

Yeast

A Newsletter for Persons Interested in Yeast

Official Publication of the International Commission on Yeasts
of the International Union of Microbiological Societies (IUMS)

JUNE 2003

Volume LII, Number I

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Editorial

Professor J.F.T. Spencer

Congratulations to Frank Spencer, who was awarded an Honorary Professorship by the Universidad Nacional de Tucuman, Argentina, in December 2002. A reception was held in his honour to celebrate the occasion.

I. Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602. Communicated by A. Bakalinsky <alan.bakalinsky@orst.edu>.

Recently published.

1. Martin, O., Brandriss, M.C., Schneider & G., Bakalinsky, A.T. 2003. Improved anaerobic use of arginine by *S. cerevisiae*. *Appl. Env. Micro.* 69:1623-1628.

Anaerobic arginine catabolism in *S. cerevisiae* was genetically modified to allow assimilation of all four rather than just three of the nitrogen atoms in arginine. This was accomplished by bypassing normal formation of proline, an unusable nitrogen source in the absence of oxygen, and causing formation of glutamate instead. A *pro3 ure2* strain expressing a

PGK1 promoter-driven *PUT2* allele encoding 1-pyrroline-5-carboxylate dehydrogenase lacking a mitochondrial targeting sequence produced significant cytoplasmic activity, accumulated twice as much intracellular glutamate, and produced twice as much cell mass as the parent when grown anaerobically on limiting arginine as sole nitrogen source.

II. 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. 2003. Beginnings of microbiology and biochemistry: the contribution of yeast research. *Microbiology* 149:557-567.
2. Barnett, J.A. 2003. A history of research on yeasts 5: the fermentation pathway. *Yeast* 20:509-543.
3. Barnett, J.A. 2003. A history of research on yeasts 6: the main respiratory pathway. *Yeast* (in preparation).

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

Recent publication.

1. Caridi A. 2003. Effect of protectants on the fermentation performance of wine yeasts subjected to osmotic stress. *Food Technol. Biotechnol.* 41:145-148.

During alcoholic fermentation of must from dried grapes, yeasts are subjected to very high sugar concentrations, besides other environmental stresses, and they modify their metabolic behaviour giving low ethanol yield and abnormally high acetic acid production. To investigate the protective effect of catechin, inositol, and SO₂ on wine yeasts, three thermotolerant strains of *Saccharomyces cerevisiae*, selected for winemaking of must from dried grapes, and three strains of *Saccharomyces* selected for the production of wines were inoculated in a sample of must at very high osmotic strength. A significant (p<0.01 or p<0.05) relationship between the addition

of 100 mg/L of catechin, inositol or SO₂ to the grape must and the change in the metabolic behaviour of the yeasts was observed. Compared to the control and depending on strain and protectant, the fermentation rate after 3 days increased up to 55 %, the ethanol content of the wines increased up to 16 %, the unitary succinic acid production increased up to 55 %, the unitary acetic acid production decreased up to 53 %, and the unitary glycerol production decreased up to 69 %. So by adding catechin, inositol or SO₂ to the grape must it is possible to minimise the abnormal fermentation performance that wine yeasts exhibit in winemaking of must from dried grapes.

2. Caridi A., Micari P., Caparra P., Cufari A., Sarullo V. 2003. Ripening and seasonal changes in microbial groups and in physico-chemical properties of the ewes' cheese Pecorino del Poro. *Int. Dair. J.*, 13:191-200.

Three batches of Pecorino del Poro, ewes' cheese made from raw milk, were examined throughout a 28-day ripening time at three different seasons. High logarithmic counts per gram of cheese for mesophilic coccal-shaped lactic acid bacteria (6.70-12.45), mesophilic lactobacilli (4.82-11.73), thermophilic coccal-shaped lactic acid bacteria (2.30-9.90), and thermophilic lactobacilli (2.95-8.15) were found. Coccal-shaped lactic acid bacteria were the dominant microorganisms throughout ripening. The microorganisms used as an indicator of hygiene during manufacture of the cheeses, coliforms and *Escherichia coli*, were

considerably lower, as were enterococci and yeasts. Coliforms and *E. coli* decreased sharply throughout ripening. Physico-chemical parameters such as pH (5.07-7.03), dry matter (46.34-72.79%), ether extract (31.35-51.84% of dry matter), crude protein (29.93-44.73% of dry matter), and chloride content (2.36-4.11% of dry matter) were also determined. Probably, the use of selected autochthonous mesophilic lactococci as a starter would control or suppress the growth of undesirable microorganisms. The results obtained suggest the need for improvements in milking and dairy conditions.

3. Corte V., Ragusa M., Caridi A., Cufari A. 2002. Confirmation of the ability of some yeasts to compensate the fixed acidity content of acid-deficient musts during winemaking in large volumes (in Italian). *Industrie delle Bevande*, 31:438-441.

Cryotolerant strains of *Saccharomyces bayanus* and hybrid strains - obtained via spore-conjugation of a cryotolerant strain of *Saccharomyces bayanus* and a non-cryotolerant strain of *Saccharomyces cerevisiae*, are able to yield organic acids during winemaking, so compensating, in some measure, the fixed acidity of acid-deficient musts. With the present study the AA demonstrate that, also on volumes of 1,000 hL, this fermentative behaviour is maintained; at least for wines of the Sicilian

cultivars Inzolia, Grecanico, and Grillo. However a certain weakening of the acidifying action was observed, more evident for the hybrid strain. In the wine produced from Inzolia cultivar, comparing the analytical values obtained on small volumes (10 L) and on large volumes (1,000 hL), the following reductions were observed: 5% of total acidity, 30% of malic acid, and 4% of succinic acid. The possible causes of this reduction have been considered.

4. Caridi A., Cufari A. 2003. Novel cost-effective method of screening yeasts for aptitude to adsorb phenolic compounds during winemaking. 23rd International Specialized Symposium on Yeasts "Interactions between Yeasts and other Organisms", 26-29 August 2003, Budapest, Hungary (accepted).

A quick and simple method to screen wine yeasts in Petri dishes regarding their aptitude to adsorb phenolic compounds from grapes has been developed. Two media, prepared utilising grape-skin or grape-seed as basic ingredient, were inoculated with 173 strains of *Saccharomyces*, 18 per plate. The biomass colour was examined after seven days of incubation at 25°C: a white biomass was explained as zero or low adsorption of phenolic compounds; a hazel biomass as high adsorption. On grape-skin agar 11% of the strains produced a white biomass, 82% grey, and only 7% hazel; on grape-seed agar, 14% white, 65% grey, and 21% hazel. A few strains showed a different

adsorption degree between phenolic compounds from skins and from seeds. To check the correspondence between the biomass colour and the activity on phenolic compounds, the Folin-Ciocalteu index of the biomass of 11 strains that showed the most widely differing behaviour was determined. The differences between white and hazel strains were highly significant ($P < 0.001$), thus demonstrating that the hazel strains can adsorb more phenolic compounds than the white strains. This screening method allows the differentiation of yeasts regarding their ability to interact with phenolic compounds; times and costs of testing are notably low.

5. Caridi A., Cufari A., Lovino R., Palumbo R., Tedesco I. 2003. Influence of yeast on polyphenol composition of wine. First Congress of European Microbiologists "FEMS 2003", 29 June - 3 July 2003, Ljubljana, Slovenia (accepted).

In red wine production, the type and quantity of polyphenols play a major role in wine quality. Anthocyanins, flavonols, catechins and other flavonoids contribute to the different characteristics of wine, particularly color and astringency; in addition, recently it has been shown that they possess a wide range of antioxidant and pharmacological effects. The objective of this research was to examine the influence of yeast used for winemaking on the type and quantity of phenolic compounds, elucidating possible relationships with the antioxidant capacity of wine. Two strains of *Saccharomyces*, previously selected for enology and for their different interaction with grape polyphenols, were employed. A sample of *Gaglioppo* must from Calabrian black grapes was utilized. This variety was employed because it has limited anthocyan content so it requires

careful handling to protect the phenolic compounds. Winemakings were carried out in triplicate using stainless steel vessels of 600 l, containing about 400 l of must with grape skins and seeds. Twenty-seven physicochemical and polyphenolic parameters were determined in the red wines produced. Among the polyphenolic parameters, very significant ($p < 0.01$) differences were observed for color intensity, total polyphenols, and non-anthocyanic flavonoids. Moreover, significant ($p < 0.05$) differences were observed for OD_{520} and monomeric anthocyanins. This research elucidates for the first time how yeasts interact with specific polyphenols, so varying the wine concentration of these compounds. This behavior modifies significantly the physicochemical and antioxidant characteristics of wines and, probably, also the sensorial and volatile constituents.

IV. Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorobyovy Hills, Moscow 119899, Russia. Communicated by I.Yu.Chernov <yes@soil.msu.ru>.

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

1. Polyakova A.V., Chernov I.Yu. 2002. A new yeast species, *Candida aurita* sp. nov., from oligotrophic bogs of Western Siberia. *Microbiology* 71:331-334.

Five anamorphous yeast strains of ascomycetous affinity with a specific mode of budding were isolated from high bog soils of the Bakcharskoe Bog (Tomsk region of Russia). According to their morphological and physiological properties, these strains belong to the genus *Candida* but differ from all

species described previously. The level of DNA-DNA homology with species similar in the assimilation spectrum was as low as 7%. Based on these data, the new species *Candida aurita* sp. nov. is described.

2. Bab'eva I.P., Lisichkina G.A., Reshetova I.S., Danilevich V.N. 2002. *Mrakia curviuscula* sp. nov.: a new psychrophilic yeast from forest substrates. *Microbiology* 71:449-455.

Seven strains with similar characteristics from the laboratory collection of yeasts isolated from forest substrates collected in the central part of European Russia corresponded to none of the known yeast species. Based on the study of their life

cycle, physiological characteristics, and the nucleotide composition of their DNA and taking into account the data of PCR analysis with universal primers, the strains were ascribed to a new psychrophilic yeast species, *Mrakia curviuscula* sp. nov.

3. Glushakova A.M., Zheltikova T.M., Chernov I.Yu. 2003. Yeast communities in home dust and sources of their formation. *Microbiology* (in press, in Russian).

Yeasts are a constant part of the home dust community together with bacteria, mycelial fungi and some species of microarthropods. We managed to identify 15 yeast species in dust samples from 38 Moscow flats. They belong to the following genera: *Candida*, *Cryptococcus*, *Debaryomyces*, *Rhodotorula*, *Sporobolomyces*, and *Trichosporon*. Most frequent were typical epiphytic species able to a long-time inactive persistence. Among them *Cryptococcus diffluens* and *Rhodotorula mucilaginosa* were the most abundant. Room plants are supposed to be a direct source of the dust yeasts community, though they are less

inhabited than natural ones. Besides typical epiphytic yeasts facultative pathogenic ones are present in dust. They are *Candida catenulata*, *C. guilliermondii*, *C. haemulonii*, *C. rugosa*, and *C. tropicalis*. All these species are regularly mentioned as agents of candidiasis. We didn't manage to show true dependence between the abundance of different yeast species in dust and the peculiarities of the flats researched. No dependence was demonstrated between yeast community and the presence of patients suffering from atopic dermatitis.

4. Maximova I.A., Chernov I.Yu. 2003. Structure of yeasts communities in forest ecosystems. *Microbiology* (in press, in Russian).

The results of lasting investigation of yeasts living in soil, on plant surface and plant residues in subboreal forests of European part of Russia quantity and species composition have been summarised in this paper. Yeasts quantity and species diversity have shown to decrease in range of substrates equivalent to succession in plant residues decomposition. There is a certain number of predominant yeast species adapted to biogeocenotic layer for the each stage of plant residues decomposition succession. The yeast population in forests was

shown to be characterized by a higher species diversity compared to the biogeocenosis of other geographic zones. It reveals in higher species quantity found in similar number of substrates without clear dominated species on biogeocenotic stage and higher yeast population differentiation in habitats. The features of yeast populations in forests are the high diversity of ascomycetous affinity, anamorph stages of *Taphrinales* and *Tremellales* and the presence of typical pedobiont species except *Lipomyces* sp.

5. Glushakova A.M., Chernov I.Yu. 2003. Seasonal dynamics of yeast communities on leaves of *Oxalis acetosella* L. *Microbiology* (in press, in Russian).

The yeast population was analyzed on all the year round green leaf plant *Oxalis acetosella* L. during a year. The number and species diversity of epiphytic yeasts were shown to change regularly in a yearly circle. Yeasts were practically absent on young spring leaves. But on mature ones having finished active growth period their number gradually increased during autumn. The maximum number was found in the November-February period after the snow coverage stabilized. It began to decrease in spring again. The greatest species diversity was also observed in autumn time when more species were found in a sample and there were no clear dominants. It was the first time, when regular seasonal trends of abundance for some epiphytes were disclosed.

Clear autumn increase was observed for *Cystofilobasidium capitatum*, *Rhodotorula fujisanensis*, *Leucosporidium scottii*, and *Cryptococcus flavus*. At the same time the maximum abundance of the most common species on leaves *C. laurentii* was in January. Relative abundance of the most typical "red yeasts" species *Rhodotorula glutinis* and *Sporobolomyces roseus* reliably didn't change during a year. The relative abundance of the eurybiont species *C. albidus* was somewhat variable per month but demonstrated no regularity. The results obtained demonstrate the necessity of analyzing plant material during the whole growth period to reveal the taxonomic specificity of yeast population on definite plant species.

V. Russian Collection of Microorganisms, Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino 142292, Russia. Communicated by W.I. Golubev.

Recent publications.

1. Golubev, W.I. 2003. The genus *Cryptococcus* Vuillemin and its type species *Cryptococcus neoformans* (Sanfelice) Vuillemin. In: An Update on Systematics and Nomenclature of Fungi (Dyakov Y.T., Sergeev Y.V., eds.) Moscow, Natl. Acad. Mycol. 357-381 (In Russian).

The taxonomy of the yeast genus *Cryptococcus* has undergone profound changes over the past 20 years which led to the present demarcation of this anamorphic taxon with only basidiomycetous affinities. Major developments, from ultra-

structural to molecular studies, are briefly reviewed. Considerable attention has been given to *Cr. neoformans* as one of the most common opportunistic pathogens in patients with AIDS.

2. Golubev, W.I., Pfeiffer, I., Churkina, L.G. & Golubeva, E.W. 2003. Double-stranded RNA viruses in a mycotoxinogenic strain of *Cystofilobasidium infirmominiatum*. *FEMS Yeast Res.* 3:63-68.

The viral particles (about 30 nm in diam.) that contain dsRNAs (2.0 and 6.3 kbp) encapsidated by a coat of protein were detected in a mycotoxin-secreting strain of *Cystofilobasidium infirmominiatum* isolated from plants in an oak forest (Moscow region). The mycotoxin with a molecular mass above 15 kDa is fungicidal (maximum activity at pH 4.5) and active mainly against

some species of the Cystofilobasidiales and Filobasidiales (*Cryptococcus aerius* clade). Curing by incubation at elevated temperature resulted in the concomitant loss of dsRNAs and mycotoxinogenic activity, and cured derivatives became sensitive to the mycotoxin produced by the parent strain.

3. Golubev, W.I., Gadanho, M., Sampaio, J.P. and Golubev, N.W. 2003. *Cryptococcus nemorosus* sp. nov. and *Cryptococcus perniciosus* sp. nov., related to *Papiliotrema* Sampaio et al. (Tremellales). *Int J Syst Evol Microbiol* 53:905-911.

Three mycocinogenic strains representing the genus *Cryptococcus* were isolated on glucuronate agar from plants and turf collected in the Prioksko-terrasny biosphere reserve (Russia). These isolates fit the standard description of *Cryptococcus laurentii*, but differ from its type strain in both their mycocin-sensitivity profiles and the killing patterns of their mycocins. Sequence analyses of the D1/D2 domain of the 26S rDNA and of the internal transcribed spacer region confirmed

that these isolates represent two novel species, for which the names *Cryptococcus nemorosus* sp. nov. (type strain VKM Y-2906) and *Cryptococcus perniciosus* sp. nov. (type strain VKM Y-2905) are proposed. Morphological, physiological and biochemical characteristics, as well as mycocinotyping and molecular analyses, show a close affinity these two novel anamorphic species and the teleomorphic species *Papiliotrema bandonii* (Tremellales).

4. Kulakovskaya, T.V., Kulakovskaya, E.V. and Golubev, W.I. 2003. ATP leakage from yeast cells treated by extracellular glycolipids of *Pseudozyma fusiformata*. *FEMS Yeast Res.* 3:401-404.

The ustilaginaceous yeast *Pseudozyma fusiformata* secreted glycolipids which were lethal to many yeast-like fungi more active at pH of about 4.0 and in the temperature range of 20-30°C. Purified glycolipids enhanced non-specific permeability of the cytoplasmic membrane in sensitive cells, which resulted in ATP leakage and susceptibility of the cells to staining with bromocresol purple. Cells of *Saccharomyces cerevisiae* lost the

ability to acidify the medium. Basidiomycetous yeasts were more sensitive to the glycolipids than ascomycetous ones. The minimal effective glycolipid concentration was 0.13 and 0.26 mg/ml for *Cryptococcus terreus* and *Filobasidiella neoformans*, while for *Candida albicans* and *Saccharomyces cerevisiae* it was 1.0 and 2.6 mg/ml.

VI. Department of Biology, Faculty of Medicine, Masaryk University, Jostova 10, 66243 Brno, Czech Republic. Communicated by M. Kopecká.

Papers published in journals.

1. Kopecká M., Gabriel M., Takeo K., Yamaguchi M., Svoboda A., Hata K. 2003. Analysis of microtubules and F-actin structures in hyphae and conidia development of the opportunistic human pathogenic black yeast *Aureobasidium pullulans*. *Microbiology (UK)* 149:865-876.
2. Kopecká M., Gabriel M., Takeo K., Yamaguchi M., Svoboda A., Hata K. 2003. Development of antifungals against black yeast. *Microbiology Today* 30:87-87.

Abstracts of lectures.

3. M. Gabriel M., Kopecká M., Takeo K., Yamaguchi M., Svoboda A., Nakase T., Sugita T. 2003. The Cytoskeleton during the Conidiogenesis. In: XI. Cytoskeletální klub, Vranovská Ves 23-25, 4:18.
4. David M., Gabriel M., Kopecká M. 2003. The first findings on the microtubule cytoskeleton in *Malassezia pachydermatis*. In: XI. Cytoskeletální klub, Vranovská Ves 23-25, 4:21.

VII. Department of Food Sciences, via Marangoni 97, 33100 Udine, Italy. Communicated by M. Manzano <marisa.manzano@dsa.uniud.it>.

Recent publication.

1. G. Santovito, B. Salvato, M. Manzano and M. Beltramini. 2002. Copper adaptation and methylotrophic metabolism in *Candida boidinii*. *Yeast* 19:631-640.

The importance of the antioxidant enzyme superoxide dismutase (CuZnSOD) in metabolic switch from normotrophic to methylotrophic conditions was studied in the facultative methylotrophic yeast *Candida boidinii*. Copper adaptation was performed to qualify *C. boidinii* as a suitable cellular system to study the effect of induction of CuZnSOD, and other biochemical components along the copper detoxification system, on the methanol adaptation. Copper adaptation results in the induction of CuZnSOD, peroxidase activity as well as of glutathione. The effects at metabolic level of the exposure to both copper and methanol were also studied: the results suggest that the effect on antioxidant enzyme levels as a function of the change of trophic condition are predominant with respect to the effects of copper administration. Thus, the methanol-dependent induction of such enzymes is likely to provide a sufficient protection for the cells

against toxic effects depending on copper administration. Administration of copper under methylotrophic conditions decreases the growth rate in spite of the high levels of antioxidant enzymes that are elicited by copper treatment. The adaptation to methanol metabolism was studied also after methanol-independent induction of CuZnSOD, glutathione and catalase levels, obtained by exposure to high copper concentrations in glucose-containing medium. The metabolic changes induced by copper are persistent over several re-inocula in normo-cupric glucose medium, thus allowing the study of the glucose-to-methanol switch on cells exhibiting high levels of antioxidant enzyme activities. Under such conditions the lag time observed during the transition from normotrophic to methylotrophic conditions is strongly reduced.

VIII. Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@wur.nl>.

The following papers appeared since December 2002.

1. Middelhoven, W.J. 2003. Identification of clinically relevant *Trichosporon* species. *Mycoses* 46:7-11.

A dichotomous identification key to pathogenic species of the basidiomycetous genus *Trichosporon* Behrend is provided. It is based on growth tests with carbon sources not traditionally used in yeast taxonomy, viz. uric acid, ethylamine, L-4-hydroxyproline, tyramine and L-phenylalanine as sources of carbon and nitrogen, and polygalacturonate, quinate,

4-ethylphenol, 2,3-dihydroxybenzoate and orcinol as carbon sources. Of the standard growth tests, assimilation of L-rhamnose and the maximum growth temperature proved to be useful. In addition to medically relevant species, other species able to grow at 37 °C were treated as well.

2. Middelhoven, W.J. and Kurtzman, C.P. 2003. Relation between phylogeny and physiology in some ascomycetous yeasts. *Antonie van Leeuwenhoek* 83:69-74.

The question of whether yeasts with similar physiological properties are closely related has been examined using recently published phylogenetic analyses of 26S domain D1/D2 rDNA nucleotide sequences from all currently recognized ascomycetous yeasts. When apparently unique metabolic pathways are examined, some relationships between physiology and rDNA phylogeny are evident. Most *Candida* and *Pichia* species that are able to assimilate methanol as the sole carbon source are in a clade delimited by *C. nanospora* and *C. boidinii*. Exceptions are *P. capsulata* and *P. pastoris* which are phylogenetically separated from the other methanol-assimilating yeasts. Yeasts subject to the petite mutation, resulting in respiratory deficiency, belong to three different clades, viz. a *Saccharomyces* clade delimited by *S. cerevisiae* and *S. rosini*, the *Dekkera/Brettanomyces* clade, and some *Schizosaccharo-*

myces species ('Archiascomycete' clade). However, petite mutants were also found in *Zygosaccharomyces fermentati* and some other more distantly related species. Yeasts able to assimilate n-hexadecane, uric acid or amines as sole carbon source are broadly distributed over the ascomycetous phylogenetic tree. However, species that assimilate adenine as sole carbon source are closely related. Most of these species also assimilated glycine, uric acid, n-hexadecane, putrescine and branched-chain aliphatic compounds such as isobutanol, leucine and isoleucine. Among the Saccharomycetales, species utilizing all or the great majority of these eight compounds are in the *Stephanoascus/Arxula/Blastobotrys* clade. *Candida blankii*, which is distantly related to this clade, proved to be an exception and assimilated six of eight of these compounds.

IX. 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. Effect of increased glycerol production on yeast activity and wine bouquet. *Vinohrad* 40:17-18.

Increasing the formation of glycerol to 10-20 g/L might positively influence properties and partially wine aroma. Reactions of wine yeasts may lead to acetaldehyde and acetic acid formation. Genetic manipulations intervene into the yeast metabolism and prevent the formation of these compounds supposed that genetic background of yeasts was changed. when manipulated yeast strains are used a strict regime of behaviour

control of the yeasts has to be applied. Though genetically manipulated organisms have to follow national and international laws prior to be authorized for human needs or from the environmental viewpoint, wine researchers and experts in the wine industry should realize that wine consumers ought to be informed on the benefit of gene technics and technology and on their harmlessness for human health.

2. E. Minárik. 2002. Importance of enzymes in wine production. *Vinič a Víne* 2: (Suppl. 7, in Slovak).

Grape mash treatment by enzyme preparations shows important technological advantage in improving pressing conditions and quick self clarification by settling. The sediment volume is, however, larger. Alcoholic and malolactic fermentations require some more time compared with grape

must without enzyme treatment (smaller inner surface of fermenting medium). Sensory properties of resulting wines are usually raised though the aroma rested unchanged as the terpene content is not increased by the treatment.

3. E. Minárik. 2002. Wine yeast nutrients in red wine production. *Vinič a Víne* 2: (Suppl. 8, in Slovak).

Providing adequate nutrients to accomplish complete fermentation and first class red wine quality is an important factor. A new preparation Opti Red® containing d-amino acids, vitamins, minerals and other biofactors provides complete

alcoholic fermentation without residual sugar in the wine. Opti Red® is now available on the European market. The dose of 30 g/hL is advised by the producer (Lallemand, Montréal).

4. E. Minárik. 2003. Volatile sulphur compounds in grape wines. *Vinič a Víne* 3: Suppl. 7, in Slovak).

Sulphur compounds in wine do not belong to the best known characteristics that are usually completely controlled. Although the compounds have sometimes pleasant properties in low concentration, they cause unpleasant properties at higher levels. Dimethyl sulphide belongs to the most widespread sulphur compounds occurring in wine. 10-20 µg/L is not detectable by smell or taste. Levels over 20 µg/L display a

cabbage mouth feeling or odour. Methanthiol (methylmercaptane) occurs more often in white wine. Ethanthiol (ethylmercaptane) prevails on the other hand in red wine. Practical aspects are elucidated: yeast nutrition and selected starter strains may decrease the formation of volatile sulphur compounds (hydrogen sulphide included).

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

The following are publications for 2003 or papers in press.

1. Naumova E.S., Korshunova I.V., Jespersen L., Naumov G.I. 2003. Molecular genetic identification of *Saccharomyces sensu stricto* strains from African sorghum beer. *FEMS Yeast Research* 3:177-184.
2. Naumova E.S., Bulat S.A., Mironenko N.V., Naumov G.I. 2003. Differentiation of six sibling species in the *Saccharomyces sensu stricto* complex by multilocus enzyme electrophoresis and UP-PCR analysis. *Antonie van Leeuwenhoek* 83:155-166.
3. Spirek M., Jun Yang, Groth C., Petersen R.F., Langkjaer R.B., Naumova E.S., Sulo P., Naumov G.I., Piskur J. 2003. High-rate evolution of *Saccharomyces sensu lato* chromosomes. *FEMS Yeast Research*. 3:363-373.
4. Naumova E.S., Gazdiev D.O., Naumov G.I. 2003. Unusual molecular genetic heterogeneity of the yeast *Zygosaccharomyces californica*. *Dokl. Biol. Sciences*, 389:123-126.
5. Naumova E.S., Korshunova I.V., Naumov G.I. 2003. Molecular analysis of alpha-galactosidase *MEL* genes of the *Saccharomyces sensu stricto* complex. *Molecular Biology (Moscow)* 37(5): (in press).

XI. Department of Microbiology, Technical University of Denmark, DTU-301, DK-2800 Lyngby, Denmark. Communicated by J. Piškur <jp@im.dtu.dk>.

Recent publications.

1. R.B. Langkjær, P. F. Cliften, M. Johnston & J. Piškur. 2003. Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. *Nature* 421:848-852.

Gene redundancy has been observed in yeast, plant and human genomes, and is thought to be a consequence of whole-genome duplications. Baker's yeast, *Saccharomyces cerevisiae*, contains several hundred duplicated genes. Duplication(s) could have occurred before or after a given speciation. To understand the evolution of the yeast genome, we analysed orthologues of some of these genes in several related yeast species. On the basis of the inferred phylogeny of each set of genes, we were able to deduce whether the gene duplicated and/or specialized

before or after the divergence of two yeast lineages. Here we show that the gene duplications might have occurred as a single event, and that it probably took place before the *Saccharomyces* and *Kluyveromyces* lineages diverged from each other. Further evolution of each duplicated gene pair - such as specialization or differentiation of the two copies, or deletion of a single copy - has taken place independently throughout the evolution of these species.

2. Z. Gojkovic, L. Rislund, B. Andersen, M. P.B. Sandrini, P.F. Cook, K.D. Schnackerz & J. Piškur. 2003. Dihydropyrimidine amidohydrolases and dihydroorotases share the same origin and several enzymatic properties. *Nucleic Acids Res.* 31: 1683-1692.

Slime mold, plant and insect dihydropyrimidine amidohydrolases (DHPases, EC 3.5.2.2), which catalyze the second step of pyrimidine and several anticancer drug degradations, were cloned and shown to functionally replace a defective DHPase enzyme in the yeast *Saccharomyces kluyveri*. The yeast and slime mold DHPases were over-expressed, shown to contain two zinc ions, characterized for their properties and compared to those of the calf liver enzyme. In general, the kinetic parameters varied widely among the enzymes, the mammalian DHPase having the highest catalytic efficiency. The ring opening was catalyzed most efficiently at pH 8.0 and

competitively inhibited by the reaction product, N-carbamyl-b-alanine. At lower pH values DHPases catalyzed the reverse reaction, the closing of the ring. Apparently, eukaryote DHPases are enzymatically as well as phylogenetically related to the de novo biosynthetic dihydroorotase (DHOase) enzymes. Modelling studies showed that the position of the catalytically critical amino acid residues of bacterial DHOases and eukaryote DHPases overlap. Therefore, only a few modifications might have been necessary during evolution to convert the unspecialized enzyme into anabolic and catabolic ones.

XII. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nußdorfer Lände 11, A-1190 Vienna, Austria. Communicated by H. Prillinger <hansjoerg.prillinger@boku.ac.at>.

The following publication is accepted in *Mycological Progress*.

- 1 K. Bacigálová, K. Lopandic, M.G. Rodrigues, A. Fonseca, M. Herzberg, W. Pinsker & H. Prillinger. Phenotypic and genotypic identification and phylogenetic characterisation of *Taphrina* fungi on alder.

All *Taphrina* species are dimorphic with a mycelium stage biotrophic on vascular plants and a saprophytic yeast stage. European species of *Taphrina* on *Alnus* species (Betulaceae) were identified using morphological, physiological and molecular characteristics, the latter including determination of

PCR fingerprints and of nucleotide sequences from selected nuclear ribosomal DNA regions. PCR fingerprinting gives a good overview of species identification, as do nucleotide sequences, which in addition, help to clarify phylogenetic relationships. *Taphrina alni* is a homogeneous species that

exhibited more than 50% similarity in PCR fingerprinting with three different primers. Morphologically, it produces tongue-like outgrowths from female catkins of *Alnus incana*. *Taphrina robinsoniana* from *A. rugosa* and *A. serrulata* in North America is phylogenetically closely related to *T. alni*, but the two species could be separated by their PCR fingerprints, partial sequences of 26S rDNA (D1/D2) and ITS1/ITS2 sequences. *T. epiphylla* and *T. sadebeckii* are two phylogenetically closely related species. *T. epiphylla* causes witches brooms in crowns of *A. incana*. In addition, *T. epiphylla* forms slightly yellow white-grey leaf spots in midsummer on *A. incana*. Yellow white-grey leaf spots up to 10 mm on *A. glutinosa* are

characteristic for *T. sadebeckii*. Both species can be separated well by PCR fingerprinting. Different from *T. epiphylla*, *T. sadebeckii* is genotypically more heterogeneous. Only two out of three different primers showed similarity values above 50% in different European strains of *T. sadebeckii*. Although genetic variability was not detected in complete sequences of the 18S ribosomal DNA of *T. sadebeckii*, ITS1/ITS2 sequences appeared to be more heterogeneous too. *Taphrina tosquetii* is a genotypically homogeneous species causing leaf curl on *Alnus glutinosa*. It was not possible to distinguish the yeast phases from different *Taphrina* species on *Alnus* using morphological and physiological characteristics only.

XIII. CNRS, UMR 5503, 5 rue Paulin Talabot. 31106 Toulouse Cedex 01, France. Communicated by P. Strehaiano <Pierre.Strehaiano@ensiacet.fr>.

In the area of the industrial use of yeast, the Microbial Systems Engineering team of the Chemical Engineering Laboratory (CNRS UMR 5503) pursues four avenues of studies.

a. Utilization of immobilised yeast cells in winemaking.

Our lab in association with the Proenol Company (Portugal) has developed a new way to obtain dry cells of yeasts entrapped in alginate gel. These entrapped cells are used to solve some problems met in winemaking such as sparkling

wine making, treatment of stuck fermentations, deacidification of musts and wine by entrapped cells of *Schizosaccharomyces pombe*, control of fermentation of sweet wines. The following papers have been published on that subject.

1. Silva S., Ramon Portugal F., Silva P., Abreu S., Teixeira da Silva M. & Strehaiano, P. 2003. Malic acid consumption by dry immobilized cells of *Schizosaccharomyces pombe*. Am. J. Enol. Vitic. 54:50-55.
2. Silva S., Ramon Portugal F., Silva P., Teixeira Da Silva M. et Strehaiano P. 2002. Production of smooth wine with immobilized yeast cells (Vinification de vin moelleux en utilisant des levures incluses). Revue des Œnologues 104:23-26.
3. Silva S., Ramon Portugal F., Silva P., Abreu S., Teixeira Da Silva M. et Strehaiano P. 2002. Malolactic fermentation of white and red musts with *Schizosaccharomyces pombe* immobilized in dry alginate beads (Démalication de moûts blancs et rouges par des levures *Schizosaccharomyces pombe* incluses dans des billes d'alginate sèches). Rev. Fr. Oenol. 196:18-22.
4. Silva S., Ramon Portugal F., Silva P., Teixeira Da Silva M. et Strehaiano P. 2002. The use of immobilized yeast cells for the treatment of stuck fermentations (Utilisation de levures incluses pour le traitement des arrêts de fermentations). J. Int. Sci. Vigne Vin 3:161-167.
5. Strehaiano P., Taillandier P., Silva S. et Nepveu F. 2002. Yeasts and the control of acidity in musts and wines (Levures et maîtrise de l'acidité des moûts et des vins). Revue des Œnologues 105:23-27.

b. Behaviour of yeast populations.

b1. Mathematical modelling of Killer effect.

The killer effect is well known from a fundamental point of view. However, its actual efficiency in industrial fermentations is not well recognized. Our objective is to obtain a good description and modelling of the behaviour of mixed

cultures (killer plus sensitive yeast) in relation to the initial ratio of these two kinds of cells. On this subject the following papers have been published.

6. S. Pommier, C. Albasi, J.P. Riba, M.L. Delia. 2002. A new membrane tool for the quantification of microorganisms interaction dynamics: application to yeast killer system. Desalination 149:243-245.

b2. Behaviour of *Brettanomyces* in the fermentation industry.

Contaminations of alcoholic fermentation by *Brettanomyces* strains are quite frequent in winemaking. But very little is known on the growth kinetics of this yeast and its

sensitivity to the environmental conditions. On this subject the following papers have been published.

7. Aguilar Uscanga M.g., Delia M.L., Strehaiano P. 2003. *Brettanomyces bruxellensis* : Effect of oxygen on growth and acetic acid production. Appl. Microbiol. Biotechnol. 61:157-162.
8. Medawar W., Strehaiano P., Delia M.L. 2003. Yeast growth: Lag phase modelling in alcoholic media. Food Microbiol. (in press).

c. Production of hybrid yeast.

In winemaking some interest arises for some "hybrid" strains of *S. cerevisiae* and *S. uvarum*. In order to produce these strains it is necessary to define their growth characteristics and

to analyse the best way for producing them in relation to their expected fermentative abilities.

9. A. Serra, P. Strehaiano, P. Taillandier. 2003. Characterization of the metabolic shift of *Saccharomyces bayanus* var. *uvarum* by continuous aerobic culture. *Appl. Microbiol. Biotechnol.* (in press).

d. Yeast and lactic acid bacteria interactions.

The antagonism between *S. cerevisiae* and *Oenococcus oeni* is studied in order to improve the control of the malolactic fermentation. The focus is on the population dynamics during

fermentations. On this subject the following papers have been published.

10. C. Albasi, P. Tataridis, P. Taillandier, P. Strehaiano. 2002. A new tool for the quantitative study of microbial interactions in liquid media: application to lactic bacteria of wine (Un nouvel outil d'étude quantitative des interactions microbiennes en milieu liquide: application aux bactéries lactiques oenologiques). *Sciences des aliments* 22:189-198.
11. P. Taillandier, P. Tataridis, C. Albasi, P. Strehaiano 2002. A study of antagonisms between yeasts and lactic acid bacteria in the control of malolactic fermentation (Etude des antagonismes entre levures et bactéries lactiques et entre souches de bactéries lactiques pour la maîtrise de la fermentation malolactique). *Revue des Oenologues* 105:37-42.

XIV. Section of Fungal Genomics, Wageningen University, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands. Communicated by H. Visser <hans.visser@wur.nl>.

The following papers have been published recently.

1. H. Visser, C.A.G.M. Weijers, A.J.J. van Ooyen & J.C. Verdoes. 2002. Cloning, characterization and heterologous expression of epoxide hydrolase-encoding cDNA sequences from yeasts belonging to the genera *Rhodotorula* and *Rhodospiridium*. *Biotechnol. Lett.* 24:1687-1694.

Epoxide hydrolase-encoding cDNA sequences were isolated from the basidiomycetous yeast species *Rhodospiridium toruloides* CBS 349, *Rhodospiridium toruloides* CBS 14 and *Rhodotorula araucariae* CBS 6031 in order to evaluate the molecular data and potential application of this type of enzymes. The deduced amino acid sequences were similar to those of the known epoxide hydrolases from *Rhodotorula glutinis* CBS 8761,

Xanthophyllomyces dendrorhous CBS 6938 and *Aspergillus niger* LCP 521, which all correspond to the group of the microsomal epoxide hydrolases. The epoxide hydrolase encoding cDNAs of the *Rhodospiridium* and *Rhodotorula* species were expressed in *Escherichia coli*. The recombinant strains were able to hydrolyze *trans*-1-phenyl-1,2-epoxypropane with high enantioselectivity.

2. H. Visser, M. de Oliveira Villela Filho, A. Liese, C.A.G.M. Weijers & J.C. Verdoes. 2003. Construction and Characterisation of a Genetically Engineered *Escherichia coli* Strain for the Epoxide Hydrolase-Catalysed Kinetic Resolution of Epoxides. *Biocatal. Biotrans.* 21:33-40.

The *Rhodotorula glutinis* epoxide hydrolase, Eph1, was produced in the heterologous host *Escherichia coli* BL21(DE3) in order to develop a highly effective epoxide hydrolysis system. A 138-fold increase in Eph1 activity was found in cell extracts of the recombinant *E. coli* when compared to cell extracts of *Rhodotorula glutinis*, despite the formation of Eph1 inclusion bodies. Optimisation of cultivation conditions and co-expression of molecular chaperones resulted in a further increase in activity

and a reduction of the inclusion bodies formation, respectively. Compared to *Rhodotorula glutinis* cells and cell extracts, a total increase in Eph1 activity of over 200 times was found for both *Escherichia coli* cells and crude enzyme preparations of these cells. The improved conditions for recombinant Eph1 production were used to demonstrate the Eph1-catalysed kinetic resolution of a new Eph1 substrate, 1-oxaspiro[2.5]octane-2-carbonitrile.

3. H. Visser, J.C. Verdoes & A.J.J. van Ooyen. 2003. Fermentation and carotenoid analysis of the yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). Pages 309-313, *In: Non-Conventional Yeasts in Genetics, Biochemistry and Biotechnology*. K. Wolf, K. Breunig, G. Barth (Eds.), Springer-Verlag Berlin Heidelberg.
 4. J.C. Verdoes, H. Visser and A.J.J. van Ooyen. 2003. Metabolic engineering of the carotenoid biosynthetic pathway in *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). Pages 315-322, *In: Non-Conventional Yeasts in Genetics, Biochemistry and Biotechnology*. K. Wolf, K. Breunig, G. Barth (Eds.), Springer-Verlag Berlin Heidelberg.
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XV. University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Room 705, Piscataway, New Jersey 08854-5635, U.S.A. Communicated by M.J. Leibowitz.

Recent publication.

Y. Zhang, Z. Li, D.S. Pilch & M.J. Leibowitz. 2002. Pentamidine inhibits catalytic activity of group I intron Ca.LSU by altering RNA folding. *Nucl. Acids Res.* 30:2961-2971.

The antimicrobial agent pentamidine inhibits the selfsplicing of the group I intron Ca.LSU from the transcripts of the 268 rRNA gene of *Candida albicans*, but the mechanism of pentamidine inhibition is not clear. We show that preincubation of the ribozyme with pentamidine enhances the inhibitory effect of the drug and alters the folding of the ribozyme in a pattern varying with drug concentration. Pentamidine at 25 μ M prevents formation of the catalytically active F band conformation of the precursor RNA and alters the ribonuclease T1 cleavage pattern of Ca.LSU RNA. The effects on cleavage

suggest that pentamidine mainly binds to specific sites in or near asymmetric loops of helices P2 and P2.1 on the ribozyme, as well as to the tetraloop of P9.2 and the loosely paired helix P9, resulting in an altered structure of helix P7, which contains the active site. Positively charged molecules antagonize pentamidine inhibition of catalysis and relieve the drug effect on ribozyme folding, suggesting that pentamidine binds to a magnesium binding site(s) of the ribozyme to exert its inhibitory effect.

XVI. Department of Applied Microbiology, Chemical Center, University of Lund, P.O. Box 124, S-22100 Lund, Sweden. Communicated by B. Hahn-Hägerdal.

Research activities on yeast at the department of Applied Microbiology, Lund University, Sweden, concern (i) the construction and development of pentose fermenting strains of *S. cerevisiae*, and (ii) stereo-selective bioconversions with yeast as reducing biocatalyst.

Xylose fermentation. A bi-functional enzyme with xylose reductase (XR) and xylitol dehydrogenase (XDH) activity (Anderlund et al. 2001) has been expressed in *S. cerevisiae*.

In collaboration with the New University of Lisbon, Portugal, a high capacity xylose transporter from *C. intermedia* has been characterized (Gárdonyi et al. 2003) and the control of xylose transport on xylose consumption has been established (Gárdonyi et al. 2003).

In collaboration with the University of Stellenbosch, South Africa, mutants of the xylose isomerase (XI) enzyme from *Thermus thermophilus* have been generated (Lönn et al. 2002) and the influence of the mutated enzymes on xylose fermentation in *S. cerevisiae* has been investigated (Lönn et al. 2003).

In strains of *S. cerevisiae* expressing XR and XDH the oxidative pentose phosphate pathway flux has been modulated to reduce NADPH-dependent reduction of xylose to xylitol (Jeppsson et al. 2002; Jeppsson et al. 2003). This resulted in

enhanced ethanol yield.

A new CRE/loxP expression vector for repeated genomic integration in *S. cerevisiae* has been developed (Johansson and Hahn-Hägerdal, 2002a). It has been used to elucidate the control of the pentose phosphate pathway over xylose consumption (Johansson and Hahn-Hägerdal, 2002b).

In collaboration with the University of Stellenbosch, South Africa, mutant strains of xylose fermenting *S. cerevisiae* with superior growth and fermentation characteristics have been developed (Wahlbom et al. 2003a) and the molecular basis for the altered properties have been investigated (Wahlbom et al. 2003b).

Stereo-selective bioconversion. Stereo-selective reducing yeast biocatalysts have been isolated in collaboration with the University of the Free State, South Africa (Botes et al. 2002). Baker's yeast *S. cerevisiae* has been metabolically engineered to improve the stereo-selective bio-reduction of bicyclo[2.2.2]octane-2-6dione (Katz et al. 2002).

Literature cited:

1. Anderlund, M., Rådström, P., Hahn-Hägerdal, B. 2001. Expression of bifunctional enzymes with xylose reductase and xylitol dehydrogenase activity in *Saccharomyces cerevisiae* alter product formation during xylose fermentation. *Metabolic Engineering* 3:226-235.
2. Botes, A.L., Harvig, D., van Dyk, M.S., Sarvary, I., Frejd, T., Kats, M., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F. 2002. Screening of yeast species for the stereo-selective reduction of bicyclo[2.2.2]octane-2-6dione. *J Chem Soc Perkin Trans* 1:1-5.
3. Gardonyi, M., Hahn-Hägerdal, B. 2003. The *Streptomyces rubiginosus* xylose isomerase is misfolded when expressed in *Saccharomyces cerevisiae*. *Enzyme Microb Tech* 32:252-259.
4. Gardonyi, M., Jeppsson, M., Liden, G., Gorwa-Grauslund, M.F., Hahn-Hägerdal B. 2003. Control of xylose consumption by xylose transport in recombinant *Saccharomyces cerevisiae*. *Biotechnol Bioeng* 82:818-824.
5. Gardonyi, M., Österberg, M., Rodrigues, C., Spencer-Martins, I., Hahn-Hägerdal, B. 2003. High capacity xylose transport in *Candida intermedia* PYCC 4715. *FEMS Yeast Res* 3:45-52.
6. Jeppsson, M., Johansson, B., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F. 2002. Reduced Oxidative Pentose Phosphate Pathway Flux in Recombinant Xylose-Utilizing *Saccharomyces cerevisiae* Strains Improves the Ethanol Yield from Xylose. *Appl Environ Microbiol* 68:1604-1609.

7. Jeppsson, M., Träff, K., Johansson, B., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F. 2003. Effect of enhanced xylose reductase activity on xylose consumption and product distribution in xylose-fermenting recombinant *Saccharomyces cerevisiae*. FEMS Yeast Res 3:167-175.
8. Johansson, B., Hahn-Hägerdal, B. 2002a. Over-production of pentose phosphate pathway enzymes using a new CRE-*loxP* expression vector for repeated genomic integration in *Saccharomyces cerevisiae*. Yeast 19:225-231.
9. Johansson, B., Hahn-Hägerdal, B. 2002b. The non-oxidative pentose phosphate pathway controls the fermentation rate of xylulose but not of xylose in *Saccharomyces cerevisiae* TMB3001. FEMS Yeast Res 2:277-282.
10. Katz, M., Sarvary, I., Frejd, T., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F. 2002. An improved stereoselective reduction of a bicyclic diketone by *Saccharomyces cerevisiae* combining process optimization and strain engineering. Appl Microbiol Biotechnol 59:641-648.
11. Lönn, A., Gárdonyi, M., van Zyl, W., Hahn-Hägerdal, B., Cordero Otero, R. 2002. Cold adaptation of xylose isomerase from *Thermus thermophilus* through random PCR mutagenesis. Eur J Biochem 269:157-163.
12. Lönn, A., Träff-Bjerre, K.L., Cordero Otero, R.R., van Zyl, W.H., Hahn-Hägerdal, B. 2003. Xylose isomerase activity influences xylose fermentation with recombinant *Saccharomyces cerevisiae* strains expressing mutated *xylA* from *Thermus thermophilus*. Enzyme Microb Tech 32:567-573.
13. Wahlbom, C.F., Cordero Otero, R.R., van Zyl, W.H., Hahn-Hägerdal, B., Jönsson, L.J. 2003. Molecular analysis of a *Saccharomyces cerevisiae* mutant with improved ability to utilize xylose shows enhanced expression of proteins involved in transport, initial xylose metabolism, and the pentose phosphate pathway. Appl Environ Microbiol 69:740-746.
14. Wahlbom, C.F., van Zyl, W.H., Jönsson L.J., Hahn-Hägerdal, B., Otero, R.R. 2003. Generation of the improved recombinant xylose-utilizing *Saccharomyces cerevisiae* TMB 3400 by random mutagenesis and physiological comparison with *Pichia stipitis* CBS 6054. FEMS Yeast Res 3:319-326.

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

Recent publications.

1. T. Wartmann, C. Bellebna, E. Böer, O. Bartelsen, G. Gellissen and G. Kunze. 2003. The constitutive *AHSB4* promoter – a novel component of the *Arxula adenivorans*-based expression platform. Appl. Microbiol. Biotechnol. (in press).

An *Arxula adenivorans*-*AHSB4* gene, encoding histone H4, was isolated and characterized. The gene includes a coding sequence of 363 bp, disrupted by a 51 bp intron similar to the situation in other fungal *H4* genes. The identity of the gene was confirmed by a high degree of homology of the derived amino acid sequence with that of other H4 histones. The gene is strongly and constitutively expressed maintaining this expression profile under salt stress conditions. The *AHSB4* promoter was tested for suitability in heterologous gene expression using genes encoding the intracellular GFP and the secreted HSA for assessment. Plasmids incorporating respective expression cassettes were used to transform the host strain *A. adenivorans*

LS3, which forms budding cells at 30 °C, and strain 135 which forms mycelia under these conditions. Transformants of both types were found to harbour a single copy of the heterologous DNA. A strong constitutive expression was observed culturing in both, salt-containing and salt-free media, as expected from the expression profile of the analyzed *AHSB4* gene. In initial 200 mL shake flask cultures, maximal HSA levels of 20 mg L⁻¹ culture medium were achieved. This productivity could be increased to 50 mg L⁻¹ in strains harbouring two copies of the expression cassette. The *AHSB4* promoter thus provides an attractive component for constitutive heterologous gene expression under salt-free and salt stress conditions.

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

Publications since our last communication include the following.

1. B. Christensen; A. K. Gombert; J. Nielsen. 2002. Analysis of flux estimates based on ¹³C-labeling experiments. Eur. J. Biochem. 269:2795-2800.
2. M. M. dos Santos, Gombert, A. K., Christensen, B., Olsson, L., and J. Nielsen. 2003. Identification of in vivo enzyme activities in the co-metabolism of glucose and acetate by *Saccharomyces cerevisiae* using ¹³C-labeled substrates. Eukaryotic Cell. In press.

3. J. Förster; A. K. Gombert; J. Nielsen. 2002. A novel approach to combine metabolome analysis with *in silico* pathway analysis for elucidating the function of orphan genes. *Biotechnol. Bioeng.* **79**:703-712.
4. J. Förster; I. Famili; P. Fu; B. Ø. Palsson; J. Nielsen. 2003. Genome-scale reconstruction of the *Saccharomyces cerevisiae* metabolic network. *Genome Res.* **13**:244-253.
5. H. B. Klinke, L. Olsson, A. B. Thomsen and B. K. Ahring. 2003. Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **81**:738-747.
6. L. Krejci, B. Song, W. Bussen, R. Rothstein, U. H. Mortensen & P. Sung. 2002. Interaction with Rad51 is indispensable for recombination mediator function of Rad52. *J. Biol. Chem.* **277**:40132-41.
7. Lei, F., Olsson, L. and S. B. Jørgensen. 2003. Multiple steady-states in aerobic continuous cultivations of *Saccharomyces cerevisiae*. *Biotechnol. and Bioeng.* **82**: 766-777.
8. M. Lisby, U. H. Mortensen & R. Rothstein Co-localization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nature Cell Biol.* (in press).
9. J. Nielsen; L. Olsson. 2002. An expanded role for microbial physiology in metabolic engineering and functional genomics: Moving towards systems biology. *FEMS Yeast Res.* **2**:175-181.
10. K. Møller; C. Bro; J. Piskur; J. Nielsen; L. Olsson. 2002. Steady-state and transient-state analysis of aerobic fermentation in *Saccharomyces kluyveri*. *FEMS Yeast Res.* **2**:233-244.
11. M. D. W. Piper; P. Daran-Lapujade; C. Bro; B. Regenber; S. Knudsen; J. Nielsen; J. T. Pronk. 2002. Reproducibility of oligonucleotide microarray transcriptome analysis: an interlaboratory comparison using chemostat cultures of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **277**:37001-37008.
12. C. Roca, and L. Olsson. 2003. Increasing ethanol productivity during xylose fermentation by cell recycling of recombinant *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **60**:560-563.
13. J. Zaldivar; A. Borges; B. Johansson; H. P. Smits; S. G. Villas-Boas; J. Nielsen; L. Olsson. 2002. Fermentation performance and intracellular metabolite patterns in laboratory and industrial xylose fermenting *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **59**:436-442.

Recent PhD theses.

14. Jochen Förster. 2002. Pathway analysis of the metabolic network of *Saccharomyces cerevisiae*.
15. Christophe Roca. 2002. Engineering of *Saccharomyces cerevisiae* for improved xylose fermentation.
16. Margarida Moreira dos Santos. 2003. Metabolic engineering of the redox metabolism of *Saccharomyces cerevisiae*.

XIX. Food Science and Technology Department, Biotechnical Faculty University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. Communicated by P. Raspor.

Original scientific paper.

1. Čadež, N., Raspor, P., de Cock, A.W.A.M., Boekhout, T., Smith, M.Th. 2002. Molecular identification and genetic diversity within species of the genera *Hanseniaspora* and *Kloeckera*. *FEMS yeast research.* **1**:279-289.

Three molecular methods: RAPD-PCR analysis, electrophoretic karyotyping and RFLP of the PCR-amplified ITS regions (ITS1, ITS2 and the intervening 5,8S rDNA) were studied for accurate identification of *Hanseniaspora* and *Kloeckera* species as well as for determining inter- and intra-specific relationships of 74 strains isolated from different sources and/or geographically distinct regions. Of these three methods, PCR-RFLP analysis of ITS regions with restriction enzymes *DdeI* and *HinI* is proposed as a rapid identification method to discriminate unambiguously between all six *Hanseniaspora* species and the single non-ascospore forming apiculate yeast species *Kloeckera lindneri*. Electrophoretic

karyotyping produced chromosomal profiles by which the 7 species could be divided into four groups sharing similar karyotypes. Although most of 60 strains examined exhibited a common species-specific pattern, a different degree of chromosomal length polymorphism and a variable number of chromosomal DNA fragments were observed within species. Cluster analysis of the combined RAPD-PCR fingerprints obtained with one 10-mer primer, two microsatellite primers and one minisatellite primer generated clusters which with a few exceptions are in agreement with the groups as earlier recognized in DNA-DNA homology studies.

2. Recek, M., Čadež, N., Raspor, P. 2002. Identification and characterization of yeast isolates from pharmaceutical waste water. *Food technol. biotechnol.* **40**:79-84.

3. Raspor, P., Čuš, F., Povhe Jemec, K., Zagorc, T., Čadež, N., Nemanič, J. 2002. Yeast population dynamics in spontaneous and inoculated alcoholic fermentations of Zametovka must. Food technol. biotechnol. 40:95-102.

Induced fermentation which is more rapid and more reliable than spontaneous one, and which assures predictable wine quality is nowadays prevalent in the Slovene large-scale wine production. However, spontaneous fermentation strengthens the diversity in wine production and offers opportunities for innovation. In 1999 vintage, spontaneous and induced fermentations of Zametovka grape must (*Vitis vinifera* L.) took place. The diversity of yeast species and strains in both investigated fermentations was observed by applying physiological and molecular tests. During the fermentations 648 yeast isolates were isolated and 358 isolates were identified. The distinction in the actual beginning of both fermentations, yeast growth kinetics and yeast population dynamics were the main differences between spontaneous and induced fermentations. The detectable yeast population diversity was higher in the

spontaneous process. The yeast isolates from spontaneous fermentation isolated in large numbers were identified as *Candida stellata*, *Hanseniaspora uvarum* and *Saccharomyces cerevisiae*; other identified species were *Saccharomyces bayanus*, *Pichia kluyveri*, *Pichia membranifaciens* and *Torulaspota delbrueckii*. In the induced fermentation the dominant species was *Saccharomyces cerevisiae*, other species found in lower numbers were *Candida stellata*, *Hanseniaspora uvarum* and *Debaryomyces hansenii* var. *hansenii*. From the grape must at the spontaneous fermentation 15 different strains of *Saccharomyces cerevisiae* and three different strains of *Saccharomyces bayanus* were isolated, in case of induced fermentation only two strains of *Saccharomyces cerevisiae* starter culture were observed.

Summaries of recently completed dissertations.

4. Skrt, Mihaela. Isolation and characterization of low molecular weight chromium binding species from yeast *Candida intermedia*. Doctoral dissertation.

Yeasts responses to the heavy-metal ion stress. One of the possible mechanisms is heavy-metal ion transport into microbial cells, where heavy-metal ions can form different complex compounds. Transport mechanisms for Cr(III) ions are not known and Cr(III)-binding compounds are still not identified. Biochemistry of Cr(III) in the yeast is also unknown. It is known that heavy-metal ion can bind to different low-molecular-weight compounds. Low-molecular-weight chromium-binding species were isolated from yeast *C. intermedia*. To study the effect of Cr(III), low-molecular-weight chromium-binding species were isolated from yeast biomass, harvested from different bioprocesses. For yeast biomass production, chemically defined medium was used and Cr(III) ions were supplemented in 3 mM, 5 mM and 20 mM concentrations in the beginning of the bioprocess or in the middle of the exponential growth phase. Yeast biomass was also harvested from bioprocesses, where Cr(III) ions were supplemented in the middle of the exponential growth phase and temperature was elevated at the end of the exponential growth phase. Low-molecular-weight chromium binding species were isolated with mechanical disruption of yeast cells, ethanol precipitation and separation on anion exchange chromatography gel DEAE-Sepharose Fast Flow or on CIM-DEAE. Chromium concentrations in elution fractions and different isolation steps fractions were determined with atomic absorption spectroscopy.

Chromium rich elution fractions from ion-exchange chromatography were collected, concentrated and further separated with gel filtration on Superdex 30 pg or Superdex Peptide chromatography gel. SDS PAGE analysis of isolated species after ethanol precipitation revealed that Cr(III) ions are responsible for new species with molecular weight of 45 kDa, 29 kDa, 16,9 kDa and 14 kDa. These species also appeared in control species isolated when temperature was elevated above optimal growth temperature in the middle exponential growth phase. Amino acid analysis of low-molecular-weight chromium binding species revealed higher levels of histidine, serine, glycine, threonine and leucine in comparison with control species. XANES and EXAFS analysis also revealed that chromium is bounded in Cr(III) form and that is coordinated with four oxygen atoms and two sulphure atoms. With IR spectroscopy two IR frequencies were identified, one at 525,7 cm^{-1} for $\nu(\text{Cr-O})$ and at 435 cm^{-1} for $\nu(\text{Cr-N})$. With gel filtration low-molecular-weight chromium binding species were isolated with molecular weight ~22600 Da, ~10300 Da, ~5800 Da, ~3200 Da, ~1800 Da, ~850 Da, ~350 Da and species with molecular weight lower than 123 Da. Absorption maximum for these fractions is in range 194 nm-266 nm. Structure identification of these chromium binding species was not possible.

5. Jamnik, Polona. The response of yeast *Candida intermedia* to Cr(VI) as a stress factor. Doctoral dissertation.

Changes in the chemical or physical conditions of the cell that impose a negative effect on growth demand rapid cellular responses, which are essential for survival. Stress response of the yeast *Candida intermedia* - ZIM 156 exposed to Cr(VI) was investigated. Cultures were grown in a bioreactor ($V_e = 2.5$ l, $T = 28$ °C, air supply 2.5 l/min; 200 rpm) in a chemical defined medium. In the mid-exponential phase Cr(VI) as potassium dichromate was added to give concentrations of 50, 100, 300 and 500 $\mu\text{mol Cr(VI)/l}$. Cr(VI) concentration that induces stress responses was determined by monitoring the yeast growth with some bioprocess parameters (OD_{650} , pH, pO_2) during the whole bioprocess. Furthermore, increase in the ROS level in the cell after Cr(VI) addition was investigated by using the dye dichlorofluorescein, which is sensitive to oxidants. Enzymatic specifically superoxide dismutase and catalase as well as non-enzymatic (glutathione level in the reduced form) antioxidant defence systems were investigated. In addition, a

profile of soluble proteins and heat shock proteins (Hsp104, Hsp70) were studied using SDS-PAGE, 2-D electrophoresis and Western blotting. Samples were taken at 0, 0.5, 1, 2 and 4 hours after Cr(VI) addition. Monitoring of some bioprocess parameters during yeast growth specifically pO_2 showed that Cr(VI) addition in concentration of 100 and partially 50 $\mu\text{mol/l}$ increased metabolism intensity which is connected to induced stress responses. Oxidation of dichlorofluorescein indicated increased intracellular oxidant level, specifically at 100 μM Cr(VI) concentration. No significant differences in catalase and superoxide dismutase activity were found between the control cells and cells exposed to the both Cr(VI) concentrations, which indicate that catalase and superoxide dismutase do not participate in defence systems. Intracellular glutathione content in reduced form increased significantly in the cells exposed to 100 $\mu\text{mol Cr(VI)/l}$. Chromium(VI) stress resulted in variation in protein synthesis, which indicate that Cr(VI) might regulate the

expression of some genes. At 100 µM Cr(VI) concentration induction of Hsp104 was observed in contrast to 50 µM. This indicates that Cr(VI) in this concentration causes protein aggregation. Hsp104 assists their resolubilization and so contributes to cell survival. Slight changes in Hsp70 synthesis were observed only at 50 µM Cr(VI) concentration. General analysis of soluble proteins with SDS-PAGE showed variations in synthesis, specifically as repression at 100 µmol Cr(VI)/l.

The synthesis of particular proteins declined 0.5 h after Cr(VI) addition, while synthesis of some proteins decreased more gradually. The most pronounced changes were observed 4 hours after Cr(VI) addition. 2-D electrophoresis of proteins after 4-hour exposure to 100 µM Cr(VI) showed the same. Furthermore, more inducible proteins with different molecular weights than at 50 µM Cr(VI) concentration were also observed.

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

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

1. Sampaio, J.P., Gadanho, M., Bauer, R. and Weiß, M. 2003. Taxonomic studies in the Microbotryomycetidae: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders Leucosporidiales and Sporidiobolales. *Mycol. Progr.* **2**: 53-68.

The subclass Microbotryomycetidae (Basidiomycota, Urediniomycetes) comprises a remarkably diverse assemblage of fungi. This group includes phytoparasites, mycoparasites and probably also saprobes that show a wide range of ecological preferences. In order to study the phylogenetic relationships within the Microbotryomycetidae, and to develop a more natural classification system, mitosporic and meiosporic taxa were investigated using an integrated approach. Sequence data of 26S rDNA D1/D2 domains were analyzed using several procedures, including the Bayesian Markov chain Monte Carlo method of

phylogenetic inference. Ultrastructural markers such as the type of septal pore and presence / absence of colacosomes were investigated and micromorphological and nutritional properties were compared. In this study the current concept of the genus *Leucosporidium* and its apparent polyphyletic nature were addressed, as well as the relationships of this genus with the Microbotryales and *Mastigobasidium*. The classification of the anamorphic species closely related to *Leucosporidium*, and the concepts of the order Sporidiales and family Sporidiobolaceae were also reviewed.

2. Inácio, J., Pereira, P., Carvalho, M., Fonseca, A., Amaral-Collaco, M.T. and Spencer-Martins, I. 2002. Estimation and diversity of phylloplane mycobiota on selected plants in a mediterranean-type ecosystem in Portugal. *Microb. Ecol.* **44**:344-353.
3. Rodrigues, M.G. and Fonseca, A. 2003. Molecular systematics of the dimorphic ascomycete genus *Taphrina*. *Int. J. Syst. Evol. Microbiol.* **53**:607-616.
4. Middelhoven, W.J., Fonseca, A., Carreiro, S.C., Pagnocca, F.C. and Bueno, O.C. 2003. *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. *Antonie van Leeuwenhoek* **83**:167-174.
5. Golubev, W.I., Gadanho, M., Sampaio, J.P. and Golubev, N.W. 2003. *Cryptococcus nemorosus* sp. nov. and *Cryptococcus perniciosus* sp. nov., related to *Papiliotrema* Sampaio et al. (Tremellales). *International Journal of Systematic and Evolutionary Microbiology* **53**: 905-911.

Dimorphic Basidiomycetes WWW project. New paper posted online on March 2003.

6. Sampaio, J.P. and Bauer, R. 2003. The classification of dimorphic basidiomycetes. Dimorphic Basidiomycetes WWW Project. (http://www.crem.fct.unl.pt/dimorphic_basidiomycetes).

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

The following papers, whose abstracts were given in the previous issue, are now in print.

1. Teixeira, A.C.P., M.M. Marini, J.R. Nicoli, Y. Antonini, R.P. Martins, M.A. Lachance & C.A. Rosa. 2003. *Starmerella meliponinorum*, a novel ascomycetous yeast species associated with stingless bees. *Int. J. Syst. Evol. Microbiol.* **53**:339-343.
2. Marinoni, G., J. Piskur, and M.A. Lachance. 2003. Ascospores of large-spored *Metschnikowia* species are genuine meiotic products of these yeasts. *FEMS Yeast Res.* **3**:85-90.
3. Lachance, M.A. J.M. Bowles, and W.T. Starmer. 2003. *Metschnikowia santaceciliae*, *Candida hawaiiiana*, and *Candida kipukae*, three new yeast species associated with insects of tropical morning glory. *FEMS Yeast Res.* **3**:97-103.
4. Hong, S.G., Bae, K.S., Herzberg, M., Titze, A. and Lachance, M.A. 2003. *Candida kunwiensis* sp. nov., a yeast associated with flowers and bumblebees. *Int. J. Syst. Evol. Microbiol.* **53**:367-372.

Recently published or accepted papers.

5. Starmer, W.T., R. Schmedicke, and M.A. Lachance. 2003. The origin of the cactus-yeast community. *FEMS Yeast Research* 3:441-448.

The yeast community found in decaying cactus stems and cladodes is stable in terms of species membership and is similar in composition over space and time. The ecological origins of the three core and four common species in the assemblage were inferred by mapping yeast habitats onto a phylogeny of yeasts reconstructed from rDNA sequences. The members of the community belong to distinct clades and

consequently have independent origins. The inferred evolutionary pathways of the taxa originate in either tree-flux or decaying fruit habitats and lead to decaying *Opuntia* cladode and columnar stem habitats. The reasons for the polyphyletic origins of the cactus-yeast community could be due to unique aspects of cactus chemistry, environmental extremes, vector association and interactions among the members.

6. Thanh, V.N., D.A. Haia, M.A. Lachance. *Issatchenkia hanoiensis*, a new yeast species isolated from frass of the litchi fruit borer *Conopomorpha cramerella* Snellen. *FEMS Yeast Research* (accepted February 2003).

The new ascogenous yeast species *Issatchenkia hanoiensis* was discovered in the frass of the litchi fruit borer *Conopomorpha cramerella* Snellen. The yeast forms unconjugated persistent asci containing one to two roughened ascospores. The yeast has a CoQ-7 system, which is typical for the genus *Issatchenkia*. The closest species to *I. hanoiensis* as indicated by analysis of the partial ribosomal DNA large-subunit

(D1/D2) sequence is the asexual species *Candida pseudolambica*. The two share 94.2% similarity in the sequenced region. Other species of *Issatchenkia* were also among the closest relatives of *I. hanoiensis*, the level of similarity ranging from 89.8% to 94.1%. The type culture is strain HB1.3.13=CBS 9198=NRRL Y-27509.

7. Lachance, M.A., H.M. Daniel, W. Meyer, G.S. Prasad, S.P. Gautam, and K. Boundy-Mills. In press. The D1/D2 domain of the large subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. *FEMS Yeast Research* (in press).

Ten different versions of the D1/D2 divergent domain of the large-subunit ribosomal DNA were identified among interbreeding members of the yeast species *Clavispora lusitaniae*. One major polymorphism, located in a 90-bp structural motif of the D2 domain, exists in two versions that differ by 32 base substitutions. Three other polymorphisms consist of a two-base substitution, a two-base deletion, and a single-base deletion, respectively. The polymorphisms are

independent of one another and of the two mating types, indicating that the strains studied belong to a single, sexually active Mendelian population. Several strains were heterogeneous for one or more of the polymorphisms, and one strain was found to be automicotic and capable of producing asci on its own by isogamous conjugation or by bud-parent autogamy. These observations suggest circumspection in the use of sequence divergence as the principal criterion for delimiting yeast species.

The following paper is based on a keynote lecture to be presented at ISSY23 in Budapest, August 2003.

8. Lachance, M.A. J.M. Bowles, and W.T. Starmer. 2003. Geography and niche occupancy as determinants of yeast biodiversity: the yeast-insect-morning glory ecosystem of Kīpuka Puāulu, Hawai'i. *FEMSYR* (in press).

Biodiversity theory proposes two types of hypotheses to account for the species composition of a given community. The first encompasses geographic and historical factors. For example, local species richness is thought to be affected by area, proximity to large landmasses, dispersal mechanisms, and climatic history, collectively known as biogeography. The second type, termed niche occupancy rules, deals with the intrinsic properties of the species as they affect their interaction with the habitat and with other members of the community. The yeast-insect-morning glory ecosystem is a good model to explore biodiversity theory in ascomycetous yeasts. Here we focus beetles that breed or feed in morning glories and a group of ascomycetous yeasts that are associated exclusively with them. Specifically, we analyse the community found in the vicinity of Kīpuka Puāulu, a small patch of disturbed but mature forest situated amidst lava flows on the island of Hawai'i. Major members of the yeast community include *Metschnikowia hawaiiensis*, *M. lochheadii*, and the related asexual species

Candida ipomoeae, *C. kipukae*, and *C. hawaiiiana*. These species are nearly indistinguishable from one another in terms of nutritional requirements and abilities, although their phylogenetic range is enormous. Their distribution, both global and local, is far from random. As Kīpuka Puāulu is an island within an island, the principles of island biogeography may be invoked to explain some aspects of its yeast species composition. *M. lochheadii*, *C. ipomoeae*, and *C. hawaiiiana* are recent introductions from the American continent and therefore exotic, whereas *M. hawaiiensis* and *C. kipukae* might be regarded as endemic, as they are yet to be isolated elsewhere. Vectoring by certain nitidulid beetles explains the long-range dispersal of these species. However, niche occupancy rules may account in part for the local spatial distribution of the yeasts within the island of Hawai'i and within the kīpuka itself. We have identified maximum growth temperature as a potentially critical property of the fundamental niche of these yeasts.

International Commission on Yeasts

During the 23d International Specialized Symposium on Yeasts (ISSY 23) in Budapest, the International Commission on Yeasts will convene during lunch on Wednesday 27 August 2003, under the hospitality of Professor Tibor Deák. All Commissioners are kindly invited to take part.

Suggestions for the Agenda and other messages may be sent to lex.scheffers@tnw.tudelft.nl

Lex Scheffers, Chair

Forthcoming Meetings

XXI International Conference on Yeast Genetics and Molecular Biology Goteborg, Sweden, July 7-12, 2003

Do not miss the XXI International Conference on Yeast Genetics and Molecular Biology in Göteborg (July 7-12, 2003). More than 1000 delegates have already registered and 840 abstracts have been submitted. Several new genome sequences being released at the conference, including those of *Kluyveromyces lactis* and *Yarrowia lipolytica*!

See the attractive scientific programme at our website (www.yeast2003.se)! Travelling with children? Register for daycare at yeast2003@gmm.gu.se. Traveling/flying within Europe has never been so cheap. Register and submit abstracts for poster session at www.yeast2003.se. See you this summer in Sweden!

16th Meeting on The Biology of *Kluyveromyces*. 12-14 September 2003, Cortona (Arezzo), Italy First Announcement and Call for Contributions

Dear Colleagues,

We are pleased to invite you to attend the 16th International Meeting on The Biology of *Kluyveromyces* that will be held on 12th-14th September 2003 in Cortona (Arezzo) Italy. As in previous years, the Meeting will be very informal with short talks of approximately 15-20 minutes each and we will try give each group the opportunity to present his work. The deadline for registration, hotel reservation and abstract

submission is June 6th 2003. The Registration Fee, that we hope to reduce with the help of new sponsors, will include all meals and coffee-breaks. The Fee must be paid directly at the Meeting and receipts will be issued on payment. You will find the relevant informations at the web site:

<http://151.100.42.47/meeting/homepage.htm>

Should you have any question, please contact us to the following e-mail address:

klactis2003@uniroma1.it

Claudio Falcone and Claudio Palleschi

Learning from Yeast – A Symposium Honouring Herman Jan Phaff Santiago de Compostela, Spain - September 23 and 24 2003

The Symposium is dedicated to the beloved memory of Prof. Herman Jan Phaff and is sponsored by the Ramón Areces Foundation, Madrid. Venue: Auditorium of the Universidad de Santiago, Campus Universitario, Santiago de Compostela, Spain.

Organizers: T.G. Villa and J.A. Alonso. Speakers: T.G. Villa, C. Hardisson, J.R. Villanueva, A.L. Demain, M.A. Lachance, E.A. Johnson, A. Vaughan, E. Herrero, A. Querol, S.A. Meyer, M. Gacto Fernández, and A. Martini.

Secretariat:

Departamento de Biología Celular y Molecular
Facultad de Ciencias
Universidad de A Coruña

<ecrebcm@udc.es>

Physiology of Yeasts and Filamentous Fungi - PYFF2

March 24-28 2004, Anglet, France

On behalf of the organizing committee we have the pleasure to invite you to attend the PYFF2 meeting, the second meeting on Physiology of Yeasts and Filamentous Fungi, to be held in Anglet (near Biarritz, Basque Coast, on the Atlantic ocean), France from March 24th to 28th, 2004. This event is co-sponsored by FEMS, (Federation European Microbiology Society) and EFB (European Federation of Biotechnology). This PYFF2 meeting aims to stimulate relationships between specialists, post-doc and post-graduate students active in academic and industrial researches of integrated physiology of yeasts and fungi for fundamental and applications purposes in Biotechnology.

Preliminary Scientific Program. System biology. Physiogenomics. Metabolic engineering. New technologies/tools for physiology studies. Biotechnology challenges at the post-genomic era for yeasts. Biotechnology challenges at the

PROGEP - LGC
B.P. 1301
5 Rue Paulin Talabot
31106 Toulouse Cedex I
France

post-genomic era for fungi.

Scientific committee. David Archer, Barbara Bakker, Laurent Benbadis, Eckard Boles, Jeff Cole, Jean M. François, Carlos Gancedo, Stéphane Guillouet, Santiago Gutierrez, Barbara Hahn-Hagerdhal, Lisbeth Olsson, Jack Pronk.

The pre-registration is open now on our WEB-site:
<http://pyff2.insa-tlse.fr>

The website is now fully operational with electronic paper submission activated. You can also download the PYFF2 leaflet to distribute it to your colleagues. All instruction for abstract submission, registration fees and other activities can be found on the WEB site.

Registration: Ph.D. Students: 500; Postdocs: 575; Senior scientists: 650.

Other technical information can also be obtained at:

Tel +33 (0) 5 34 61 52 89
Fax +33 (0) 5 34 63 94 35
Email Progep-PYFF2@ensiacet.fr

Eleventh International Symposium on Yeasts, ISY 11

Rio de Janeiro, August 15-20 2004

On behalf of the International Committee on Yeasts (ICY) and the Federal University of Rio de Janeiro (UFRJ), I have the honor and pleasure to announce the Eleventh International Yeast Congress (former ISY) to be held during 15-20th of August, 2004 in Rio de Janeiro, Brazil.

The Conference venue is Hotel Gloria Convention Center, a traditional five star hotel, located in the south zone of Rio, close to the city center and with a panoramic view over Guanabara Bay. The first announcement information is

available at the homepage <http://www.icy2004.com.br>. Further information can be obtained by e-mail: congress@icy2004.com.br or leda@icy2004.com.br.

The theme of the symposium will be "Yeasts in Science and Technology: the quest for sustainable development." The scientific program is under development and we would welcome your suggestions on the topics to be presented. Please, send your address to receive ICY2004 folder and poster. Welcome to Rio!

Leda Mendonça-Hagler, ICY2004 Chair

Brief News Item

Change of Address: Dr. Jorgen Stenderup

I have left the Serum Institute and taken up the responsibility of building up a new Mycology laboratory in a hospital. With time, a substantial part of mycological

diagnostics will be covered there. I shall continue to live in Copenhagen and will be using the same email address as in the past.

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