 2001. Conversion of xylose to ethanol by recombinant *Saccharomyces cerevisiae*: Importance of xylulokinase (*XKSI*) and oxygen availability. *Metab. Eng.* **3**:236-249.

11. Torkko, J.M., Koivuranta, K.T., Miinalainen, I.J., Yagi, A.I., Schmitz, W., Kastaniotis, A.J., Airene, T.T., Gurvitz, A. & Hiltunen, K. 2001. *Candida tropicalis* Etr1p and *Saccharomyces cerevisiae* Ybr026p (Mrf1'p), 2-enoyl thioester reductases essential for mitochondrial respiratory competence. *Mol Cell Biol.* **21**:6243-6253.
12. Vasara, T., Saloheimo, M., Keränen, S. and Penttilä, M. 2001. *Trichoderma reesei rho3*, a homologue of yeast *RHO3*, suppresses the growth defect of yeast *sec15-1* mutation. *Curr. Genet.* **40**:119-127.
13. Vasara, T., Salusjärvi, L., Raudaskoski, M., Keränen, S., Penttilä, M. and Saloheimo, M. 2001. Interactions of the *Trichoderma reesei rho3* with the secretory pathway in yeast and *T. reesei*. *Mol. Microbiol.* **42**:1349-1361.
14. Vasara, T., Keränen, S., Penttilä, M. and Saloheimo, M. 2002. Characterization of two 14-3-3 genes from *Trichoderma reesei*: interactions with yeast secretory pathway components. *Biochem. Biophys. Acta* (in press).

The following MSc. theses, supervised at VTT, have been presented.

15. Ritva Väänänen. 2000. Enhancing the xylose metabolism of *Saccharomyces cerevisiae*. (In Finnish). University of Kuopio, Finland.
16. Marianne Mäntymäki (2001) Characterization of transketolases of yeast *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*. Department of Biological and Environmental Science. University of Jyväskylä, Finland.
17. Mikko Putkanen (2001) Expression of the fungal L-arabinose pathway in *Saccharomyces cerevisiae*. Department of Chemical Technology, Helsinki University of Technology, Finland.

Mikko Putkonen was awarded a prize (€1000) by The Association of Finnish Chemical Societies for the best Master's thesis of 2001 in biotechnology.

XV. Japan Collection of Microorganisms, RIKEN, Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@jcm.riken.go.jp>.

The director of Japan Collection of Microorganisms (JCM) changed over from Dr. Hiroyuki Osada, who had been administratively directing JCM for the last two years, to Dr. Toshiaki Kudo. His research mainly targets bacteria and his main research subjects are: (1) Function and interaction of symbiotic microorganisms, (2) Diversity and ecological roles of yet-uncultivated microorganisms and (3) Function, adaptation and evolution of aromatic compound-degrading microorganisms.

The eighth edition of JCM Catalogue of Strains has published in March 2002. The catalogue covers 2492 yeast and fungal, 19 yeast-like alga

Prototheca, 3976 bacterial and 155 archaeal strains with indexes, media, formulae and references. The price of the catalogue is ¥5,000 and additional shipping and handling charges are requested for overseas delivery.

Orders should be addressed to: Business Center for Academic Societies Japan, 5-16-9 Honkomagome, Bunkyo-ku, Tokyo 113-8622, Japan. E-mail: <sub@bcasj.or.jp>.

Fax: +81 3 5814 5822

You can also get a wealth of information for microorganisms from the catalogue on the JCM home page at <http://www.jcm.riken.go.jp/>.

The following articles have appeared, or are in press.

1. Takashima, M. and Nakase, T. 2001. *Tilletiopsis derxii*, *Tilletiopsis oryzicola* and *Tilletiopsis penniseti*, three new species of the ustilagionomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. *Antonie van Leeuwenhoek* **80**:43-56.

2. Nagahama, T., Hamamoto, M., Nakase, T. and Horikoshi, K. 2001. *Rhodotorula lamellibrachii* sp. nov., a new yeast species from a tubeworm collected at the deep-sea floor in Sagami Bay and its phylogenetic analysis. *Antonie van Leeuwenhoek* **80**:317-323.
3. Takashima, M., Deak, T. and Nakase, T. 2001. Emendation of *Dioszegia* with redescription of *Dioszegia hungarica* and two new combinations, *Dioszegia aurantiaca* and *Dioszegia crocea*. *J. Gen. Appl. Microbiol.* **47**:75-84.
4. Fungsin, B., Hamamoto, M., Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P. and Nakase, T. 2002. *Kockovaella barringtoniae*, a new basidiomycetous yeast species isolated from a plant leaf collected in a tropical rain forest in Thailand. *Int. J. Syst. Evol. Micorbiol.* **52**:281-284.
5. Hamamoto, M., Thanh, V. N. and Nakase, T. 2002 *Bannoa hahajimensis* gen. nov., sp. nov., and three related anamorphs *Sporobolomyces bischofia* sp. nov., *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov., yeasts isolated from plants in Japan. *Int. J. Syst. Evol. Micorbiol.* **52**:1023-1032.
6. Sugita, T., Takashima, M., Shinoda, T., Suto, H., Unno, T., Tsuboi, R., Ogawa, H. and Nishikawa, A. 2002. New yeast species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. *J. Clin. Microbiol.* **40**:1363-1367.
7. Bai, F. -Y., Takashima, M., Jia, J. -H. and Nakase, T. 2002. *Dioszegia zsoitii* sp. nov., a new ballistoconidium-forming yeast species with two varieties. *J. Gen. Appl. Microbiol.* **48**:17-23.
8. Suzuki, M. and Nakase, T. 2002. A phylogenetic study of ubiquinone-7 species of the genus *Candida* based on 18S ribosomal DNA sequence divergence. *J. Gen. Appl. Microbiol.* **48**:55-65.
9. Ueno, R., Urano, N., Suzuki, M. and Kimura, S. 2002. Isolation, characterization, and fermentative pattern of a novel thermotolerant *Prototheca zopfii* var. *hydrocarborea* strain producing ethanol and CO₂ from glucose at 40C. *Arch. Microbiol.* **177**:244-250.
10. Hamamoto, M., Nagahama, T. and Tamura, M. Systematic study of basidiomycetous yeasts - Evaluation of the ITS regions of rDNA to delimitate species of the genus *Rhodosporidium*. *FEMS Yeast Research* (accepted).

XVI. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze <kunzeg@ipk-gatersleben.de>.

Recent publications.

1. T. Wartmann, E. Böer, A. Huarto Pico, H. Sieber, O. Bartelsen, G. Gellissen & G. Kunze. 2002. High-level production and secretion of recombinant proteins by the dimorphic yeast *Arxula adeninivorans*. *FEMS Yeast Res.* (in press).

The non-conventional dimorphic thermo- and salt-resistant yeast *Arxula adeninivorans* has been developed as a host for heterologous gene expression. For assessment of the system two model genes have been selected: the *GFP* gene encoding the intracellular green fluorescent protein, and the *HSA* gene encoding the secreted human serum albumin. The expression system includes two host strains, namely *A. adeninivorans* LS3, which forms budding cells at 30°C and mycelia at >42°C, and the strain *A. adeninivorans* 135, which forms mycelia at temperatures as low as 30°C. For expression control the constitutive *A. adeninivorans*-derived *TEF1*-promoter

and *S. cerevisiae*-derived *PHO5*-terminator were selected. The basic *A. adeninivorans* transformation/expression vector pAL-HPH1 is further equipped with the *E. coli*-derived *hph* gene conferring hygromycin B resistance and the 25S rDNA from *A. adeninivorans* for rDNA targeting. Transformants were obtained for both, budding cells and mycelia. In both cell types similar expression levels were achieved and the GFP was localised in the cytoplasm while more than 95% of the HSA accumulated in the culture media. In initial fermentation trials on a 200 ml shake flask scale maximal HSA product levels were observed after 96 h of cultivation.

2. T. Wartmann, U.W. Stephan, I. Bube, E. Böer, M. Melzer, R. Manteuffel, R. Stoltenburg, L. Guengerich, G. Gellissen & G. Kunze. 2002. Post-translational modifications of the *AFET3* gene product - a component of the iron transport system in budding cells and mycelia of the yeast *Arxula adenivorans*. Yeast (in press).

The yeast *Arxula adenivorans* is characterized by a temperature-dependent dimorphism. *A. adenivorans* grows as budding cells at temperatures up to 42°C, but forms mycelia at higher temperatures. A strong correlation exists between morphological status and iron uptake, achieved by two transport systems that differ in iron affinity. In the presence of high Fe(II) concentrations (>2 μM), budding cells accumulate iron concentrations up to sevenfold those observed in mycelia, while at low Fe(II) concentrations (<2 μM), both cell types accumulate similar amounts of iron. The copper-dependent Fe(II) oxidase Afet3p, composed of 615 amino acids, is a component of the high affinity iron transport system. This protein shares a high degree of homology with other yeast iron transport proteins, namely

Fet3p of *Saccharomyces cerevisiae*, Cafet3p of *Candida albicans* and Pfet3p of *Pichia pastoris*. Expression of the *AFET3* gene is found to be strongly dependent on iron concentration but independent of the morphological stage; however, cell morphology was found to influence post-translational modifications of the gene product. *O*-glycosylation was observed in budding cells only, whereas *N*-glycosylation occurred in both cell types. The *N*-glycosylated 103 kDa glycoprotein matures into the 108.5 kDa form, further characterized by serine phosphorylation. Both *N*-glycosylation and phosphorylation occur at low iron concentrations (≤5 μM). The mature Afet3p of 108.5 kDa is uniformly distributed within the plasma membrane in cells of both morphological stages.

XVII. Center for Process Biotechnology, BioCentrum-DTU, Building 223, The Technical University of Denmark, DK-2800 Kgs Lyngby, Denmark. Communicated by L.Olsson <lo@biocentrum.dtu.dk>.

Recent publications.

1. Christensen B., T. Christiansen, A. K. Gombert, J. Thykær, J. Nielsen. 2001. A simple and robust method for estimation of the flux through the pentose phosphate pathway in micro-organisms. *Biotechnol. Bioeng.* **74**:517-523.
2. Gombert A. K. , M. M. dos Santos, B. Christensen, J. Nielsen. 2001. Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* at different conditions of glucose repression. *J. Bacteriol.* **183**:1441-1451.
3. Jørgensen, H., Olsson, L., Rønnow, B., and E. Palmqvist. 2002. Fed-batch cultivation of baker's yeast followed by nitrogen or carbon starvation: Effects on fermentative capacity and content of trehalose and glycogen. *Appl. Microbiol. Biotechnol.* In press.
4. Mortensen, U.H., N. Erdeniz, Q. Feng & R. Rothstein, A. . 2002. molecular genetic dissection of the evolutionarily conserved N-terminus of yeast Rad52. *Genetics*, in press.
5. Møller K., C. Bro, J. Piskur, J. Nielsen, L. Olsson. 2002. Steady-state and transient-state analysis of aerobic fermentation in *Saccharomyces kluyveri*. *FEMS Yeast Res.*, in press.
6. Møller K., B. Christensen, J. Förster, J. Piskur, J. Nielsen, L. Olsson. 2001. Aerobic glucose metabolism of *Saccharomyces kluyveri*: Growth, metabolite production and quantification of metabolic fluxes. *Biotechnol. Bioeng.* **77**:186-193.
7. Møller K., Olsson L., and J. Piskur. 2001. Ability for anaerobic growth was not sufficient for development of the petite-positive character in *Saccharomyces kluyveri*. *J. Bacteriol.* **183**:2485-2489.
8. Møller, K. L. D. Tidemand, J. R. Winther, L. Olsson, J. Piskur, J. Nielsen. 2001. Production of a heterologous proteinase A by *Saccharomyces kluyveri*. *Appl. Microbiol. Biotechnol.* **57**:216-219.
9. Nielsen J.. 2001. Metabolic engineering. *Appl. Microbiol. Biotechnol.* **55**:263-283.
10. Nielsen J., L. Olsson. 2002. An expanded role for microbial physiology in metabolic engineering and functional genomics: Moving towards systems biology. *FEMS Yeast Res.*, in press.

11. Nissen T. L., M. Anderlund, M. C. Kielland-Brandt, J. Nielsen, J. Villadsen. 2001. Expression of a cytoplasmic transhydrogenase in *Saccharomyces cerevisiae* results in formation of 2-oxoglutarate due to depletion of the NADPH pool. *Yeast* **18**:19-32.
12. Ostergaard S., L. Olsson, J. Nielsen. 2001. *In vivo* dynamics of galactose metabolism in *Saccharomyces cerevisiae*: Metabolic fluxes and metabolite levels. *Biotechnol. Bioeng.* **73**:412-425.
13. Ostergaard, S., K. O. Walløe, C. S. G. Gomes, L. Olsson, J. Nielsen. 2001. The impact of GAL6, GAL80, and MIG1 on glucose control of the GAL system in *Saccharomyces cerevisiae*. *FEMS Yeast Research.* **1**:47-55.
14. Zaldivar J., J. Nielsen, L. Olsson. 2001. Metabolic engineering of biocatalysts for fuel ethanol production. *Appl. Microbiol. Biotechnol.* **56**:17-34.

XVIII. Food Science and Technology Department, Biotechnical Faculty University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. Communicated by P. Raspor < peter.raspor@bf.uni-lj.si >.

Original scientific paper.

1. Batiè, M., Raspor, P. 2000. Effect of cultivation mode on a bioprocess for chromium yeast biomass enrichment. *Pflügers Arch.* **439**(3):R73-R75.

Defined cultivation media for yeast growth which contained 278.8 mM of glucose and 0.1 mM of chromium(III) added as K₂Cr(SO₄)₂ · 12 H₂O was used in batch and combined batch-fed-batch cultivation mode. In fed batch cultivation mode the rate of substrate addition remained constant during growth of yeast and corresponded to a growth rate of 0.25 h⁻¹. In both cases the growth and yeast activity was followed by on line measurement of optical density, pH and pO₂ at 30°C. At the end of the bioprocess the concentration of protein in yeast biomass was determined off line by the biuret reaction. Total and organically bound chromium was detected by ETA-AAS. Different cultivation modes affected the total cell protein concentration of yeast grown in media supplemented with chromium. In batch process the protein content represented

25.7% of dry yeast biomass, in contrast in the mixed bioprocess this value was 16.9% one the same period of time. The influence of cultivation mode on chromium uptake was seen in total chromium accumulation which reached 8.68 ± 0.16 micromol g⁻¹ d.wt. in batch and 1.92 ± 0.04 micromol of chromium g⁻¹ of dry yeast biomass in combined batch/fed-batch cultivation mode. The opposite was observed for organically bound chromium. The 60 % of total accumulated chromium was organically bound during yeast growth in combined batch/fed-batch mode. When yeast was grown in batch mode this value attained 13.5 %. Results suggested that a combined batch/fed-batch mode of cultivation was more effective over a batch system in chromium biotransformation to organically bound chromium, regardless of the lower protein ratio determined in the yeast biomass.

2. Heintel, T., Zagorc, T., Schmitt, M.J. 2001. Expression, processing and high level secretion of a virus toxin in fission yeast. *Appl. Microbiol. Biotechnol.* **56**(1/2):165-172.

The virally encoded K28 toxin of *Saccharomyces cerevisiae* kills sensitive yeast cells in a multi-step receptor-mediated fashion by cell cycle arrest and inhibition of DNA synthesis. *In vivo*, the toxin is translated as a 38 kDa preprotoxin (pptox) which is enzymatically processed to the biologically active alpha/beta heterodimer during passage through the yeast secretory pathway. Here, we demonstrate that *Schizosaccharomyces pombe*, a yeast from which no natural toxin-secreting killer strains are known, is perfectly capable of expressing a killer phenotype. Episomal as well as integrating K28 pptox gene cassettes were constructed that allowed a tightly

thiamine-regulated killer phenotype expression under transcriptional control of the *Sch. pombe* nmt1 promoter. Northern analysis of the toxin-coding transcript as well as Western analysis of the secreted toxin indicated that fission yeast is capable of expressing a correctly processed and fully functional virus toxin. Moreover, toxin secretion in recombinant *Sch. pombe* was at least ten-fold higher than in any natural and/or recombinant *Sac. cerevisiae* killer strain, indicating that pptox-derived vectors might be attractive in the fast growing field of heterologous protein expression and secretion in yeast.

3. Zagorc, T., Maráz, A., Èade, N., Povhe Jemec, K., Péter, Gabor, Resnik, M., Nemaniè, J., Raspor, P. 2001. Indigenous wine killer yeasts and their application as starter cultures in wine fermentation. *Food microbiol.* **18**:441-451.

Wine yeast strains were isolated from seven fermentations of the red wines, Refošk and Teran, produced in

the southwestern part of Slovenia. Among 613 isolated strains, 22 expressed killer activity against the supersensitive strain

Saccharomyces cerevisiae. Killer strains were isolated at different stages of wine fermentation but did not dominate in any of them. Species identification was based on the combination of RFLP analysis of an amplified rDNA region and biochemical-physiological tests. Killer isolates were identified as *Saccharomyces cerevisiae*, *Pichia anomala*, *P. kluyveri*, *P. pijperi*, *Hanseniaspora uvarum* and *Candida rugosa*. Electrophoretic karyotyping was used to differentiate strains of the same species. Fermentation properties of four *S. cerevisiae* strains that

possessed stable killer activity were characterized in fermentations of Malvasia must by studying their population dynamics and chemical composition and by sensory analysis of the produced wines. In order to compare the results, spontaneous and by commercial yeast starters induced fermentations were performed concomitantly. The local killer strain S₅12/10 showed the best fermentation properties and produced wine with favorable characteristics.

4. Povhe-Jemec, K., Èade, N., Zagorc, T., Bubiæ, V., Upec, A., Raspor, P. 2001. Yeast population dynamics in five spontaneous fermentations of Malvasia must. *Food microbiol.* **18**:247-259.

Using electrophoretic karyotyping, PCR-RFLP analysis of the rDNA spacer and physiological testing, the dynamics of the wine yeast strains present in spontaneous wine fermentation have been studied. The isolation and killer phenomena of 937 isolates were performed. The population of the non-Saccharomyces yeasts in must after sedimenting was 103 CFU/ml and belonged to the genera as follows: *Candida*, *Metschnikowia*, *Hanseniaspora*, *Rhodotorula*, *Issatchenkia* and *Debaryomyces*. However, *Saccharomyces* sp. has never been detected in fresh must. All 263 non-Saccharomyces yeasts isolated from the initial stage of the spontaneous fermentations were identified according to the karyotyping and PCR-RFLP analysis of the rDNA spacer. Based on the chromosome length polymorphism among 649 isolates from the subsequent phases of fermentation, it was possible to distinguish 46 different

electrophoretic patterns of *Saccharomyces cerevisiae*. The most abundant strains in all fermentors represented karyotypes L1, L4, L12, P6. The sequential substitution of *S. cerevisiae* strains along the fermentation agreed with different fermentation phases. At the slow fermentation rate in almost all fermentors karyotype L4 was most abundant. Later, at the beginning of the tumultuous fermentation the most frequent karyotype became L1 followed by karyotype L4. Finally, along the fermentation process, pattern L4 was clearly replaced by karyotype L1 followed by pattern L12. Despite the same fermentation source (grape must) differences among five spontaneous fermentations were observed. The population dynamics of *Saccharomyces cerevisiae* yeasts, especially the dynamics of the major *S. cerevisiae* strains (L1, L4, L12) were quite similar in all five fermentors in opposite to the non-Saccharomyces populations.

Review.

5. Raspor, P., Batiè, M., Jamnik, P., Josiæ, D., Milaèiè, R., Paš, M., Recek, M., Reia-Dereani, V., Skrt, M. 2000. The influence of chromium compounds on yeast physiology. *Acta Microbiol. Immunol. Hungar.*, **47**(2/3):143-173.

Chromium is an element which trace levels are required in yeast metabolism, but when accumulated in excess, becomes toxic. The form and concentration of chromium in the macro- and microenvironment of the yeasts are important. Chromium(VI) with its negative charge enters cell easily than chromium(III), which is positively charged in physiological conditions. However, all the transport mechanisms are still not clearly defined. Since toxicity is connected with chromium concentration, its manifestation in metabolism is proportional with amount, which can enter the cell and interfere with biological functions. DNA was showed to be damaged by chromium(VI) in vivo and chromium(III) in vitro. Single-stranded and double-stranded brakes are induced by chromium. During cultivation in batch, feed-batch and continuous processes

reduction of RNA, proteins and biomass yield are present. However, there are yeasts which tolerate higher concentrations of chromium and are more suitable for bioprocessing. Their fast screening can be conducted by the gradient agar plate method, followed by cell viability/ mortality method, which select adequate yeast. Yeast biomass showed to be able to uptake high amount of chromium by biosorption and bioaccumulation. The ratio between total and organically bound chromium was good. However, the function of chromium in glucose tolerance factor and role of chromium in yeast metabolism remains in focus of current research. The question, if metallothionein-like complex, which is able to bind chromium, belongs to so called glucose tolerance factor is active in glucose metabolism in yeast remains to be opened for further research.

Book chapters.

6. Raspor, P., Smole Moina, S., Èade, N. 2001. Identification of Yeasts From Grape/must/wine System. In: Spencer, J. F.T., Ragout De Spencer, A.L. *Food microbiology protocols, (Methods in biotechnology, 14)*. Totowa (New Jersey): Humana Press, 243-257.

1.	INTRODUCTION	2.3.1.	Isolation of DNA
2.	MATERIALS	2.3.2.	PCR amplification of rDNA
2.1.	Isolation and enumeration of yeasts	2.3.3.	Restriction analysis of amplified rDNA
2.3.	PCR-RFLP of rDNA	2.4.	Confirmative physiological testing

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|--------|---|--------|--|
| 2.5. | Electrophoretic karyotyping | 3.3.2. | PCR amplification of rDNA |
| 2.5.1. | Isolation of chromosomal DNA | 3.3.3. | Restriction analysis of amplified rDNA |
| 2.5.2. | Pulsed field gel electrophoresis (PFGE) | 3.3.4. | Analysis of species specific restriction patterns of ribosomal DNA (PCR-ribotypes) |
| 3. | METHODS | 3.5. | Electrophoretic karyotyping with PFGE |
| 3.1. | Isolation and enumeration of yeasts | 3.5.1. | Isolation of chromosomal DNA |
| 3.2. | Morphological characterization | 4. | NOTES |
| 3.3. | PCR – RFLP of rDNA | 5. | References |
| 3.3.1. | DNA isolation | | |

2. Raspor, P., Èade, N., Povhe-Jemec, K., Smole-Moina, S., Zagorc, T. 2000. How to approach to biodiversity of complex yeast community in the must/wine system?. In: International School for Molecular Biology, Microbiology and Science for Peace, International Training Course, August 23-27, 2000, Keszthely, Hungary [printed materials]. [Keszthely: Hungarian Society for Microbiology; Jerusalem: UNESCO-Hebrew University of Jerusalem, 2000], p. [1-9].

The problem of yeast determination in specific environments is rather old but it still offers many new dimensions to be elucidated. The new molecular methods which are becoming of age in last years sowed to be in reality worthy tool in this respect. One of most common environments in focus is wine fermentation. It has been searched many times in last century, however proper yeast kinetics is still under velum. This problem pushes many attitudes to the research laboratories with an intention to offer new view in this respect. The methodology we constructed in last years is based on step by step approach which shows many advantages over traditional approach which has been supported in many laboratories over the world in last decades. This approach offered us possibility to select yeast strains in to particular groups (*Saccharomyces* vs. non-*Saccharomyces*) and further to continue to particular direction of

identification. Classical methods of yeast determination include morphological and physiologically-biochemical tests. Besides phenotypic characterisation many molecular-biology analyses of yeast nucleic acids have been developed recently. They can give stable and unique electrophoretic profiles independently of the microbial cultivation conditions. In this respect the application of the yeast nuclear DNA analysis for identification of food-borne yeasts showed to be appropriate. Following typing methods have been found efficient: electrophoretic karyotyping, the study of the restriction fragment length polymorphism (RFLP), and two techniques based on PCR amplification of yeast DNA: the amplification with non-specific primers (AP-PCR, arbitrarily primed PCR, RAPD, random amplified polymorphic DNA analysis) and PCR ribotyping - restriction analysis of amplified ribosomal RNA genes of tested yeasts.

XIX. 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@mail.fct.unl.pt>.

The following papers have been accepted for publication.

1. Gadanho, M. and Sampaio, J.P. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodotorula*: *Rh. glutinis sensu stricto* and *Rh. dairenensis* comb. nov. FEMS Yeast Research.

The phenotypic and genetic heterogeneity of the basidiomycetous yeast species *Rhodotorula glutinis* was investigated in a group of 109 isolates. A polyphasic taxonomic approach was followed which included PCR fingerprinting, determination of sexual compatibility, 26S and ITS rDNA sequence analysis, DNA-DNA reassociation experiments and reassessment of micromorphological and physiological attributes. The relationships with species of the teleomorphic genus

Rhodospodium were studied and isolates previously identified as *Rh. glutinis* were found to belong to *R. babjevae*, *R. diobovatum* and *R. sphaerocarpum*. Other isolates included in the study were found to belong to *Rh. glutinis* var. *dairnensis*, which is elevated to the species level, or to undescribed species. The concept of *Rh glutinis sensu stricto* is proposed due to the close phenetic and phylogenetic proximity detected for *Rh. glutinis*, *Rh. graminis* and *R. babjevae*.

2. Sampaio, J.P., Weiß, M., Gadanho, M. and Bauer, R. 2002. New taxa in the Tremellales: *Bulleribasidium oberjochense* gen. et sp. nov., *Papiliotrema bandonii* gen. et sp. nov. and *Fibulobasidium murrhardtense* sp. nov. Mycologia.

Two new genera, *Bulleribasidium* and *Papiliotrema*, and three new species, *B. oberjochense*, *P. bandonii* and *Fibulobasidium murrhardtense* are described. An integrated analysis of morphological, ultrastructural, physiological and

molecular data indicates that the new taxa belong to the Tremellales (Basidiomycota). Relevant characteristics of the new genera and species are discussed and compared with those of closely related taxa.

XX. Academy of Sciences of Czech Republic, Institute of Microbiology, Vídeňská 1083, 142 20 Prague 4, Czech Republic. Communicated by J. Voríšek.

Recent publication.

1. Enzymatic activities of Ura2 and Ural proteins (aspartate carbamoyltransferase and dihydro-orotate dehydrogenase) are present in both isolated membranes and cytoplasm of *Saccharomyces cerevisiae*.

Computational analysis predicted three potential hydrophobic transmembrane α -helices within the Ura2 multidomain protein of *Saccharomyces cerevisiae*, the C-terminal sub-domain of which catalyses the second step of uridine-monophosphate biosynthesis by its L-aspartate carbamoyltransferase activity (EC 2.1.3.2). The fourth step of pyrimidine biosynthesis is catalysed by dihydro-orotate

dehydrogenase (Ural protein; EC 1.3.99.11), which was similarly characterized as a peripheral membrane protein. *Ex situ*, the activities of the investigated enzymes were associated both with isolated yeast membranes, fractionated by differential centrifugation to remove intact nuclei, and with soluble cytoplasmic proteins.

XXI. Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by M.A. Lachance <lachance@uwo.ca>.

The following paper was accepted recently.

1. M.A. Lachance and J.M. Bowles. 2002. *Metschnikowia arizonensis* and *Metschnikowia dekortorum*, two new large-spored yeast species associated with floricolous beetles. FEMS Yeast Res.

Two new haplontic heterothallic species of *Metschnikowia* were discovered in flowers and associated beetles. *Metschnikowia arizonensis* was recovered from flowers of cholla cactus (*Opuntia echinocarpa*) and a specimen of *Carpophilus* sp. (Coleoptera: Nitidulidae) found in these flowers, in Arizona. *Metschnikowia dekortorum* was isolated in specimens of the nitidulid beetle *Conotelus* sp. captured in flowers of two species of *Ipomoea* in northwestern Guanacaste Province, Costa Rica. The sexual cycle of these yeasts is typical of the large-spored *Metschnikowia* species, but the asci and spores are intermediate in size between these and other members of the genus. The physiology is consistent with that of most *Metschnikowia* species except that both species fail to utilize

lysine as sole nitrogen source. Also, *M. arizonensis* utilizes fewer carbon compounds than most species and exhibits considerable variability among strains at this level. Partial ribosomal DNA large-subunit (D1/D2) sequences suggest that *M. arizonensis* and *M. dekortorum* are moderately related sister species whose positions are intermediate between the large-spored species *Metschnikowia* and *Metschnikowia hibisci*. The type cultures are: *M. arizonensis*, strains UWO(PS)99-133.3.1 = CBS 9064 = NRRL Y-27427 (h⁺, holotype) and UWO(PS)99-103.4 = CBS 9065 = NRRL Y-27428 (h⁻, isotype); and *M. dekortorum*, strains UWO(PS)01-142b3 = CBS 9063 = NRRL Y-27429 (h⁺, holotype) and UWO(PS)01-138a3 = CBS 9062 = NRRL Y-27430 (h⁻, isotype).

International Commission on Yeasts

ISSY 22

The 22nd International Specialized Symposium on Yeasts, entitled "Yeast Fermentations and other Yeast Bioprocesses", held at Pilanesberg National Park, South Africa, was a great success. James du Preez and his colleagues are to be congratulated on presenting an interesting scientific programme for over 150 participants, who

moreover enjoyed the wonderful location amidst exciting wildlife, as well as the excellent social atmosphere of the meeting and the warm hospitality of the organizers. A meeting's report by Han de Winde will appear in FEMS Yeast Research Vol.2, Nr. 3.

Lex Scheffers, Chair, ICY

Minutes of Meeting of the Commissioners
Tuesday March 26, 2002
ISSY 22, Pilanesberg, South Africa

In attendance. Deák, T. (Hungary); du Preez, J. (S. Africa); Fleet, G.H. (Vice-Chair, Australia); Hahn-Hägerdal, B. (Sweden); Kurtzman, C. (USA); Loureiro-Dias, M. (Portugal); Naumov, G.I. (Russia); Penttilä, M.E. (Finland); Pretorius, I.S. (S. Africa); Prior, B.A. (S. Africa); Raspor, P. (Slovenia); Romano, P.R. (Italy); Scheffers, W.A. (Chair, The Netherlands); Sibirny, A.A. (Ukraine); Thevelein, J. (Belgium), van Dijken, J.P. (The Netherlands). Apologies: Spencer-Martins, I. (Portugal)

Chair's Report. Lex Scheffers (Chair ICY 2000-2004) opened the meeting and thanked James du Preez and his team for hosting a meeting with an excellent scientific program at an exceptionally outstanding venue. He noted the passing away of Herman Phaff, one of the founding members of ICY and honorary commissioner, and acknowledged that ISSY22 was dedicated to the memory of Herman Phaff. A copy of the dedication, compiled in the book of abstracts would be sent to Herman's wife, Diane.

ISSY22 Report. James du Preez (Chair of ISSY22) noted the successful response to ISSY22 with some 150 registrants from a diversity of countries, and thanked the local organising committee and the international scientific committee for their contributions. From the perspective of planning the symposium, he noted the importance of getting registrants/participants to submit abstracts by the deadline.

ISSY23 (2003) Report. Tibor Deak is chair of the organising committee for this symposium which will be held in Budapest, 26-29 August 2003. The topic of this symposium is "Interactions between yeasts and other organisms" with an emphasis on ecology, biodiversity and biochemical and molecular mechanisms of interaction. A draft program was handed out. A publicity brochure has been prepared and sent out to 44 commissioners. Graham Fleet commented that there are some 80 commissioners and that all of them should receive the brochure, in the interests of maximum publicity. A homepage for the symposium has been established and it is intended to manage symposium registrations and abstracts by internet.

ISY11 (2004). This general yeast symposium will be held in Brazil with Leda Mendonça-Hagler as Chair of the organising committee. As Leda was not at ISSY22, no report was available. Lex Scheffers agreed to follow up on progress with planning of ISY11.

ISSY24 (2005). This will be organised in Spain by Rafael Sentandreu on the topic "Morphogenesis of dimorphic fungal species; basic and applied aspects".

ISY25 (2006). This will be organised in Finland by Merja Penttilä on the topic "Physiology of yeasts and metabolic engineering". Merja indicated that she will report on planning in future years.

ISY26 (2007). A proposal was made by Patrizia Romano to organise this symposium in Italy in 2007. This was accepted by the meeting, with details of a symposium theme and venue to be discussed at future meetings.

General Discussion. Some commissioners expressed a sense of confusion between symposia within the ISSY series and ISY series and, especially, it is confusing to non-commissioners. It was suggested that Lex Scheffers clarify this to delegates in his closing remarks at ISSY22. It was also considered that "symposium" might not be the best term to describe the more general yeast conferences (i.e., the ISY series) held every four years. It was proposed that the names of such conferences be changed to **International Congress on Yeasts** rather than **International Symposium on Yeasts**. This was accepted unanimously by the commissioners. Henceforth, **International Congress on Yeasts** will be used to describe the general yeast conference that is held every four years, the next one being in Brazil, 2004.

The recruitment of new commissioners was raised, as has been discussed at most previous meetings. Graham Fleet commented that the list of commissioners had been significantly culled and re-activated during his term as Chair (1996-2000). The current list circulated to all commissioners in 2000, has the names and contact details of about 80 commissioners, the majority of whom are active and responsive. Nevertheless, there are some areas that need to be addressed, notably recruiting active commissioners from several countries (e.g., Germany, France, Switzerland, Japan, China, S.E. Asia). With the approval of the committee, Lex Scheffers and Graham Fleet agreed to work to cull inactive commissioners and recruit new commissioners where needed. They would seek advice from other commissioners where necessary.

Lex Scheffers closed the meeting by thanking James du Preez and his team for their excellent hospitality and provision of lunch for the meeting.

Minutes prepared by G.H. Fleet

Forthcoming Meetings

7th International Mycological Congress August 11-17 2002 - Oslo, Norway

Clete Kurtzman and Teun Boekhout have organized for this congress a symposium entitled **Molecular Systematics and Ecology of Yeasts**. Molecular genetic comparisons have radically changed our views on the definition of a species and the criteria for circumscription of genera and higher orders of classification. In turn, the new approaches to systematics affect our perceptions of species ecology and biodiversity. The speakers will address species and genus concepts, classification of ascomycetous and basidiomycetous yeasts from multigene comparisons and the effect that this work is having on the ecology of yeasts as well as on studies of plant and human pathogenic species.

Speakers and Topics: Steve Oliver, Manchester,

UK, What makes a species? Redundancy, recombination and reproductive isolation. Clete Kurtzman, Peoria, IL, USA, Genus relationships in the Saccharomycetales from multigene analyses. Álvaro Fonseca, Quinta da Torre, Portugal, Molecular approaches to the reappraisal of species diversity within the ascomycete genus *Taphrina*. André Lachance, London, ON, Canada, Biogeography of floricolous yeasts: is everything everywhere? Jack Fell, Key Biscayne, FL, USA, Molecular ecology of basidiomycetous yeasts in tropical marine habitats. Teun Boekhout, Utrecht, The Netherlands, Systematics of the human pathogen *Cryptococcus neoformans* in the genomics era. Rich Calderone, Washington, D.C., USA, Functional genomics in *Candida albicans* for drug target design.

For additional information, contact

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<http://www.uio.no/conferences/imc7/>

XVth International Conference on the "Biology of Kluyveromyces", Smolenice, Slovakia, 6-8 September, 2002

Deadline for Registration and Reservation of Accommodation and Transport: 30 May 2002.
Deadline for Submission of Abstract and

Registration Fee: 15 June 2002. Organizing committee: Julius Subík, Yvetta Gbelska and Margita Obernauerová.

<http://www.fns.uniba.sk/Kluyveromyces/3.php>

VIIIth International Fungal Biology Conference Guanajuato, Gto. Mexico, December 02-06, 2002

We invite all scholars and students interested in the analysis of cellular and molecular aspects of fungal growth, development, differentiation, and morphogenesis to attend the *VIII INTERNATIONAL FUNGAL BIOLOGY CONFERENCE* at Guanajuato, México, on December 1-5, 2002.

The city of Guanajuato, located in the central part of México has been named by UNESCO part of the cultural heritage of mankind due to its picturesque location, and the number of beautiful palaces, historic buildings, and churches erected during the colonial period. The city contains also important museums, and a number of unique underground streets.

The conference will take place in one of the most beautiful ex-Haciendas of Guanajuato, now turned into the picturesque four-star *Hotel Parador San Javier*.

Both city and hotel will offer an ideal background for a memorable scientific meeting.

Organization. Conference Secretariat: Jesús Aguirre and José Ruiz-Herrera.

Chairman of the International Steering Committee: Salomón Bartnicki-García.

International Scientific Committee: Ralph Dean, Louise Glass, Neil Gow, Wilhelm Hansberg, Regine Kahmann, Francis Martin, Robert Roberson, Rafael Sentandreu, Hans Sietsma Cees van den Hondel.

Local Committee: Félix Gutiérrez-Corona, Doralinda Guzmán-de-Peña, Alfredo Herrera-Estrella, Claudia León-Ramírez (*Treasurer*), Guadalupe Martínez-Cadena, Georgina Reyna-López, Cristina G. Reynaga-Peña (*Registration*), Olivia Sánchez.

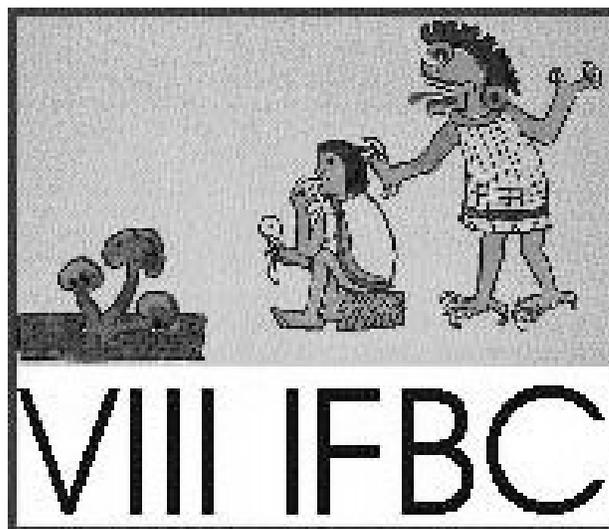
Conference Office: VIII IFBC, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato. Apartado Postal 629. Irapuato, Gto., 36500, MEXICO. Phone: +52 (462) 623-9653, FAX 624-5849. Email: ifbc@ira.cinvestav.mx Web page: www.ira.cinvestav.mx/cur-even/fungal.htm

Preliminary Program

Key Note Speakers: Regine Kahmann and Salomón Bartnicki-García

Symposia: Fungus-host interactions. *Organizers:* Francis Martin and Garry Cole. Signal transduction. *Organizer:* Ralph Dean. Sexual and asexual development. *Organizers:* Louise Glass and Wilhelm Hansberg. Yeast-like and dimorphic fungi. *Organizer:* Neil Gow. Fungal structure. *Organizer:* Robert Roberson. Fungal growth and differentiation. *Organizer:* José Ruiz-Herrera.

Workshops: Fungal cell wall synthesis and structure. *Organizer:* Rafael Sentandreu. Genomics. *Organizer:* Jeffrey Shuster. Secondary metabolism. *Organizer:* Nancy Keller. Secretion and extracellular enzymes. *Organizer:* Cees van den Hondel. Fungi in biological control. *Organizers:* Ilan Chet and Alfredo



Herrera-Estrella.

Topics For Free Communications: Fungus-host interactions, Secretion and extracellular enzymes, Signal transduction, Non-conventional yeasts, Fungal structure, Fungal growth and differentiation, Genetics, Genomics, Fungal pathogenesis, Secondary metabolism, Fungal cell wall synthesis and structure, Sexual and asexual development, Biotechnology, Evolution and phylogenetics, Fungal cytoskeleton, Regulation of fungal metabolism, Stress responses, Others.

Social Program: Welcoming reception; *Callejoneada* – a unique style of touring downtown Guanajuato at night, visiting its alleys while accompanied by live music; folk dance ballet; farewell dinner. No cost for registered attendants.

Abstract Submission: Abstracts for lectures, symposia, workshops and free communications should be submitted ONLY by filling out the corresponding forms at the Web site: www.ira.cinvestav.mx/cur-even/fungal.htm. The deadline for abstract submission is **August 31, 2002**.

Registration: Deadline for registration is **October 31, 2002**. **Registration will be limited to 250 participants.** Please fill the electronic form at the Congress Web page.

Fees

Before August 31, 2002:

Regular	USD\$385
Students	USD\$180
Accompanying persons	USD\$140

After August 31, 2002:

Regular	USD\$515
Students	USD\$260
Accompanying persons	USD\$185

Registration will cover transportation from the Leon airport (if needed, and only on Sunday Dec. 1),

identification badge, portfolio, program and proceedings, welcoming reception, admission to scientific sessions and poster presentations, luncheons for December 2-5, admission to social activities, and farewell dinner. The registration fee for accompanying persons will also cover a special sightseeing tour, but no portfolio, program, proceedings nor admission to sessions.

Transportation: The City of Guanajuato is served by the León ("Bajío") International Airport, listed by international

airlines as LEON (BJX), and located about 30 km from Guanajuato City. It is connected by direct flights to the main Mexican cities, including México City, as well as to US cities: Los Angeles, Oakland, Chicago, Dallas, Houston and Atlanta. Taxi service to Guanajuato is available at the airport. On Sunday December 1 only, a special information desk will be arranged at the airport, and a free shuttle bus will transport congress attendants to the Conference Hotel. By road, comfortable coaches connect Guanajuato to México City and other important cities in the country.

Brief News Item

Altech Medal of Excellence

This year's winner of the Medal of Excellence in the field of alcohol production technology is Dr. Charles Abbas of ADM. Dr. Abbas is recognized for his contribution to the development of yeast strains that are currently used in industrial applications at ADM. His work in biotechnology and with nonconventional yeast has resulted in the development of production processes that use a variety of feedstocks to produce organic acids, pigments, biomass and numerous biochemical products for use in food, pharmaceutical and chemical industries. He has taken advantage of the diverse metabolic activities of yeast to enhance industrial processes and provide many useful products for mankind. Dr. Abbas' work demonstrates the wide-ranging potential of the modern biorefinery as we begin to look at products and co-products for the future.

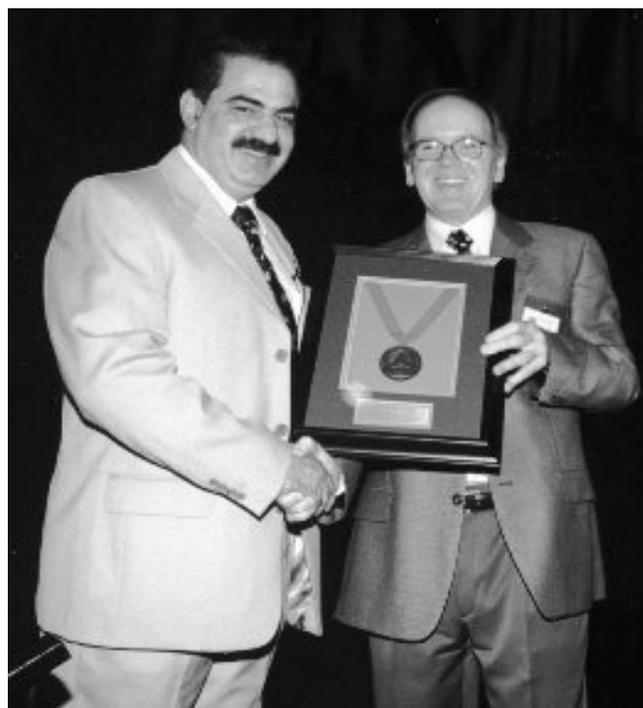
Summary of Dr. Abbas' presentation

When moving away from the conventional processes yeast are used for, *ie.* ethanol and bread-making, it is best to look

to other genera besides *Saccharomyces*, said Dr. Abbas. Dr. Abbas detailed 13 species from 8 genera capable of producing a wide array of products. He summarized by predicting: Over the next decade the commercial value of all products derived from the biotechnological applications of non-conventional yeasts will surpass those derived from current uses of *S. cerevisiae*. Rapid advances in metabolic engineering, bioinformatics and improved fermentation processes aided by increased automation will fuel much of these advances. With these developments in mind, the next decade promises to be an exciting era for non-conventional yeast biotechnology.

Products yeast make: Amino acids, carotenoids, feed additives, fermented foods, flavors, foods, fuels, nutraceuticals, organic acids, pharmaceuticals, polyols, polysaccharides, SS protein, vitamins, chemicals, enzymes.

T. Pearse Lyons



Dr. Charles Abbas (left) receiving the Altech Medal of Excellence from Dr. Lyons