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# Y E A S T

## A Newsletter for Persons Interested in Yeast

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# Editorials

## ISY 2000 - Papendal, Arnhem, The Netherlands

Congratulations are very much in order to the Organizing Committee of the 10th general ISY, held at the Papendal Sports Centre this summer. Many have already commented on the perfection of the organization, the attractiveness of the venue, and the outstanding quality of the scientific program.. I was particularly pleased with the fact that every session offered a balance of topics that would appeal to all members of the audience. The organizers were very successful with the difficult task of scheduling the afternoon concurrent sessions so as to minimize overlap of interests and maximize the diversity of topics. Job very well done indeed.

## *FEMS Yeast Research*

The new journal, *FEMS Yeast Research*, is intended to start publication soon (January 2001). A meeting of the Editorial Board was held in Papendal during the 10th ISY. The Chief Editor, Lex Scheffers, informed the members of the board that the underlying philosophy of the new journal is to provide the broadest forum to discuss all aspects of research dealing with yeasts. Quality will be the main determinant of acceptance of papers. Dr. Scheffers advises that the first issue is in preparation and is scheduled to appear in the Spring 2001.

Readers of the Yeast Newsletter are invited to submit manuscripts for subsequent issues. Relevant information *FEMS Yeast Research* can be found in page 86 of the YNL, and in more detail at the website http://www.fems-microbiology.org/. The address for submission of manuscripts is: FEMS Publications Office, Poortlandplein 6, NL-2628 BM Delft, The Netherlands.

## New items in this issue of the Yeast Newsletter

This issue of the Yeast Newsletter introduces two novel communication formats. First (p. 47), Wladys Golubev presents an exhaustive list of strains of *Xanthophyllomyces dendrorhous* (which I believe is either the teleomorph or a close relative of *Phaffia rhodozyma*) available in various international culture collections. This sort of list of yeasts of special scientific or biotechnological interest is of great potential value for the research community. Second (p. 77), after an Internet exchange with my colleagues Kurtzman and Fell, I persuaded them that it would be worthwhile to share with YNL readers our thoughts on the contentious matter of describing new species on the basis of single strains. Readers who wish to enter this debate or introduce others are invited to join the mêlée.

## Payment by Credit Card

Many readers in the past have asked to pay their subscription by credit card. We are now able to offer this option for Master Card and Visa holders. We ask that all credit card payments be made only to the Editor, and that payments be sent as soon as possible after receipt of the invoice.

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

M.A. Lachance
Editor

## I. 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>

*Xanthophyllomyces dendrorhous* is the only yeast able to synthesize astaxanthin as its principal carotenoid. Astaxanthin is used as a supplement in aquaculture and poultry industry. It is a powerful antioxidant and has been linked to reduced incidence of cancer and other degenerative diseases. These facts have generated considerable activity in *X. dendrorhous* research. However, it is necessary to note that a majority of these investigations were done on the same two-three strains. In view of different electrophoretic karyotypes and carotenoid profiles in natural isolates of *X. dendrorhous*, such data are probably insufficient for proper characterization of this species and also for the choice of strains perspective biotechnologically as strain differences could have industrial significance. Information on *X. dendrorhous* strains maintained in culture collections will be useful for zymologists engaged in research of this species.

Nearly all original isolates of *X. dendrorhous* originate from studies by H.J. Phaff et al. (1972), Univ. California, Davis (UCD), and by W.I. Golubev and I.P. Bab'eva (1977), Russian Collection of Microorganisms (VKM) and Dept. Soil Biology, Moscow State Univ. (KBP).

**Collection resources of *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*, *Rhodomyces dendrorhous*)**

| Initial number | Strain numbers in other collections | Origin (host plant, locality) |
|---|---|---|
| CBS 6938 | ATCC 74438 = ATCC 96594 = CCRC 22365 = JCM 9684 = PR 219 = SzMC 1456 = VKM Y-2793<br>Mutants: CBS 797.91, CBS 303.93 | *Betula* sp., Finland |
| KBP 2600 | VKPM Y-1651 | Forest litter, Novgorod region, Russia |
| KBP 2601 | | Forest litter, Novgorod region, Russia |
| KBP 2604 | VKPM Y-1652 | *Betula* sp., Novgorod region, Russia |
| KBP 2605 | VKPM Y-1653 | *Betula* sp., Novgorod region, Russia |
| KBP 2607 | VKPM Y-1654 | *Betula* sp., Komi Republic, Russia |
| KBP 2609 | VKPM Y-1655 | *Betula* sp. Komi Republic, Russia |
| KBP 2610 | VKPM Y-1622 = VKPM Y-1656 | *Betula* sp., Novgorod region, Russia |
| KBP 2612 | VKPM Y-1657 | *Betula* sp., Novgorod region, Russia |
| KBP 2613 | VKPM Y-1623 = VKPM Y-1658 | *Betula* sp., Novgorog region, Russia |
| KBP 2614 | VKPM Y-1659 | *Betula* sp., Moscow region, Russia |
| KBP 2615 | | *Betula* sp., Moscow region, Russia |
| KBP 2616 | VKPM Y-1660 | *Betula* sp., Moscow region, Russia |
| KBP 2617 | VKPM Y-1661 | *Betula* sp., Moscow region, Russia |
| KBP 2618 | VKPM Y-1662 | *Betula* sp., Moscow region, Russia |
| KBP 2619 | VKPM Y-1663 | *Betula* sp., Moscow region, Russia |
| KBP 2621 | VKPM Y-1664 | *Betula* sp., Moscow region, Russia |
| KBP 2623 | VKPM Y-1665 | *Betula* sp., Moscow region, Russia |
| KBP 2625 | VKPM Y-1666 | *Betula* sp., Moscow region, Russia |
| KBP 2627 | VKPM Y-1667 | *Betula* sp., Moscow region, Russia |
| KBP 2629 | VKPM Y-1668 | *Betula* sp., Moscow region, Russia |
| KBP 2630 | VKPM Y-1669 | *Betula* sp., Moscow region, Russia |
| KBP 2631 | VKPM Y-1670 | *Betula* sp., Moscow region, Russia |
| KBP 2632 | VKPM Y-1671 | *Betula* sp., Moscow region, Russia |
| KBP 2633 | VKPM Y-1672 | *Betula* sp., Moscow region, Russia |
| KBP 2636 | VKPM Y-1673 | *Betula* sp., Moscow region, Russia |
| UCD 67-202 | ATCC 24229 = JCM 9682 = VKM Y-2788 | *Cornus brachypoda*, Honshu, Japan |
| UCD 67-203 | ATCC 24201 = VKM Y-2789 | *Cornus brachypoda*, Honshu, Japan |

| | | |
|---|---|---|
| UCD H11-A | | *Cornus brachypoda*, Honshu, Japan |
| UCD 67-210 | ATCC 24202 = ATCC 36587 = ATCC MYA-131 = CBS 5905 = CCRC 21346 = CCY 77-1-1 = CECT 1690 = DSM 5626 = DBVPG 7009 = IFO 10129 = IGC 4172 = IHEM 5758 = JCM 9680 = MUCL 31142 = NCYC 874 = NRRL Y-10921 = VKM Y-2274<br>Mutants: DSM 6560, DSM 6561 | *Fagus crenata*, Honshu, Japan |
| UCD 67-383 | ATCC 24203 = CBS 5908 = CCRC 22367 = JCM 9683 = VKM Y-2790 | *Alnus japonica*, Honshu, Japan |
| UCD 67-385 | ATCC 24230 = CBS 7919 = NRRL Y-17810 = VKM Y-2791<br>Mutants: ATCC 66270, ATCC 66272, ATCC 96815, ATCC 96816, ATCC 96220, ATCC 96221, NRRL Y-17811 | *Betula tauschii*, Honshu, Japan |
| UCD 67-484 | ATCC 24261 = VKM Y-2792<br>Mutants: CBS 224-87, CBS 225-87, CBS 215-88, DBT 415, DSM 6559 | *Betula maximowicziana*, Honshu, Japan |
| UCD 68-653C | ATCC 24228 = IFO 10130 = VKM Y-2814 | *Betula papyrifera*, Alaska, USA |
| UCD 316A | | *Betula* sp., Honshu, Japan |
| UCD 612 | | *Ulmus japonica*, Hokkaido, Japan |
| VKM Y-2059 | ATCC 96814 = NRRL Y-17268 = VKPM Y-902 | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2266 | NRRL Y-10922 = NRRL Y-17296 = UCD 76-18 | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2267 | | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2268 | ATCC 96813 = NRRL Y-17269 = UCD 76-19 | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2269, VKM Y-2270, VKM Y-2271, VKM Y-2272 | | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2273 | ATCC 96812 = NRRL Y-17270 | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2786 | CBS 7918 = JCM 9681 = NRRL Y-17832 | *Betula verrucosa*, Moscow region, Russia |
| VKM Y-2806 | | *Betula verrucosa*, Moscow region, Russia |
| VKPM Y-990 | | *Betula* sp., Mordovian Republic, Russia |

## II. Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR USA 97331-6602. Communicated by A. Bakalinsky <bakalina@ava.bcc.orst.edu>.

The following papers, whose abstracts have appeared in a previous issue of the Yeast Newsletter, have now been published.


1. Park, H., Lopez, N.I., and Bakalinsky, A.T. 1999. Use of sulfite resistance in *S. cerevisiae* as a dominant selectable marker. Curr. Genet. **36**:339-344.

2. Park, H. and Bakalinsky, A.T. 2000. *SSU1* mediates sulfite efflux in *S. cerevisiae*. Yeast **16**:881-888.

## III. 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. J A Barnett. 2000. A History of Research on Yeasts 2: Louis Pasteur and his Contemporaries, 1850-1880. Yeast **16**:755-771.

2. J A Barnett & F W Lichtenthaler 2001. A history of research on yeasts 3. Emil Fischer, Eduard Buchner and their contemporaries, 1880-1900. Yeast (in the press).

3. In preparation: A history of research on yeasts 4: cytology.

## IV. Culture Collection of Yeasts, Institute of Chemistry, Dúbravská cesta 9, 842 38 Bratislava, Slovakia. Communicated by E. Breierova <chememi@savba.sk>.

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

1. Sláviková, E. and Košíková, B. 2000. Modification of lignin by *Geotrichum klebahnii.* World J Microbiol. Biotechnol. (in press).

$^{13}C$ NMR spectroscopic analysis indicates that the yeast-like species *Geotrichum klebahnii* is an efficient microorganism for lignin biodegradation. This strain modified beechwood lignin even if it was the only carbon source by $C_\alpha$ - $C_\beta$ side chain cleavage, $C_\alpha$ - oxidations, aromatic ring cleavage and reductive reactions. The obtained results outline prospective application of *G. klebahnii* for biotechnological pretreatment of lignicellulosic materials.

2. Chorvatovičová, D., Machová, E., Šandula, J., Kogan, G. 1999. Protective effect of the yeast glucomannan against cyclophosphamide-induced mutagenicity. Mutation Res. **444**, 117-122.

Glucomannan (GM) isolated from *Candida utilis* with molecular weight 30 kDa was administered either intraperitoneally or orally prior to cyclophosphamide (CP) injection and its effect on the frequency of micronuclei was evaluated in polychromatic erythrocytes of mouse bone marrow. GM administration by either route decreased significantly ($p< 0.002$) the clastogenic effect of CP. The protective effect was concentration-dependent, with a higher decrease achieved by 200 mg/kg than by 100 mg /kg b. wt. (body weight). The fact that GM was effective also at oral administration is indicative of the passage of GM molecules though the wall of the gastrointestinal tract. The important characteristics of GM isolated from *C. utilis* , such as good water solubility, relatively small molecular weight (30 kDa), and antimutagenic effect exerted also at oral administration, appear to be promising features for its prospective use as a natural protective agent.

## V. Biology Department, Tennessee State University, 3500 John Merritt Blvd., Nashville, Tennessee 37209, USA. Communicated by P.F. Ganter <pganter@TNSTATE.EDU>.

The following is an abstract of a paper in press.

1. P.F. Ganter and M. de Barros Lopes. 2000. The use of anonymous DNA markers in assessing worldwide relatedness in the yeast species *Pichia kluyveri* Bedford and Kudrjavzev. Can. J. Microbiol. (in press).

*Pichia kluyveri*, a sexual ascomycetous yeast from cactus necroses and acidic fruit, is divided into three varieties. We used physiological, RAPD, and AFLP data to compare 46 *P. kluyveri* strains collected worldwide to investigate relationships among varieties. Physiology did not place all strains into described varieties. Although the combined AFLP and RAPD data produced a single most parsimonious tree, separate analysis of AFLP and RAPD data resulted in significantly different trees (by the partition homogeneity test). We then compared the distribution of strains per band to an expected distribution. This suggested we could separate both the AFLP and RAPD datasets into bands from rapidly and slowly changing DNA regions. When only bands from slowly changing regions (from each dataset) were included in the analysis, both

the RAPD and AFLP datasets supported a single tree. This second tree did not differ significantly from the cladogram based on all of the DNA data, which we accepted as the best estimate of the phylogeny of these yeast strains. Based on this phylogeny, we were able to demonstrate the strong influence of geography on the population structure of this yeast, confirm the monophyly of one variety, question the utility of maintaining another variety, and demonstrate that the physiological differences used to separate the varieties did not do so in all cases.

## VI. Sojo University, Ikeda 4-22-1, Kumamoto 860-0082, Japan. Communicated by N. Gunge <gunge@bio.soj.o-u.ac.jp>.

The following are abstracts of three recent publications on yeasts.

1. H. Takata, K. Fukuda, F. Meinhardt, and N. Gunge. 2000. Telomere sequences attached to nuclearly migrated yeast linear plasmid. Plasmid **43**:137-143.

The yeast linear plasmid pCLU1, derived from pCKLI, has terminal proteins (Tps) covalently attached at the 5' ends of inverted terminal repeats (ITRs) and replicates in the cytoplasm, presumably using the TP as a primer for DNA synthesis. In *Saccharomyces cerevisiae*, under certain conditions, pCLUI migrated into the nucleus and replicated in either linear or circular form. The linear-form plasmid lacked Tps, instead it carried host-telomere repeats at the ITR ends. The present study showed that (I) the added telomere was primarily composed of the repeated tracts of TGTGTGGGTGTGG, which was complementary to the RNA template of yeast telomerase, (2) the telomeric addition occurred at the very end of the ITRs, and (3) the sequence composition of the added telomeres: was diverse among individual plasmids, but symmetrically identical at both ends of each plasmid. A similar mode of telomere addition was also observed in cells defective in the *KAn52* gene.

2 N. Gunge, H. Takata, K. Fukuda, S. Iwao, and Miyakawa. 2000. Relocation of a cytoplasmic yeast linear plasmid to the nucleus is associated with circularization via nonhomologous recombination involving inverted terminal repeats. Mol. Gen. Genet. **263**:846-853.

The linear plasmid pCLUI from the yeast *Kluyveromyces lactis* normally replicates in the cytoplasm, with the aid of the helper linear plasmid pGKLZ, using terminal protein (TP) as a primer. However, it relocates to the nucleus when selection is applied for the expression of a plasmid-borne nuclear marker. Migration to the nucleus occurred in *K. lactis* at a frequency of about $10^{-3}$/cell ten or more times higher than the rate observed in *Saccharomyces cerevisiae*. The nuclear plasmids existed only in a circularized form in *S. cerevisiae* a telomere-associated linear form is also found. Sequence analysis showed that circularization in *K. lactis* was caused by non-homologous recombination between the inverted terminal repeat (ITR) at the ends of the linear form and non-specific internal target sites in pCLU1. No sequence similarity existed among the junction sites, indicating that the free ITR end plays a crucial role in circularization. In *S. cerevisiae*, circular plasmids were generated not only by nonhomologous recombination, but also by homologous recombination between short direct repeats within pCLUI. Circularization via the ITR end was observed independently of RAD52 activity. Sequences highly homologous to ARS core elements, 5'-ATTTATTGTTTT3' for *K .lactis* and 5'-(AIT)TTTAT(TIG)TTT(A:'T)-3' for *S. cerevisiae*, were detected at multiple sites in the nuclear forms of the plasmids.

3. T. Fukuda, K. Fukuda, A. Takahashi, T. Ohnishi, T. Nakano C, M. Satoc, and N. Gunge. 2000. Analysis of deletion mutations of the *rpsL* gene in the yeast *Saccharomyces cerevisiae* detected after long-term flight on the Russian space station Mir Mutation Research **7644**:1-8

Using the yeast *Saccharomyces cerevisiae* on board the Russian space station Mir, we studied the effects of long-term space flight on mutation of the bacterial ribosomal protein L gene (*rpsL*) cloned in a yeast-*Escherichia coli* shuttle vector. The mutation frequencies of the cloned *rpsL* gene on the Mir and the ground (control) yeast samples were estimated by transformation of *E. coli* with the plasmid DNAs recovered from yeast and by assessment of the conversion of the *rpsL* wild-type phenotype ($Sm^S$) to its mutant phenotype ($Sm^R$). After a 40-day space flight, some part of space samples gave mutation frequencies two to three times higher than those of the ground samples. Nucleotide sequence analysis showed no apparent difference in point mutation rates between the space and the ground mutant samples. However, the greater part of the Mir mutant samples were found to have a total or large deletion in the *rpsL* sequence, suggesting that space radiation containing high-linear energy transfer (LET) might have caused deletion-type mutations.

## VII. Laboratorio de Ecologia Microbiana e Taxonomia, and Laboratorio de Leveduras, Coleção de Culturas Dept. Microbiol. Geral, Inst. Microbiol Prof. Paulo de Goes, CCS, Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, 21941-590, Brasil. Communicated by Allen N. Hagler and Leda Cristina Mendonça-Hagler <immgalh@microbio.ufrj.br>.

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


1. Santos, E. A., de Oliveira, R. B., L. C. Mendonça-Hagler, and A. N. Hagler. 1996. Yeasts associated with cashew, caja, umbu, and mango fruits typical of the semiarid region of northeastern Brazil. Rev. Microbiol. **27**(1):33-40.

2. Valente, P., G. A. Lemos, F. C. Gouveia, D. Pimentel, D. van Elsas, L. C. Mendonça-Hagler, A. N. Hagler. 1996. PCR amplification of the ITS region for differentiation of *Saccharomyces* cultures. FEMS Microbiol. Lett. **137**:253-256.

3. Morais, P. B., C. A. Rosa, J. Abranches, L. C. Mendonça-Hagler & A. N. Hagler. 1996. Yeasts vectored by *Drosophila quadrum* (calloptera group) in tropical rain forests. Rev. Microbiol. **27**:87-91.

4. Abranches, J., P. B. Morais, C. A. Rosa, L. C. Mendonça-Hagler, and A. N. Hagler. 1997. The incidence of killer activity and extracellular proteases in tropical yeast communities. Can. J. Microbiol. **43**(4):328-336.

5. Valente, P., F. C. Gouveia, G. A. Lemos, D. Pimentel, D. van Elsas, L. C. Mendonça-Hagler, A. N. Hagler. 1997. PCR amplified ITS length variation within the yeast genus *Metschnikowia*. J. Gen. Appl. Microbiol: **43**:179-181.

6. Hagler, A. N., L. C. Mendonca-Hagler and J. B. Silva. 1997. Ascomycetous yeast communities in coastal forest ecosystems of southeast Brazil. Progress in Microbial Ecology. pp. 189-195.

7. Mendonça-Hagler, L. C., A. N. Hagler, C. A. G. Soares, F. V. de Araujo, & P. R. Peres Neto. 1997. Yeasts as a Model of Microbial Diversity in Coastal Marine Habitats in Rio de Janeiro, Brazil. Progress in Microbial Ecology. pp. 237-244.

8. Soares, C. A., M. Maury, F. Pagnocca, F. A. Araujo, A. N. Hagler, and L. C. Mendonça-Hagler. 1997. Yeast communities in dark muddy intertidal estuarian sediments of Rio de Janeiro, Brazil. J. Gen. Appl.. Microbiol. **43**:265-272.

9. Araujo, F. V., R. J. Madeiros, L. C. Mendonça-Hagler, & A. N. Hagler. 1998. A preliminary note on prevalent species in yeast communities of bromeliad - tank waters in different tropical ecosystems of Rio de Janeiro, Brazil. Rev. Microbiologia. **29**:118-121.

10. Abranches, J., H. N. Nóbrega, P. Valente, L. C. Mendonça, & A. N. Hagler. 1998. A preliminary note on yeasts associated with rodents and marsupials of Atlantic Forest fragments in Rio de Janeiro, Brazil. Rev. Microbiologia **29**:170-173.

11. Abranches, J., P. Valente, H. N. Nóbrega, F. A. S. Fernandez, L. C. Mendonça-Hagler, and A. N. Hagler. 1998. Yeast diversity and killer activity dispersed by fecal pellets from marsupials and rodents in a southeast Brazilian tropical habitat mosaic. FEMS Microbiol. Ecol. **26**:27-33.

12. Azeredo, L. A. I., E. A. Gomes, L. C. S. Mendonca-Hagler, & A. N. Hagler. 1998. Yeast communities associated with sugar-cane (*Saccharum officinarum* Linneau) in Rio de Janeiro, Brazil. Internatl. Microbiol. **1**:205-208.

13. Ramos, Jesus P., P. Valente, A. N. Hagler, O. Leoncini. 1998. Restriction analysis of the ITS region for characterization of *Debaryomyces* species. J. Gen Appl. Microbiol. **44**:399-404.

14. Abranches, J., M. J. S. Vital, W. T. Starmer, L. C. Mendonça Hagler & A. N. Hagler. 2000. Yeast community and mycocin producers of guava fruit in Rio de Janeiro, Brazil. Mycologia **92**:16-22.

The following graduate theses were recently defended.

15. Fabio Castro Gouveia - M.Sc. 1998. Estudo sobre a região espaçadora interna transcrita de rDNA em leveduras dos gêneros *Saccharomyces* e *Metchnikowia*. Inst. Microbiol. Prof. Paulo de Goes, Univ. fed. Rio de Janeiro.

16. Fábio Vieira de Araujo - D.Sc. 1999. Comunidades de leveduras associadas a sedimentos de manguezais e a bromélias em ecossistemas costeiros do Rio de Janeiro, Brasil. Inst. Microbiol. PPG., UFRJ.

17. Rodrigo Jesus Medeiros - M.Sc., 1999. Aspectos ecológicos da produção de micocinas (Toxinas "killer") por leveduras de manguezal e mata Atlantica. PPG Ecologia UFRJ.

18. Jaqueline Abranches Lemos - D.Sc. 2000. Comunidades de leveduras Associadas a pequenos mamíferos e ao fruto *Psidium guajava* e as interações levedura-levadura em habitas tropicais. Inst. Microbiol. PPG., UFRJ.

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

For recent acquisitions, please consult the catalogue on the JCM home page at http://www.jcm.riken.go.jp/. The following articles have appeared, are in press or accepted.

1. Takashima, M., Hamamoto, M. and Nakase, T. 2000. Taxonomic significance of fucose in the class Urediniomycetes: distribution of fucose in cell wall and phylogeny of urediniomycetous yeasts. Syst. Appl. Microbiol. **23:** 63-70.

The carbohydrate compositions of cell wall were determined in the strains of class Urediniomycetes, mainly ballistoconidium-forming yeasts and related taxa. The major component of cell wall was mannose, and glucose was included as the second component, but xylose was not detected in any strain. Out of 41 strains examined, 39 contained galactose, 14 contained arabinose and 12 contained rhamnose. As a minor component, fucose was detected in 30 strains but not in 11 strains. A phylogenetic tree based on 18S rDNA sequences indicated that the fucose-lacking strains, *Erythrobasidium hasegawianum, Rhodotorula aurantiaca, R. lactosa, R. minuta, Sakaguchia dacryoidea, Sporobolomyces coprosmae, S. elongatus, S. foliicola, S. gracilis, S. kluyverinielii* and *S. oryzicola,* constituted a distinct cluster from those strains which contained fucose. This cluster corresponded to one of the five subclusters, the *Erythrobasidium* cluster, in the phylogenetic tree of class Urediniomycetes. The carbohydrate composition of cell wall is believed to reflect the phylogenetic relationships among basidiomycetous fungi. The presence or absence of fucose in cell wall should be regarded as an important phenotypic characteristics in the taxonomy of basidiomycetes.

2. Takashima, M. and Nakase, T. 2000. Four new species of the genus *Sporobolomyces* isolated from leaves in Thailand. Mycoscience **41:** 357-369.

Thirteen undescribed strains of ballistoconidium-forming yeasts, isolated from leaves collected in the suburbs and along the southeast seacoast of Bangkok, Thailand, were divided into four different groups in the genus *Sporobolomyces* on the basis of morphological, physiological, and chemotaxonomical characteristics, and analyses of the sequences of 18S rDNA and internal transcribed spacer regions. DNA-DNA reassociation experiments with related species revealed that these four groups were four new distinct species. *Sporobolomyces nylandii* sp. nov., *S. poonsookiae* sp. nov., *S. blumeae* sp. nov. and *S. vermiculatus* sp. nov. are proposed for these strains.

3. Bai, F.-Y., Takashima, M., Hamamoto, M. and Nakase, T. 2000. *Sporobolomyces yunnanensis* sp. nov., a Q-10($H_2$)-containing yeast species with a close phylogenetic relationship to *Erythrobasidium hasegawianum*. Int. J. Syst. Evol. Microbiol. (in press).

4. Fungsin, B., Hamamoto, M., Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P. and Nakase, T. 2000. *Bensingtonia thailandica* sp. nov., a new basidiomycetous yeast species isolated from plant leaves in Thailand (accepted by IJSEM).

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

Recent publications include the following.

1. Aristidou, A. and Penttilä, M. 2000. Metabolic engineering applications to renewable resource utilization. Curr. Opinion Biotechnol. **11**:187-98.

2. Aristidou, A., Richard, P., Ruohonen, L., Toivari, M., Londesborough, J. and Penttilä, M. 2000. "Redox balance in fermenting yeast." European Brewing Convention Monograph (Symposium on Yeast Physiology) **28**:161-170.

3. Bagnat, M., Keränen, S., Shevchenko, A., Shevchenko, A. and Simons, K. 2000. Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. Proc. Natl. Acad. Sci. USA. **97**:3254-3259.

4. Granström, T., Aristidou, A., Jokela, J. and Leisola, M. 2000. Growth characteristics and metabolic flux analysis of *Candida milleri*. Biotechnol. Bioengin. **70**:197-207.

5. Kaukonen, J, Juselius, J.K., Tiranti, V., Kyttälä, A., Massimo Z., Comi, G.P., Keränen, S., Peltonen, L. and Suomalainen, A. 2000. Role of ANT1 in mtDNA maintenance. Science **289**:782-785.

6. Ossig, R., Schmitt, H.D., de Groot, B., Riedel, D., Keränen, S., Ronne, H., Grubmüller, H. and Jahn, R. 2000. Exocytosis requires asymmetry in the central layer of the SNARE complex. EMBO J. **19**:6000-6010.

7. Reinman, M and Londesborough, J. 2000. Rapid mobilization of intracellular trehalose by fermentable sugars: a comparison of different strains. *in* Brewing Yeast Fermentation Performance (ed. Smart, K.) pp 20-26, Blackwell Science Ltd, Oxford, UK.

8. Richard, P., Toivari, M.H. and Penttilä, M. 1999. Evidence that the gene YLR070 of *Saccharomyces cerevisiae* encodes a xylitol dehydrogenase. FEBS Letters **457**:135-138.

9. Richard, P., Toivari, M.H. and Penttilä, M. 2000. The role of xylulokinase in *Saccharomyces cerevisiae* xylulose metabolism. FEMS Microbiology Letters **190**:39-43.

10. Riento, K., Kauppi, M., Keränen, S. and Olkkonen, V.M. 2000. Munc18-2, a functional partner of syntaxin 3, controls apical membrane trafficking in epithelial cells." J. Biol. Chem. **275**:13467-13483.

11. Virkajärvi, I. and Pohjala, N. 2000. Primary fermentation with immobilized yeast: some effects of carrier materials on the flavour of the beer. J. Inst. Brewing **106**:311 - 318.

The following PhD thesis, based on work carried out at VTT, has been successfully defended.

12. Toikkanen, Jaana. 1999. Functional studies on components of the secretory pathway of *Saccharomyces cerevisiae*. Department of Biosciences, Division of Genetics, University of Helsinki. (Available as VTT Publications 389; 92 p. + appendix 61 p).

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

13. Hurme, Jonne. 1999. The physiology of xylose utilization by recombinant yeast in batch and chemostat cultures. Department of Chemical Technology, Helsinki University of Technology, Finland.

14. Märtnes, Inga. 1999. The role of cofactor balancing in genetically modified strains of *Saccharomyces cerevisiae* capable of xylose fermentation. Department of Biotechnology, Fachhochshule Wihenstephan, Freising, Germany.

15. Reinman, Mikko. 1999. Comparison of trehalose metabolism in brewer's and baker's yeasts. (Finnish language) Department of Biosciences; Division of Biochemistry, University of Helsinki, Finland.

16. Vainio, Anu. 1999. Physiology studies of *Trichoderma reesei* in chemostat cultivations: the role of the catabolite repressor protein CREI. Department of Biological & Environmental Science, University of Jyväskylä, Finland.

17. Markkula, Tuomas. 2000. Fermentation of high gravity and very high gravity worts by Finnish brewer's yeasts. (Finnish language) Department of Chemical Technology, Helsinki University of Technology, Finland.

18. Muurman, Jolanda. 2000. Enzymology studies of redox recycling enzymes in recombinant yeast. Department of Biotechnology, Van Hall Institute, Leeuwarden, The Netherlands.

19. Pitkänen, Juha-Pekka. 2000. Metabolic Flux analysis of xylose utilization in genetically engineered *Saccharomyces cerevisiae* with $^{13}C$-labeled substrates. Department of Chemical Technology, Helsinki University of Technology, Finland.

20. Rautio, Jari. 2000. Effects of carbon catabolite repression on maltose metabolism in brewing fermentations. Department of Biochemistry, University of Oulu, Finland.

## X. Department of Food Science, University of Udine, Italy. Communicated by M. Manzano <marisa.manzano@dsa.uniud.it>.

Publications 1992-2000.

1. Romandini P., Tallandini L., Beltramini M., Salvato B., Manzano M., de Bertoldi M., Rocco G. 1992. Effects of copper and cadmium on growth, superoxide dismutase and catalase activities in different yeast strains. Comparative Biochem. and Physiol. **103c**(2):255-262

2. Manzano M., Romandini,P;, de Bertoldi M., Beltramini M., Salvato B., Cozzani I. 1992. Interaction among heavy metals and methanol affecting superoxide dismutase activity in *Saccharomyces cerevisiae*. Comp. Biochem and Physiol. **105c**(2):175-178.

3. Manzano M., Sarais I., Comi G., de Bertoldi M. 1993. Copper, cadmium and methanol as factor affecting the growth of *Candida boidinii*. M.A.N. **11**:443-446.

4. Sarais I., Manzano M., de Bertoldi M., Romandini P., Beltramini M., Salvato B., Rocco G.P. 1994. Adaptation of a *Saccharomyces cerevisiae* strain to high copper concentrations. BioMetals **7**:221-226.

The following papers have been recently published.

5. G. Comi, M. Maifreni, M. Manzano, C. Lagazio, L. Cocolin 2000. Mitochondrial DNA restriction enzyme analysis and evaluation of the enological characteristics of *S. cerevisiae* strains isolated from grapes of the wine producing area of Collio (Italy). Int. J. Food Microbiol. **58**:117-121.

A total of 70 strains of Saccharomyces cerevisiae were isolated from different grapes from the Collio Region. Chemical parameters and mitochondrial DNA (mtDNA) restriction patterns were determined. Higher alcohols were the main useful for differentiating between strains, whereas the mtDNA analysis demonstrated a high genetic variability between strains. A weak correlation was observed when the dendrograms obtained from the chemical and genetic results were compared.

6. Manzano M., Cocolin L. Citterio B., Conte L., de Bertoldi M., Comi G., Santovito G., Beltramini M., Salvato B. 2000. Biochemical responses in a *Candida famata* strain adapted to high copper concentrations. Biometals (in press).

A strain of *Candida famata* was adapted to high copper concentration (1.26 mM) and a number of biochemical parameters have been tested, in order to get information on the mechanisms of metal toxicity and detoxification as well as on the metabolic responses to the treatment. The cytoplasmic levels of superoxide dismutase, peroxidase and glutathione were found significantly increased with respect to control cells, in contrast to catalase which is not affected. The activities of enolase and of triosephosphate isomerase are found to decrease as a consequence of the exposure to copper. Statistically significant differences in the content of some amino acids are found between copper-treated and control cells.

## XI. Departamento de Genética, División de Biotecnología Industrial. Centro de Ingeniería Genética y Biotecnología. POBox 6162, CP 10600, Havana, Cuba. Communicated by J. Menéndez <javier.menendez@cigb.edu.cu>.

The following doctoral dissertations were successfully defended.

1. Menéndez, J. 1998. Study of the pyruvate carboxylase genes from *Saccharomyces cerevisiae* and *Pichia pastoris.* Centro de Ingeniería Genética y Biotecnología. Havana, Cuba.

2. García, B. 1999. Isolation and characterization of the dextranase-encoding gene from *Penicillium minioluteum* and its expression in *Pichia pastoris*. Centro de Ingeniería Genética y Biotecnología. Havana, Cuba.

3. Paifer, E. 1999. Isolation and expression of the alfa-amylase gene from *Bacillus licheniformis* in the yeast *Pichia pastoris*. Centro de Ingeniería Genética y Biotecnología. Havana, Cuba.

We report the following publications (1995-2000).

4. Paifer, E., Menéndez, J., Basabe, L., Yong, V., Rodriguez, L. and Delgado, J. 1995. Isolation of the β-galactosidase gene from *Kluyveromyces fragilis.* Biotecnología Aplicada **1:**42-45.

5. Basabe, L., Cabrera, N., Yong, V., Menéndez, J., Delgado, J. and Rodríguez, L. 1996. Isolation and characterization of mutants as an approach to a transformation system in *Kluyveromyces marxianus.* Current Genetics **30:**89-92.

6. Roca, H., García, B., Rodríguez, E., Mateu, D., Coroas, L., Cremata, J. A., García, R., Pons, T. and Delgado, J. 1996. Cloning of the *Penicillium minioluteum* gene encoding dextranase and its expression in *Pichia pastoris*. Yeast **12:**1187-1200.

7. Menéndez, J., Delgado, J and Gancedo, C. 1998. Isolation of the *Pichia pastoris PYC1* gene encoding pyruvate carboxylase and identification of a suppressor of the *pyc* phenotype. Yeast **14:**647-654.

8. Menéndez, J. and Gancedo, C. 1998. Regulatory regions in the promoters of *Saccharomyces cerevisiae PYC1* and *PYC2* genes encoding isoenzymes of pyruvate carboxylase. FEMS Microbiology letters **2:**345-352.

9. Montesino, R., Quintero, O., García, R. and Cremata, J. A. 1998. Glycosylation profiling of heterologous protein expressed in the methylotrophic yeast *Pichia pastoris*. In Methods in Molecular Biology. *Pichia* protocols. Ed. James Cregg and David Higgins. Humana Press, Vol. 103, Chap. **8:**95-105.

10. Montesino, R., García, R., Quintero, O. and Cremata, J. A. 1998. Variation in N-linked oligosaccharide structures on heterologous proteins secreted by the methylotrophic yeast *Pichia pastoris*. Protein Expression and Purification **14:**197-207.

11. Gómez, C., Menéndez, J. and García, B. 1999. Expression of the *Dex* gene in the yeast *Kluyveromyces lactis*. Biotecnología Aplicada **16:**97-102.

12. Montesino, R., Nimtz, M., Conradt, H., Quintero, O., García, R., Falcón V. and Cremata, J. A. 1999. Characterization of the oligosaccharides assembled on the *Pichia pastoris*-expressed recombinant Aspartic protease. Glycobiology **9:**1037-1043.

13. García, B., Rodríguez, E., Rivero, T., Hidalgo Y and Menéndez, J. 2000. Dextranase expression in two different host-vector systems of the methylotrophic yeast *Pichia pastoris.* Biotecnología Aplicada **17**:11-15.

14. Beldarraín, A., Acosta, N., Montesino, R., Mata M. and Cremata, J. A. 2000. Characterization of *Mucor pusillus* rennin expressed in *Pichia pastoris*. Enzymatic, spectroscopic and calorimetric studies. Biotechnology and Applied Biochemistry **31:**77-84.

15. Paifer, E., Figueroa, N., Montesino, R. and Cremata, J. A. 2000. Characterization of a recombinant alfa-amylase expressed in *Pichia pastoris*. Biotecnología Aplicada. In press.

16. Menéndez, J., García, B. and Hidalgo, Y. 2000. A new method for the selection of multi-copy transformants of *Pichia pastoris*, using 3-amino-1,2,4 triazol. Biotechnique, submitted.

17. Delfín, J., Perdomo, W., García, B. and Menéndez, J. 2000. Isolation and sequence of the *MIG1* homologue from the yeast *Candida utilis.* Yeast, submitted

18. Betancourt, L., H., García, R., González, J., Montesino, R., Quintero, O. and Cremata, J. A. 2000. Dextranase from *Penicillium minioluteum* expressed in *Pichia pastoris*: Two host cells with minor differences in N-Glycosylation processing. Glycobiology, submitted.

## XII. Research Institute for Viticulture and Enology, Matúškova 25, 833 11 Bratislava, Slovakia Communicated by E. Minárik.

The following two contributions were recently published.

1. F. Malík. 2000. Active dry wine yeasts. Part II. Vinohrad **38**:109-110 (in Slovak).

The author deals with the transformation of properties of wine yeasts caused by fluid drying in active dry wine yeast elaboration. Information is complemented by the evaluation of the quality of individual preparations. In regard to the Office International de la Vigne et du Vin (O.I.V.) in Paris, the following criteria are recommended: dry matter min. 82%, viable yeast cells $>10^9 \cdot g^{-1}$, number of foreign yeast species < 0.01%, number of filamentous fungi $< l.g^{-1}$, number of bacteria $< 10^5 \cdot g^{-1}$. Finally, drying versus wine yeast properties are discussed.

2. F. Malík. 2000. The phenomenon of modern enology - pure wine yeast starters. Vinohrad **38**:30-33 (in Slovak).

Morphologically, biochemically, and technologically defined pure wine yeast starters have an invaluable position and task in modern winemaking. Their use ensures the production of wines characterized by an excellent grape bouquet. Genetically manipulated wine yeast strains and the possibility of their use in the vinification process, mainly in the fermentation of grape must and in the refermentation of wine in sparkling wine production are discussed in detail. The future of those yeast strains remains, however, uncertain and subject to discussion. Practical aspects are discussed in detail.

## XIII. Department of Molecular and Cellular Biology, University of California, Berkeley, California, 94720. Communicated by R.K. Mortimer <robertkm@uclink4.berkeley.edu>.

The following are my most recent publications.

1. Prior, B., Baccari, C. and Mortimer, R. 1999. Selective breeding of *Saccharomyces cerevisiae* to increase the levels of glycerol in wines. J. Int. Sci. Vigne Vin **33**:57-65.

2. Johnston, J., Baccari, C. and Mortimer, R. 2000. Genotypic characterization of strains of commercial wine yeasts by tetrad analysis. Res. in Microbiol. **151**: 583-590.

3. Cavalieri, D., McGovern, P., Mortimer, R. and Polsinelli, M. 2000. Ancient Wine Yeast. submitted to Nature.

4. Mortimer, R. 2000. Evolution and variation of the yeast (*Saccharomyces*) Genome. 2000. Genome Res. April, 403-409.

5. Mortimer, R. K. 2000. *Kloeckera apiculata* concentrations control the rates of natural fermentations. Riv. de Viticolt. e Enol. (accepted for publication).

6. Mortimer, R. K. 2000. *Saccharomyces cerevisiae*. Encycl. of Genet., S.A. Brenner, editor. accepted for publication.

7. Mortimer, R,. K. 2000. *Saccharomyces* chromosomes Encycl. of Genet., S.A. Brenner, editor. accepted for publication.

8. Cavaliere, D., Barberio, C., Casalone, E., Pinzauti, F., Sebastiani, E., Mortimer, R. and Polsinelli, M. 2000. Metodi genetici e moleculari per lostudio della biodiversita in populazioni di *S. cerevisiae*. Biodiversita, Atti del Convegno Nazionale Alghero, 8-11 Setembre, 1998, 1001-1004.

9. Mortimer, R. K, Mel, H. C. and Blakeley, E. 2000. Cornelius A. Tobias. In Memoriam, University of California, Berkeley.

10. Mortimer, R. K. 2000. Origin and diversity of the yeast *Saccharomyces cerevisiae*. in preparation for Methods in Yeast Genetics and Molecular Biology.

## XIV. 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>.

G.I.N. thanks the Organizing Committee of the ISY2000 for the invitation to give a lecture. The following are publications for 2000.

1. G.I. Naumov. 2000. New variety *Saccharomyces bayanus* var. *uvarum* comb. nov. revealed by genetic analysis. Microbiology (Engl. Transl.) **69**(3):338-342.

2. G.I. Naumov. 2000. Wild European species *Zygofabospora krassilnikovii* is an ancestor of the dairy yeast *Z. lactis*. Dokl. Biol. Sciences **372**:321-324.

3. G.I. Naumov, I. Masneuf, E.S. Naumova, M. Aigle, D. Dubourdieu. 2000. Association of *S. bayanus* var. *uvarum* with some French wines: genetic analysis of yeast populations. Research in Microbiol. **151**(8):683-691.

4. G.I. Naumov, E.S. Naumova, I. Masneuf, M. Aigle, V.I. Kondrat'eva, D. Dubordieu. 2000. Natural polyploidization of some cultured yeast *Saccharomyces* sensu stricto: auto- and allotetraploidy. Syst. Appl. Microbiol. System. Appl. Microbiol. **23**(3):442-229.

5. G.I. Naumov, S.A. James, E.S. Naumova, E.J. Louis, I.N. Roberts. 2000. Three new species in the *Saccharomyces* sensu stricto complex: *Saccharomyces cariocanus, Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. Int. J. Syst. Evol. Microbiol. **50**:1931-1942.

6. Naumov G.I., Naumova E.S., Aigle M., Masneuf I., Belarbi A. 2000. Genetic reidentification of the pectinolytic yeast strain SCPP as a *Saccharomyces bayanus* var. *uvarum.* Appl. Microbiol. Bitechnol. (in press).

Using genetic hybridization analysis, pulsed-field gel electrophoresis of chromosomal DNA and PCR/RFLP analysis of the *MET*2 gene, we reidentified 11 Champagne yeast strains. Two of them, SCPP and SC4, were found to belong to *Saccharomyces bayanus* var. *uvarum* and the remaining strains to *S. cerevisiae*. Strain SCPP (CLIB 2025) of *S. bayanus* var. *uvarum* is known as a producer of three pectinolytic enzymes.

7. E.S. Naumova, N.G. Tokareva, I.P. Bab'eva, G.I. Naumov. 2000. Molecular and genetic analyses of populations of the yeasts *Komagataea* (*Williopsis*) *pratensis*. Microbiology (Engl. Transl.) (in press).

Using UP-PCR, dot blot hybridization and izozyme electrophoresis, we compared 15 isolates of the yeast *Komagataea pratensis* from two geographic regions. It is shown that all the strains belonged to the same biological species. Two populations studied differ on PCR-profiles with the microsatellites primers $(CAC)_5$ and $(GACA)_4$, as well as on some physiological properties.

8. G.I. Naumov G.I., E.S. Naumova. 2000. Taxonomic and evolutionary genetics of the yeast *Zygofabospora* (*Kluyveromyces*) *lactis.* Tenth Int. Symp. on yeasts, 27 August-1 September 2000, Papendal, Arnhem, The Netherlands, 185-186.

9. G.I. Naumov. 2000. *Zygofabospora krassilnikovii* Kudriavzev, a wild European species, is an ancestor of the dairy yeast *Zf. lactis* (Dombrowski) G. Naumov. XIII$^{th}$ Meeting on the Biology of *Kluyveromyces*. Leiden (The Netherlands), September 1-3, 2000, 27.

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

Some recent publications involving yeast from my group follow.

1. H. Golias, G.J. Dumsday, G.A. Stanley and N.B. Pamment 2000. Characteristics of cellulase preparations affecting the simultaneous saccharification and fermentation of cellulose to ethanol , Biotechnol. Letts **22**:617-621.

The cellulase, Spezyme CP from Genencor, widely used for the simultaneous saccharification and fermentation (SSF) of cellulose to ethanol, contained enough β-glucosidase to prevent cellobiose accumulation in SSF's with a conventional non-cellobiose fermenting *Saccharomyces cerevisiae* strain. The product also contained substances inhibitory to the growth of the recombinant ethanologen *Klebsiella oxytoca* P2, emphasising the need to check for inhibition effects in SSF experimentation.

These findings are relevant to attempts to evaluate novel recombinant cellobiose-fermenting microbial strains.

2. A.R. Barber, H. Hansson and N.B. Pamment. 2000. Acetaldehyde stimulation of the growth of *Saccharomyces cerevisiae* in the presence of inhibitors found in lignocellulose-to-ethanol fermentations. J. Ind. Microbiol. Biotechnol. 25:104-108.

The addition of small quantities of acetaldehyde to fermentations containing inhibitory concentrations of furfural, acetate and other compounds typically present in lignocellulosic hydrolysates significantly reduced the lag phase of yeast growth and stimulated ethanol production. Similar effects were observed when acetaldehyde (0.06 g/l) was added to fermentations of a birch wood hydrolyzate produced by steam/acid pretreatment. Acetaldehyde addition appears to have potential as a low-cost alternative (or adjunct) to current procedures for medium detoxification in lignocellulose-to-ethanol fermentations, particularly those in which high inhibitor concentrations are generated through recycling of the culture broth.

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

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

1. M. Špírek, A. Horváth, J. Piškur, and P. Sulo. 2000. Functional co-operation between the nuclei of *Saccharomyces cerevisiae* and mitochondria from other yeast species. Curr. Genet. (in press).

We elaborated a simple method that allows the transfer of mitochondria from collection yeasts to *Saccharomyces cerevisiae*. Protoplasts prepared from different yeasts were fused to the protoplasts of the *ade2-1*, *ura3-52*, *kar1-1*, $\rho^0$ strain of *S. cerevisiae* and were selected for respiring cybrids on plates containing 5-fluoroorotic acid and a non-fermentable carbon source. The identity of putative cybrids was assessed by restriction analysis of mitochondrial. DNA, pulse field electrophoresis and tetrad analysis. In the comprehensive screening, only mitochondrial genomes from synonymous species (*S. italicus*, *S. oviformis*, *S. capensis* and *S. chevalieri* exhibited complete compatibility with S. cerevisiae nuclei. The closely related *S. douglasii* mitochondrial genome could also partially restore respiration-deficiency in $\rho^0$ *S. cerevisiae*, whereas mitochondrial genomes from phylogenetically less related species could not.

2. P. Kristoffersen, G.B. Jensen, K. Gerdes, and J. Piškur. 2000. Bacterial toxin-antitoxin gene system as containment control in yeast cells. Appl. Environ. Microbiol. (in press).

The potential of a bacterial toxin-antitoxin gene system for use in containment control in eukaryotes was explored. The *Escherichia coli relE* and *relB* genes were expressed in the yeast *Saccharomyces cerevisiae*. Expression of the *relE* gene was highly toxic to yeast cells. However, expression of the *relB* gene counteracted the effect of *relE* to some extent, suggesting that toxin-antitoxin interaction also occurs in *S. cerevisiae*. Thus, bacterial toxin-antitoxin gene systems also have potential applications in the control of cell proliferation in eukaryotic cells, especially in those industrial fermentation processes in which the escape of genetically modified cells would be considered highly risky.

3. C. Groth, R.F. Petersen, and J. Piškur. 2000. Diversity in organization and the origin of gene orders in the mitochondrial DNA molecules of the genus *Saccharomyces*. Mol. Biol. Evol. (in press).

Sequencing of the *Saccharomyces cerevisiae* nuclear and mitochondrial genomes provided a new background for studies on the evolution of the genomes. In this study, mitochondrial genomes of a number of *Saccharomyces* yeasts were mapped by restriction enzyme analysis, the orders of the genes were determined, and two of the genes were sequenced. The genome organization, i.e., the size, presence of intergenic sequences, and gene order, as well as polymorphism within the coding regions, indicate that *Saccharomyces* mtDNA molecules are dynamic structures and have undergone numerous changes during their evolution. Since the separation and sexual isolation of different yeast lineages, the coding parts have been accumulating point mutations, presumably in a linear manner with the passage of time. However, the accumulation of other changes may not have been a simple function of time. Larger mtDNA molecules belonging to *Saccharomyces sensu stricto* yeasts have acquired extensive intergenic sequences, including guanosine-cytosine-rich clusters, and apparently have rearranged the gene order at higher rates than smaller mtDNAs belonging to the *Saccharomyces sensu lato* yeasts. While within the *sensu stricto* group transposition has been a predominant mechanism for the creation of novel gene orders, the *sensu lato* yeasts could have used both transposition- and inversion-based mechanisms.

4. R.F. Petersen, G. Marinoni, M.L. Nielsen, and J. Piškur. 2000. Molecular approaches for analyzing diversity and phylogeny among yeast species. In: Ernst, JE and Schmidt, A (eds). Dimorphism in human pathogenic and apathogenic yeasts. Contrib. Microbiol. Basel, Karger, **5**:15-35.

5. Z. Gojkovic, K. Jahnke, K.D. Schnackerz, and J Piškur. 2000. *PYD2* encodes 5,6-dihydropyrimidine amidohydrolase, which participates in a novel fungal catabolic pathway. J. Mol. Biol. **295**:1073-1087.

Most fungi cannot use pyrimidines or their degradation products as the sole nitrogen source. Previously, we screened several yeasts for their ability to catabolise pyrimidines. One of them, *Saccharomyces kluyveri*, was able to degrade the majority of pyrimidines. Here, a series of molecular techniques have been modified to clone pyrimidine catabolic genes, study their expression and purify the corresponding enzymes from this yeast. The *pyd2-1* mutant, which lacked the 5,6-dihydropyrimidine amidohydrolase (DHPase) activity, was transformed with wild-type *S. kluyveri* genomic library. The complementing plasmid contained the full sequence of the *PYD2* gene, which exhibited a high level of homology with mammalian DHPases and bacterial hydantoinases. The organisation of *PYD2* showed a couple of specific features. The 542-codons open reading frame was interrupted by a 63 bp intron, which does not contain the *Saccharomyces cerevisiae* branch-point sequence, and the transcripts contained a long 5' untranslated leader with five or six AUG codons. The derived amino acid sequence showed similarities with dihydroorotases, allantoinases and uricases from various organisms. Surprisingly, the *URA4* gene from *S. cerevisiae*, which encodes dihydroorotase, shows greater similarity to *PYD2* and other catabolic enzymes than to dihydroorotases from several other non-fungal organisms. The *S. kluyveri* DHPase was purified to homogeneity and sequencing of the N-terminal region revealed that the purified enzyme corresponds to the PYD2 gene product. The enzyme is a tetramer, likely consisting of similar if not identical subunits each with a molecular mass of 59 kDa. The *S. kluyveri* DHPase was capable of catalysing both dihydrouracil and dihydrothymine degradation, presumably by the same reaction mechanism as that described for mammalian DHPase. On the other hand, the regulation of the yeast *PYD2* gene and DHPase seem to be different from that in other organisms. DHPase activity and Northern analysis demonstrated that *PYD2* expression is inducible by dihydrouracil, though not by uracil. Apparently, dihydrouracil and DHPase represent an important regulatory checkpoint of the pyrimidine catabolic pathway in *S. kluyveri.*

6. R.B. Langkjaer, M.L. Nielsen, P. R. Daugaard, W. Liu and J. Piškur. 2000. Yeast chromosomes have been significantly reshaped during their evolutionary history. J. Mol. Biol. (in press).

The structure of the first eukaryotic genome, belonging to *Saccharomyces cerevisiae*, has been deduced; however, very little is known about its origin. In order to trace events that led to the current state of the *Saccharomyces* nuclear genomes, random fragments of genomic DNA from three yeasts were sequenced and compared to a *S. cerevisiae* database sequence. Whereas *S. cerevisiae* and *Saccharomyces bayanus* show perfect synteny, a significant portion of the analysed fragments from *Saccharomyces servazii* and *Saccharomyces kluyveri* show a different arrangement of genes when compared to *S. cerevisiae*. When the sequenced fragments were probed to the corresponding karyotype, a group of genes present on a single chromosome of *S. servazzii* and *S. kluyveri* had homologues scattered on several *S. cerevisiae* chromosomes. Apparently, extensive reorganisation of the chromosomes has taken place during evolution of the *Saccharomyces* yeasts. In addition, while one gross duplication could have taken place, at least a few genes have been duplicated independently at different time-points in the evolution.

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

The following book chapter is in press.

1. Prillinger, H., Lopandic, K., Schweigkofler, W., Deak, R., Aarts, H,J.M, Bauer, R., Maraz, A. Molecular phylogeny and systematics of fungi with special reference to the Asco- and Basidiomycota. In: Chemical Immunology: Molecular aspects of Fungal Allergy and Pathogenicity. Edited by Breitenbach, M. & Crameri, R.. S. Karger AG, Basel.

The following is the summary of an invited lecture presented at the University of Tokyo (Annual meeting 2000 of the Japan Society for Microbial Systematics) - Microbial Systematics, Today and Tomorrow (at Sanjo Kaikan, Hongo, Tokyo). A similar lecture was presented at Osaka (Nov. 2nd) upon invitation from IFO. I plan also to give three similar lectures in Egypt (Universities of Cairo, Assut, El Menia) from Nov. 15 to 25.

## 2. H. Prillinger, K. Lopandic, W. Schweigkofler, K. Sterflinger. 2000. Molecular phylogeny and systematics of the fungi with special reference to the Asco- and Basidiomycota.

Dedicated to my three Japanese friends and "kings of the morning country", Dr. T. Nakase, Prof. J. Sugiyama, and Prof. Y. Yamada.

Already in 1965 Zuckerkandl & Pauling argued that sequence comparisons of informational macromolecules permit the evaluation of evolutionary relatedness, thereby fomenting a phylogenetic revolution, especially in prokaryotic and lower eukaryotic organisms. Molecular comparisons show that life on this planet divides into three primary groupings. Woese & al. (1990) introduced a new taxon called "domain" which is above the level of kingdoms. The three domains, Archea, Bacteria, and Eucarya are very dissimilar, the differences that separate them being of more profound nature than differences that separate typical kingdoms, such as animal and plants. Most eukaryotic diversity is nested within the densely branched crown of the phylogenetic tree (Sogin & al., 1996). Major clades that branch near a common point include the kingdoms (Fig. 1.) Zoobionta or Animalia (Metazoa + unicellular relatives), Chlorobionta or green Plants (Chlorophyta, Bryophyta, Vascular Plants), Mycobionta or true Fungi (Chytridio-, Zygo-, Asco-, and Basidiomycota), Heterokontobionta or Stramenopila (Diatoms, brown algae, golden brown algae, Oomycota, Hyphochytridiomycota, slimenets), Rhodobionta (red algae) and Alveolobionta or Alveolates (ciliates, dinoflagellates, apicomplexans; not shown in Fig. 1.). In the phylogenetic trees based on complete sequences of the 18S rDNA the phagotrophic plasmodial slime molds (Myxomycota) and cellular slime molds (Dictyosteliomycota) diverge prior to the terminal radiation of eukaryotes (Bruns & al., 1992). In contrast, parsimony analysis of amino acid sequences of elongation factor (EF-1α), a protein involved in the translation of messenger RNA, strongly supports a monophyletic origin of the Dictyosteliomycota and Myxomycota, and the amoeboflagellate protostelid *Planoprotostelium* (kingdom Myxobionta or Mycetozoa). Based on complete 18S ribosomal DNA sequencing the plant parasitic slime mold *Plasmodiophora brassicae* (Plasmodiophoromycota), a severe pathogen of crucifers, may be more closely related to the Alveolobionta than to any other fungi (Castlebury & Domier, 1998).

Evidence from complete 18S rDNA sequence divergence (Fig. 1.) put an end to the discussion of the kingdom Mycobionta or Eumycota (Cavalier-Smith, 1981, Jahrmann & Prillinger, 1983) and corroborated the existence of four naturally related phyla or divisions the Chytridiomycota, the Zygomycota, the Ascomycota, and the Basidiomycota. Based on complete sequences of the 18S ribosomal RNA gene and the amino acid sequence of the elongation factor 1α, the Mycobionta and Zoobionta appeared as a sister group (Hasegawa & al., 1985, Baldauf & Palmer, 1993). Our phylogenetic analysis of the **Chytridiomycota** and **Zygomycota** (Fig. 1.) corroborates the view of Sugiyama (1998) that both phyla are not monophyletic and instead suggest that losses of flagella occurred in several lineages during the course of fungal evolution. The yeast form, denoted by the term "coccal" (i. e. a unicellular organism having a rigid cell wall outside its plasma membrane) occupies a basal position in the Zygo-, Asco-, and Basidiomycota (Oberwinkler, pers. comm.) but seems to be derived in the Chytridiomycota (e.g. *Basidiobolus*; Prillinger, 1987).

The concept of "sexual differentiation" was introduced by Prillinger (1982, 1984, 1987). According to this concept the yeast isolates from the agarics *Asterophora lycoperdoides* and *A. parasitica* were interpreted as "sexual symbionts" and missing links in a polyphyletic evolution from mycoparasitism via primary homothallism to heterothallism. Based on partial sequences of the 26S rDNA (D1/D2-region) the yeast isolates from *A. lycoperdoides* and *A. parasitica* differed in 4 base pairs. The closest relative *Cryptococcus humicola* could be distinguished from the *Asterophora* yeasts in 2 base pairs (Schweigkofler, 1998). Based on complete sequences of the 18S rDNA and partial sequences of the 26S rDNA the genus *Cryptococcus* appeared heterogenous with respect to the type species *C. neoformans* (Fell & al. 2000). We therefore have included *C. humicola* and the two genotypically distinct yeast isolates from the agarics *A. lycoperdoides* and *A. parasitica* in the new genus *Asterotremella* as *A. humicola, A. lycoperdoides*, and *A. parasitica*. As a conclusion the trinity system *Russula, Asterophora* and *Asterotremella* is remarkable, and may be fundamental in the evolution of sexuality, especially primary homothallism and subsequently heterothallism in fungi. Similarly yeast isolates from the Basidiomycota *Ganoderma adspersum, Marasmius ramealis*, and *Polyporus ciliatus* were genotypically identified to belong to the plant pathogenic fungus *Ustilago maydis* and the spermatia-trichogyne-like fertilization reaction observed between the yeasts and the mycelia of the different polypores and the agaric can be traced back to mycoparasitic interactions. From our data we have to conclude that this fertilization reaction has evolved polyphyletically within the Ascomycota and rust fungi.

Phylogenetic trees inferred from 18S rDNA sequence divergence indicate the existence of two distinct phyla or divisions among the higher fungi, the **Ascomycota** (Fig. 2.) and the **Basidiomycota** (Fig. 3.). The molecular phylogenies do not support the existence of the Deuteromycetes as a distinct taxon within the Mycobionta. Molecular characters offer the potential for combining the dual classification into one natural classification (Reynolds & Taylor, 1993). Similarly the Gastromycetes appear as an artificial taxon. Gastroid genera and species have evolved polyphyletically within the Ascomycota (Fig. 3.: Eurotiales: *Elaphomyces*, Pezizales: *Tuber*) and Basidiomycota (Fig. 4.: Gomphales: *Geastrum*, *Phallus*, Boletales: *Scleroderma*, Agaricales: *Calvatia, Crucibulum, Lycoperdon*; compare Hibbett & Thorn, 2000). Yeasts occur in all three classes of the Ascomycota and Basidiomycota. The

**dimorphism** seems to be of fundamental importance for a rapid evolution of the Asco- and Basidiomycota.

Based on complete or nearly complete 18S ribosomal DNA sequencing (Fig. 3.), the qualitative and quantitative monosaccharide pattern of purified cell walls (Fig. 3.; Lopandic, 1998), the ultrastructure of septal pores (Bauer pers. comm.), and urease activity the Ascomycota can be separated in three distinct classes: the **Hemiascomycetes**, the **Protomycetes**, and the **Euascomycetes**. In our FITCH-tree the Protomycetes and Euascomycetes appear as a sister group with good bootstrap support. The Hemiascomycetes occupy a basal position. The lack of fruit bodies and urease activity beside some additional cytological and molecular characters (Prillinger & al., 2000) corroborate the basal position of the Hemiascomycetes. Different from the Protomycetes and Euascomycetes mannose and glucose are common as cell wall sugars beside glucose, mannose, and galactose. The Hemiascomycetes include the classical yeasts except *Saitoella* and *Schizosaccharomyces*. *Eremothecium ashbyi* and *E. gossypii* are phytoparasitic, filamentous fungi on cotton, which have to be included within the Saccharomycetaceae (Prillinger & al., 1997). Based on coevolution the Hemiascomycetes may be traced back by parasitic *Metschnikowia* species with their crustacean host at least to the Cambrian (500 million years ago; Prillinger & al., 1997). Cell wall sugars (glucose, mannose, galactose and glucose, mannose, galactose, rhamnose) and urease activity (+) from unicellular and dimorphic Protomycetes and Euascomycetes corroborate the sister group relationship of both classes in our investigations. Within the Protomycetes (Fig. 3.) unicellular (*Pneumocystis, Saitoella, Schizosaccharomyces*), dimorphic (*Protomyces, Taphrina*) as well as filamentous fungi with fruiting bodies (*Neolecta*) are found. Morphological and ultrastructural data of *Mixia osmundae* (Bauer & Oberwinkler pers. comm.), cell wall sugars of *Taphrina vestergrenii* (Prillinger & al., 1990) and 5S ribosomal DNA data from Gottschalk & Blanz (1985) and Walker (1985) suggest the Protomycetes be ancestral to the Basidiomycota, especially the Urediniomycetes. Within the Euascomycetes fruiting bodies (apothecia, cleistothecia, perithecia, and pseudothecia) dominate, yeast-like fungi (*Aureobasidium, Eremascus, Symbiotaphrina*), however, occur sporadically. Although the perithecia of *Ceratocystis* and *Ophiostoma* species resemble closely morphologically and exhibit a similar ascospore discharge, both genera are phylogenetically distinct and belong to the separate orders Microascales and Ophiostomatales (Fig. 3.). Similarly genera of the bitunicate Ascomycota cluster at least in three different orders. Humanpathogenic species commonly appear within the Chaetothyriales which are close to the cleistothecial Eurotiales (Fig. 3.). Plantpathogenic and endophytic species are common within the Dothideales and Pleosporales, which form a phylogenetic sister group distinct from the Chaetothyriales. Black yeasts as well as rock inhabiting and rock destroying fungi, however, can be found within all three orders (Sterflinger, 2000). The typical clump-like in situ morphology of rock inhabiting fungi is interpreted as an adaption to **extreme environmental conditions** (high UV-radiation, high temperatures, low water availability) that occurred evolutionary in distinct orders of the Euascomycetes (Sterflinger, 1998).

In the Basidiomycota the qualitative and quantitative monosaccharide pattern of purified cell walls correlates well with complete sequences of the 18S ribosomal DNA to separate the Basidiomycota in three distinct classes (Fig. 3.; Prillinger & al., 1993a). The *Microbotryum*-type (dominant amounts of mannose, glucose, galactose, fucose, sporadically rhamnose) correlates with the **Urediniomycetes**, the *Ustilago*-type (dominant amounts of glucose, mannose, galactose) with the **Ustilaginomycetes** and the *Tremella*-type (glucose, mannose, xylose, galactose may be present) with the **Hymenomycetes**. Xylose, however, may disappear by reduction in some derived genera of the Hymenomycetes (*Schizophyllum commune*, yeast stages from agarics of fungus-growing ants; Müller & al., 1998). *Rhodotorula yarrowii* is so far the only basidiomycetous fungus which has xylose in its cell walls, but dominant amounts of mannose, however, suggest an Urediniomycetes affinity (Boekhout & al., 2000). Within the derived Hymenomycetes yeast stages are common in the Tremellomycetidae but are rare within the Hymenomycetidae. There are only reports from some *Collybia* species (Prillinger & al., 1993b) and agarics from fungus growing ants (Mueller & al., 1998). The classical Homobasidiomycetes were separated by Hibbet & Thorn (2000) tentatively by molecular data in eight major clades. In accordance with Oberwinkler (1977) these clades commonly comprise resupinate, bracket-like, club-shaped or coralloid, pileate, and gasteroid fruiting bodies with various types of hymenia (e.g. corticoid, toothed, poroid, agaricoid, boletoid, gleba chambers). Similar to Oberwinkler (1977) we used distinct orders to circumscribe these clades: Agaricales, Boletales, Cantharellales, Gomphales, Hymenochaetales, Polyporales, Russulales, and Thelephorales (Fig. 3.; Prillinger & al., 2000). Different from Hibbett & al. (1997) the Schizophyllales appear as an additional distinct group in our phylogram (Fig. 3.).

Within the Polyporales we were able to detect a monogenic mutant in *Polyporus ciliatus*. This mutant with a sporulating *Phlebia*-like hymenium appeared spontanously after apomictic propagation of a haploid, pileate fruiter strain (Prillinger, 1986). In different crossing experiments we were able to obtain compatible mating types and a sporulating dikaryon, which exhibits the resupinate *Phlebia* phenotype. These genetical data agree well with the concept of Oberwinkler (1977) and phylogenetic results presented in Fig. 3. In addition these data confirm the polygenic control of fruit body formation suggested by Prillinger & Six (1983) and exclude the existence of a „fruiting initiation gene“ in *Polyporus ciliatus*, which was postulated by Esser & al. (1977).

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## XVIII. Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal. Communicated by J. P. Sampaio <jss@mail.fct.unl.pt>.

Forthcoming publications.

1. Sampaio, J.P., Gadanho, M. and Bauer, R. 2001. Taxonomic studies on the genus *Cystofilobasidium*: description of *Cystofilobasidium feraegula* sp. nov., and clarification of the status of *Cystofilobasidium lari-marini.* Int. J. Syst. Evol. Microbiol. **51** (in press).

A new species of the genus *Cystofilobasidium* is described as *C. feraegula* sp. nov. The new taxon represents the teleomorphic stage of *Cryptococcus feraegula* and was obtained in mating experiments using three strains deposited in the Portuguese Yeast Culture Collection (mating types A1) and a recent isolate (mating type A2). *Cystofilobasidium feraegula* is characterized using an integrated approach encompassing morphological studies, investigation of the ultrastructure of the septal pore, comparative study of physiological traits, determination of DNA base composition and DNA reassociation experiments, and PCR fingerprinting. During the course of this study a close similarity was detected between *C. lari-marini* and *C. capitatum*. DNA-DNA reassociation experiments gave high homology values, which indicate that *C. lari-marini* must be regarded as a synonym of *C. capitatum*.

2. Sampaio, J.P., Gadanho, M., Santos, S., Filomena, L. D., Pais, C., Fonseca, Á. and Fell, J.W. 2001. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *R. kratochvilovae* and related anamorphic species. Int. J. Syst. Evol. Microbiol. (accepted).

The phenotypic and genetic heterogeneity of the basidiomycetous yeast species *Rhodosporidium kratochvilovae* was investigated in a group of recent isolates and collection strains. A polyphasic taxonomic approach was followed which included micromorphological studies, nuclear staining, determination of sexual compatibility, physiological characterization, comparison of electrophoretic isoenzyme patterns, PCR fingerprinting, determination of molar % of G+C, DNA-DNA reassociation experiments and 26S and ITS rDNA sequence analysis. The results allowed a more natural circumscription of the species, both from the genetic and phenotypic perspectives. The relationships with anamorphic species of the genus *Rhodotorula* were studied and isolates previously identified as *Rh. glutinis* were found to belong to *R. kratochvilovae*. Other isolates included in the study were found to represent members of *Rh. glutinis* var. *dairenensis. Rhodosporidium kratochvilovae* was found to include heterothallic strains, besides those already known to be self-sporulating. A total of 17 isolates, which were found to belong to this species, comprised heterothallic, self-sporulating and anamorphic strains. It is anticipated that integrated polyphasic studies of basidiomycetous yeasts will provide a more coherent classification system and the basis for accurate identification schemes, which in turn are essential for detailed ecological studies.

## XXIX. Fermentations and bioreactors - group, Laboratoire de Génie Chimique CNRS UMR 5503, École Nationale Supérieure d'Ingénieurs de Génie Chimique, 18 Chemin de la Loge, 31078 Toulouse Cedex 4, France. Communicated by P. Strehaiano <Pierre.Strehaiano@ensigct.fr>.

Our group is specialised in stoichio-kinetic studies of microbial growth and production reactions. For several years, the group has worked on pure strain cultivation. The aim is the production of microorganisms or metabolites from single sugars or natural substrates. The team has also an experience on mixed populations studies. In the field of enology, yeast-yeast and yeast-bacteria interactions were analysed on from kinetic point of view, in particular the killer effect and malo-lactic fermentation. Industrial alcoholic fermentation was also studied through a contamination phenomenon by yeast.

Recent publications


1. Strehaiano P., Delia M.L., Taillandier P. 1999. Numération des levures et mesure de la viabilité en vinification. Revue des Oenologues **91**:17-20.

2. Taillandier P., Ramon-Portugal F., Seiller I., Favarel J.L., Nepveu F., Strehaiano P. 1999. Effet de 5 souches de levures sur l'évolution de l'acidité pendant la fermentation alcoolique. Revue Francaise d'Oenologie **178**:48-24.

3. Ramon-Portugal F., Seiller I., Taillandier P., Favarel J.L., Nepveu F., Strehaiano P. 1999. Kinetics of consumption and production of organic acids during alcoholic fermentation by *Saccharomyces cerevisiae*. Food Technol. Biotechnol. **37**(4):235-240.


This paper presents a study dealing with the production and consumption kinetics of the main organic acids, which are present during the alcoholic fermentation. A *Saccharomyces cerevisiae* strain was used. This strain is normally used for winemaking. The experiments were carried out using a synthetic liquid medium. The initial malic acid concentration and the initial pH value were the studied parameters. The kinetics of malic acid consumption and global acid organic production were quantified. The results show that a decrease of pH value favors the malic acid consumption, while an increase of the initial malic acid concentration increases the consumed amount. The acid malic specific consumption rate shows that its assimilation was microbial growth independent while the others organic acids specific production rate show that their excretion was strongly associated to the microbial growth. The pH evolution during the fermentation was followed and explained by the evolution of the global organic acids production.


4. Aranda J., Delia M.L., Riba J.P. 2000. Kinetic study and modelling of the xylitol production using *Candida parapsilosis* in oxygen-limited conditions. Bioprocess Engin. **22**(3):219-225.


Kinetic studies are presented for xylitol production and growth of the yeast *Candida parapsilosis* ATCC 28474. The oxygen supply influence on xylitol production from xylose was investigated. No metabolic activity was detected in anaerobic conditions. In contrast, it was found that under low aeration rates (0.1-0.2 vvm), xylitol is produced. Xylitol production decreases when air flow to reactor is augmented. An unstructured model is proposed for the kinetic behaviour analysis of yeast growing in batch culture. A simplex method was used for the estimation of model parameters.The parameter confidence intervals were also calculated.

5. Aguilar Uscanga M.G., Delia M.l, Strehaiano P. In press. Estudio cinetico sobre el crecimiento de *Brettanomyces bruxellensis* en glucosa a diferentes concentraciones iniciales de acido acetico. Microbiologie-Aliments-Nutrition.

We have been interested in the influence of acetic acid on the growth and the consumption of glucose by *Brettanomyces bruxellensis*. The cultures were run under both anaerobic and aerobic conditions. The interference of the initial pH and the effect of acetic acid were tested. The presence of the organic acid disturbs the growth and the fermentative activity of this yeast when its concentration exceeds 2.0 g/l. *Brettanomyces bruxellensis* proves more sensitive than *Saccharomyces cerevisiae* to the acetic acid presence. The pH (4.5 and 2.5) does not exert a significant influence on the behavior of this yeast. A slight acidity (pH: 3.5 to 3.0) seems to stimulate the fermentative activity. Nevertheless, when the acidity becomes stronger (pH = 2.0) the metabolic activities of the yeast are strongly affected.

6. Salgado Manjarrez E., Albasi C., Riba J.P. In press. A two reservoir hollow fiber bioreactor for the study of mixed population dynamics : design aspect and validation of the approach. Biotechnol. Bioengin.

A two-reservoir, membrane bioreactor for carrying out studies of mixed population dynamics in batch fermentations is presented. Mixing requirements and design aspects for the validity of the approach are presented and discussed. Equations describing mixing times between the reservoirs are presented and compared to the experimental results. The validity of the approach is demonstrated by the study of an amensalistic type interaction, the protein mediated killer phenomenon between two *Saccharomyces cerevisiae* strains. The validation consisted in the comparison between the results obtained in actual mixed culture and the results obtained by keeping the strains separated. A good agreement was found, which demonstrate the viability of the designed bioreactor.

Conference presentations.

7. Strehaiano P., Taillandier P., Gilis J.F., Tataridis P., Delia M.L. 1999. Etude des interactions entre *Saccharomyces cerevisiae* et *Oenococcus oeni.* 12th International Enological Symposium, 31 May - 2 June 1999, Montréal, Canada pp. 280-303.

Wine is a complex medium where different microbial species and genera must live together. The ratios of these different parts of the population are controlled by different kinds of interactions. Competition for the substrate is quite well known while the much more complex amensalism is less studied. Only few qualitative studies are available. In a first step, by using 11 different yeast strains (for alcoholic fermentation) and 24 bacterial strains (for malo-lactic fermentation) we have shown that these microbes interact very often: yeasts are shown able to inhibit lightly or strongly the growth of bacterial strains. After that, we have developed a method to quantify the intensity of this inhibitory effect; so it is possible to compare the sensitivity of a bacteria to different yeast strains as well as to compare the ability of a yeast strain to inhibit the growth of different bacterial strains. Also, it is established that the temperature and the pH of the medium act on the inhibition activity of yeast strains. Last, we have also shown that some interactions can exist between bacterial strains of the MLF. So, it is well known that the inoculation of wine with selected bacterial strains for the MLF is not always successful. This work demonstrates that one of the causes of this failure may be the inhibition of these bacteria by the yeast used for the alcoholic fermentation.

8. Seiller I., Ramon-Portugal F., Taillandier P., Favarel J.L., Nepveu F., Strehaiano P. 1999. Effet de la fermentation alcoolique sur l'acidité du moût: comparaison de 5 souches de *Saccharomyces cerevisiae*. Comm. 6ème Symp. International d'Oenologie, Bordeaux, France, 10-12 juin 1999. Actualités Oenologiques 99, Lonvaud-Funel (Coord.), Lavoisier (Ed.), pp. 283-286.

9. Gilis J.F., Seiller I., Delia M.L. 1999. Effets de certains paramètres physico-chimiques (pH et oxygène) sur la cinétique de croissance de *Brettanomyces*. Comm. 6ème Symp. International d'Oenologie, BORDEAUX, (France), 10-12 juin 1999. Actualités Oenologiques 99, Lonvaud-Funel (Coord.), Lavoisier (Ed.), pp.264-267.

10. Alfenore S., Delia M.L., Strehaiano P. 1999. Le mécanisme killer : étude quantitative de la relation killer/sensible. Comm. 6ème Symp. International d'Oenologie, BORDEAUX, (France), 10-12 juin 1999. Actualités Oenologiques 99, Lonvaud-Funel (Coord.), Lavoisier (Ed.), pp.259-263.

11. Salgado E., Albasi C., Riba J.P. 1999. Transfer of fermentation broth solutes in a hollow fibre reactor for the quantitative study of microbial interactions. Comm. 2nd European Congress on Chemical Engineering (ECCE2), Montpellier, France, 5-7 Oct 1999, in Congress CD-ROM.

This paper presents some preliminary studies that have been carried out in a new bioreactor which is intended to simplify the study of microbial population dynamics. The reactor consist of two reservoirs interconnected by a membrane module. Theoretical expressions regarding the dynamics of mixing are presented. Experimental determinations of the mixing time and mixing of typical fermentation media solutes (such as glucose, ethanol, glycerol and proteins) between the two reservoirs have been studied.

The following posters were presented at the 10th ISY "The rising power of yeasts in science and industry" 27 August - 1 September 2000, in Papendal, The Netherlands.

12. Alfenore S., Delia M.L., Strehaiano P. Hierarchisation of the inhibitory effect between killer and sensitive yeasts.

13. Alfenore S., Delia M.L., Strehaiano P. Environmental parameters affecting the K2 killer toxin production and its activity.

14. Delia M.L., Gilis J.F., Strehaiano P. Sugar consumption by *Brettanomyces bruxellen*sis : some kinetic observations.

15. Le Van V.M., Strehaiano P., Taillandier P. Wort assimilable nitrogen - An important index for fermentation performance estimation in malt-rice brewing.

Doctoral theses, Institut National Polytechnique, Toulouse, France.

16. Aguilar Uscanga M.G. Caractérisation cinétique et métabolique d'une souche de *Brettanomyces*

17. Salgado Manjarrez E. Conception et mise en œuvre d'un bioréacteur à membranes pour l'étude de la dynamique de populations mixtes de micro-organismes.

18. Gilis J.F. Étude de contaminations de fermentations alcooliques industrielles par les levures *Brettanomyces*.

19. Aranda Barradas J.S. Production de biomasse levurienne: influence du procédé sur les potentialités fermentaires des levures.

20. Alfenore S. Interaction de type "killer"chez *Saccharomyces cerevisiae*: Études physiologiques et cinétiques - Quantification et modélisation.

## XX. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.P. van der Walt.

The following paper was published recently.

1. van der Walt, J.P. 1999. Interpreting the automictic ascomycetous yeasts. S. African J. Sci. **95**:440-441.

Automixis, or self-fertilization, defines karyogamy in terms of two sister nuclei formed by the same cell. While the automictic alternation of generations retains the advantages of meiosis, it is strictly inbreeding whereby the stability of the environmentally successful genome is ensured. The automictic cycle admits autodiploidization but precludes heterothallism. In ascogenous yeasts the cycle is expressed in both diplontic and haplontic species. In the former it is manifested by the formation of diploid ascospores, and in the latter by the formation of asci constituted by a mother-cell and attached bud. In budding

ascomycetous diploid species that lack ascal stages (anamorphic species), the cycle implicates the formation of an undifferentiated dangeardien, manifested as a budding meioconidiophore that develops from a chlamydospore or functional teliospore. Because the meioconidiophore lacks the differentiation of the ascus (and basidium), this generative structure serves no taxonomic purpose.

## XXI. Department of Food Science and Technology, University of California, Davis, CA 95616, USA. Communicated by H.J. Phaff.

The following paper is in press.

1. Starmer, W.T., H.J. Phaff, P.F. Ganter, and M.A. Lachance. 2001. *Candida orba* sp. nov., a new cactus-specific yeast species from Queensland, Australia. Int. J. Syst. Evol. Microbiol. (in press).

A new species of yeast from decaying cladodes of *Opuntia* cactus, *Candida orba* is described. This species is a member of a four-species clade of cactophilic yeasts. The new species has only been found in one region of Queensland, Australia, where it was presumably introduced during attempts to eradicate prickly pear cactus. DNA-DNA relatedness, phylogenetic analysis, physiological differences, killer sensitivity profiles and mating reactions establish the distinctness of the taxon as a new species. *C. orba* is most closely related to *Phaffomyces thermotolerans*, a species found associated with columnar cacti in the North American Sonoran Desert. The GenBank accession number for the 26S rDNA D1/D2 sequence in this paper is AF229034. The type strain of *C. orba*, isolated from rotting cladodes of Opuntia stricta in the State of Queensland, Australia, is strain UCD-FST 84-833.1T (=CBS 8782T = NRRL Y-27336T = ATCC MYA-341). Only the $h^-$ mating type of the species has been recovered. The lack of the opposite mating type could be the result of a bottleneck during its introduction to Australia. The original geographic/host distribution of this species in the Americas is unknown.

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

The following paper, whose abstract was given in the last issue, has now been published.

1. M.A. Lachance, A. Pupovac-Velikonja, S. Natarajan, and B. Schlag-Edler. 2000. Nutrition and phylogeny of predacious yeasts. Can. J. Microbiol. **46**:495-505.

The following papers are in print or in press.

2. Lachance, M.A. J.M. Bowles, C. Mueller, and W.T. Starmer. 2000. On the biogeography of yeasts in the *Wickerhamiella* clade and description of *Wickerhamiella lipophila* sp. nov., the teleomorph of *Candida lipophila*. Can. J. Microbiol. **46**:1145-1148.

We describe the new yeast species *Wickerhamiella lipophila*, the teleomorph of *Candida lipophila*, a haploid heterothallic yeast previously isolated from insects associated with morning glories in Hawaii. Both mating types were recovered in the eastern region of Maui, and a single strain was found in the Waimea region of Kauai. We reexamined the mating compatibility of the several strains of *Candida lipophila* previously collected on the island of Hawaii and found them to be fertile mating types that had been overlooked because of the unpredictability of mating and ascus formation. The type culture of *Candida lipophila* [UWO(PS)91-681.3 = CBS 8458, $h^+$] is transferred to the genus *Wickerhamiella*, and strain UWO(PS)00-340.1 (CBS 8812, $h^-$) is designated as isotype. Also found on Maui and Kauai were strains of *Candida drosophilae* that produced a strong extracellular protease. An update on the global distribution of members of the *Wickerhamiella* clade is given.

3. Lachance, M.A., Kaden, J.E., Phaff, H.J., and Starmer, W.T. 2000. Phylogenetic structure of the *Sporopachydermia cereana* species complex. Int. J. Syst. Evol. Microbiol. (in press).

A large number of isolates previously referred to as members of the "*Sporopachydermia cereana* species complex" were examined by various DNA characterization methods, leading to the conclusion that the complex is in fact made up of 10 species, one of which contains three varieties. The sequences of the internal transcribed spacer (ITS) region and the D1/D2 divergent domains of the large subunit ribosomal DNA were determined for representatives of each taxon, and specific primers based on differences in the ITS were designed for rapid identification of five of the taxa. Whereas the data provide additional elements for the calibration of the ITS as a criterion for species delineation, the emerging pattern is that the ITS region does not function as well as the D1/D2 domains as an evolutionary clock. Some taxa appear to be specific for the geographic regions where they were isolated, and the distribution of many taxa is mutually exclusive.

4. Lachance, M.A., J.M. Bowles, M.M. Chavarria Diaz, and D.H. Janzen. 2001. *Candida cleridarum*, *Candida tilneyi*, and *Candida powellii*, three new yeast species isolated from insects associated with flowers. Int. J. Syst. Evol. Microbiol. (in press).

Three new asexual yeast species were isolated from various floricolous insects. *Candida cleridarum* was the dominant species in clerid beetles collected in flowers of various cacti in Arizona and southern California. The sequence of the D1D2 domains of the large subunit ribosomal DNA showed that it is a sister species to *Candida fragi* (0.9% base difference), a yeast isolated once from fermenting strawberries. *Candida tilneyi* and *Candida powellii* were recovered from bees and from nitidulid beetles in flowers of two morning glory (*Ipomoea*) species in northwestern Costa Rica. *C. tilneyi* is most closely related to *Candida geochares*, but differs in the D1D2 sequence by 4.7% base substitutions. *C. powellii* is a relative of *Candida batistae* and *Candida floricola*, with sequence differences of 5.9% and 6.9%, respectively. In all cases, the new species are phenotypically similar to their nearest relatives, but with sufficient differences to allow conventional identification. The type strains are: *Candida cleridarum*, strain UWO(PS)99-101.1 (CBS 8793); *Candida tilneyi*, strain UWO(PS)99-325.1 (CBS 8794); and *Candida powellii*, strain UWO(PS)99-325.3 (CBS 8795).

5. Lachance, M.A., J.M. Bowles, S. Kwon, G. Marinoni, W.T. Starmer, and D.H. Janzen. 2001. *Metschnikowia lochheadii* and *Metschnikowia drosophilae*, two new yeast species isolated from insects associated with flowers. Can. J. Microbiol. (in press).

Two new haplontic heterothallic species of *Metschnikowia* were isolated from floricolous insects and flowers. *Metschnikowia lochheadii* was recovered from insects found in various flowers on the Hawaiian Islands of Kauai and Maui, and from *Conotelus* sp. (Coleoptera: Nitidulidae) in northwestern Guanacaste Province, Costa Rica. The morphology, physiology, and sexual cycle are typical of the large-spored *Metschnikowia* species and the partial ribosomal DNA large subunit (D1D2) sequences suggest that the new species is most closely related to *Candida ipomoeae*. *M. lochheadii* is nearly indistinguishable from its ascogenous relatives and conjugates freely with *Metschnikowia continentalis*, forming sterile asci. It also exhibits asymmetric mating with *Metschnikowia hawaiiensis*. *Metschnikowia drosophilae* was found in morning glory (*Ipomoea* sp.) flowers and associated *Drosophila bromeliae* on Grand Cayman Island. Its nutritional profile is atypical of the genus, being the only species that does not utilize sucrose or maltose as carbon sources, and one of the few that does not utilize melezitose. D1D2 sequences show that *Metschnikowia drosophilae* is a sister species to *Candida torresii*, to which it bears considerable similarity in nutritional profile. The type cultures are: *Metschnikowia lochheadii*, strains UWO(PS)00-133.2 = CBS 8807 ($h^+$, holotype) UWO(PS)99-661.1 = CBS 8808 ($h^-$, isotype); and *Metschnikowia drosophilae*, strains UWO(PS)83-1135.3 = CBS 8809 ($h^+$, holotype) and UWO(PS)83-1143.1 = CBS 8810 ($h^-$, isotype).

6. Lachance, M.A. W.T. Starmer, C.A. Rosa, J.M. Bowles, J.S.F. Barker, and D.H. Janzen. In press Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Research **1**: (in press).

We studied specific yeast communities vectored by beetles, drosophilids, and bees that visit ephemeral flowers, mostly in the genus *Hibiscus* and in the families Convolvulaceae and Cactaceae, in the Neotropical, Nearctic, and Australian biogeographic regions. The communities consist mostly of yeasts in four clades centered around the genera *Metschnikowia*, *Kodamaea*, *Wickerhamiella*, and *Starmerella*. The largest geographic discontinuity occurs as a function of the nitidulid beetle species that dominate the non-pollinator insect visitors of the flowers. This partitions the New World, where the dominant beetle is in the genus *Conotelus*, from the Australian biogeographic region, dominated by species of *Aethina*. Distinct but sympatric insects may also carry radically different yeast communities.

## XXIII. Institut für Genetik und Allgemeine Biologie, Universität Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria. Communicated by M. Breitenbach <michael.breitenbach@mh.sbg.ac.at>.

At this department, 4 yeast groups are active. They are headed by M. Breitenbach (yeast mother cell specific aging), A. Bito (the yeast glyoxalase system), L. Koller (yeast ribosomes and metabolic regulation) and P. Briza (yeast sporulation). We will now give a short account of the work of the group concerned with yeast mother cell-specific aging. Other reports will follow in the next issue. We are investigating the role of reactive oxygen species (ROS) in the aging process which might be a common theme in yeast and higher cell aging. Recently we have obtained evidence that old (postmitotic) yeast mother cells isolated by a new and efficient method contain ROS that are localized in the mitochondria. These cells show all known markers of yeast apoptosis including TUNEL staining, annexin-V staining, and irregular nuclear morphology. Based on these findings we are now studying the physiology of oxygen detoxification and the influence of mutants in the known genes for oxygen detoxification on the aging process. This is aided by analyzing global transcription patterns comparing old and young cells using genomic filters.

Recent publications related to yeast mother cell-specific aging.


1. P. Laun, A. Pichova, F. Madeo, J. Fuchs, A. Ellinger, S. Kohlwein, I. Dawes, K.U. Fröhlich, M. 2001. Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. (under review).

2. Nestelbacher, R., Laun, P., Vondrakova, D., Pichova, A., Schuller, C., and Breitenbach, M. 2000. The influence of oxygen toxicity on yeast mother cell-specific aging. Exp Gerontol **35**(1)**:** 63-70.

3. Nestelbacher, R., Laun, P., and Breitenbach, M. 1999. Images in experimental gerontology. A senescent yeast mother cell. Exp Gerontol **34**(7):895-6.

4. Pichova, A., Vondrakova, D., and Breitenbach, M. 1997. Mutants in the *Saccharomyces cerevisiae RAS2* gene influence life span, cytoskeleton, and regulation of mitosis. Can J Microbiol **43**(8):774-81.


## XXIV. Northwest Biological Research Center (CIBNOR). Laboratory of Molecular Genetics. Unit of Marine Pathology. P.O. Box 128. La Paz, 23000. B.C.S., México. Communicated by N.Y. Hernández-Saavedra <nhernan@cibnor.mx>.

The following contribution was a poster presentation at the International Conference of Marine Biotechnology 2000 celebrated at Townsville (Queensland) Austalia, from September 28 to October 13.

1. Hernández-Saavedra, N. Y. and Ochoa, J. L. Isolation and characterization of the Cu,Zn superoxide dismutase from the marine yeast *Debaryomyces hansenii*: presence of two active forms of the enzyme.

**Introduction.** The superoxide dismutases (SOD) are ubiquitous enzymes in oxygen-metabolizing organisms. They are metalloproteins with different prosthetic groups; cytosoloic CuZn-SOD is found as a dimmer, whereas an extracellular form is a tetrameric protein. Its isolation for commercial applications is currently done by using bovine liver, human or bovine erythrocytes, baker yeast, and other common microorganisms. Recently the use of marine yeast has been proposed as an alternative source of CuZn-SOD enzyme, based on the high yield obtained and the unusual specific activity found in these microorganisms. This report describes the isolation and characterization of two forms of the CuZn-SOD from the marine yeast *D. hansenii*, isolated from the west coast of Baja California Sur, México.

**Methods.** Cell biomass was produced in a seawater medium. The CuZn-SOD enzyme was obtained by a modified Tsuchihashi fractionation followed by IMAC purification. CuZn-SOD from baker yeast and bovine liver were used as reference proteins. SOD activity was assayed by NBT method and protein analysis were done by SDS- and native-PAGE. Internal sequences and amino acid content carried out by the Peptide Service of the IGBMC (Strasbourg, France). Isoelectric focusing was determined on PAGE using Pharmalyte® 3-10. The effects of pH (3-10), temperature (20 °C- 95 °C and boiling time) and inhibitors (5mM NaCN, 1mM SDS, 1mM $H_2O_2$) were determined by quantifying the residual activity after treatments.

**Results and conclusions**. We isolated two cytosolic forms of CuZn-SOD from the marine yeast *D. hansenii*. Under native PAGE analysis, the initial homogenate shows the presence of 3 isoforms; two homodimers and one heterodimer, which were separated by chromatography. The homodimers (30.9 kDa) showed both an identical subunit mass of 15.9 kDa but were heterogeneous by IF PAGE, with two pI ranges: 5.14 to 4.0 and 1.6 to 1.8. The enzymes showed a remarkably strong stability at pH 6-7, retaining more than 30% activity after boiling periods of 10 min. The two homodimers differ with respect specific activity, amino acid composition, and sequence of some peptides obtained after trypsin digestion (table 1).

Table 1. Differential characteristics of two forms of CuZn-SOD presents on *D. hansenii*.

| Characteristic | CuZn-SOD 1 | CuZn SOD 2 |
|---|---|---|
| Specific activity (U/mg) | ~17 000 | ~ 8 000 |
| Number of different residues | 6 His, 2 Tyr, 9 Leu, 13 Thr | 3 His, 5 Tyr, 14 Leu, 5 Thr |
| Peptides aa 1-18 | GDSKVSGVVN | GDSNVSGVVR |
| aa 19-29 | FEQSSESDPTT | FEQTHESEPTK |
| aa 116-128 | TVVIHAGTDDL | TVVVHAGTDDY |

Such homodimers are differentially induced when the yeast is cultured with high levels of $Cu_2SO_4$ or $ClO_2$, indicating the presence of two different genes codifying two distinct proteins with apparently different regulation mechanism, and not one gene with two allelic forms.

The following two contributions were poster presentations at the XXIII National Congress of the Mexican Society of Biochemistry, celebrated at Acapulco (Guerrero) México, from November 19 to 25.

## 2. Ramírez-Serrano R. and Hernández-Saavedra, N.Y. Effect of copper ($Cu^{+2}$) addition on the copper-zinc superoxide dismutase activity of several marine yeast.

The superoxide dismutases (SOD) are generally found in eukaryotic organisms playing a major role on free radicals and ROS metabolisms. The SOD enzymes catalyzes the conversion of superoxide ion to form hydrogen peroxide, being the first antioxidant mechanism used on cellular defense. It has been reported that a significative increase on the CuZn-SOD activity as a response to high concentration of bivalent metals, suggesting that a chelating function is a second role of the enzyme. In addition, it is known that the copper ($Cu^{+2}$) modulates, at transcriptional level, the CuZn-SOD expression through the transcription factor ACE1. On this work, induction experiments were done by addition to growing cells $CuSO_4$. The effect of copper addition on 8 of 10 marine yeast strains was the increase on the CuZn-SOD activity. Responsive strains belong to *Debaryomyces, Saccharomyces, Udeniomyces, Pichia* and *Aureobasidium* genera. The activity remain unaltered on the two species of *Rhodotorula* genera tested. Changes on activity levels were monitored by non-denaturing PAGE stained with NBT, and protein synthesis by western blot and northern blot analyses.

## 3. Geraldo, R. and Hernández-Saavedra, N.Y. Subcloning of the encoding sequence of the copper-zinc superoxide dismutase from the marine yeast *Debaryomyces hansenii*, and production of the recombinant protein.

Currently, genetically manipulated microorganisms are generally used to transport and express heterologous genes to yield products with biomedical or industrial importance. Previous we have reported that the CuZn-SOD from the marine yeast *Debaryomyces hansenii* has advantageous characteristics, related to stability and activity, when compared with commercially available sources. Recently, we subcloned the encoding sequence *dh-sod1* (AFO 16383) on the expression system vectors (pPICZ and pPICZα of *Pichia pastoris* (Invitrogen). PCR amplification using specific oligonucleotides was used for the insertion of the Kozac box on the encoding sequence. To obtain the expression of heterologous products we induced cells growing on complex media thorough methanol pulses during exponential phase. The presence of recombinant protein was detected by western blotting (using polyclonal antibodies against CuZn-SOD of *D. hansenii*) and non-denaturing PAGE stained by NBT technique. By using the intracellular vector (pPICZ) the production of recombinant proteins was successful. Additional experiments must be done, to analyze the use of the extracellular vector (pPICZα) since no products were detected yet.

The following papers were submitted to be published.

## 4. Hernández-Saavedra, N.Y. Presence of two active forms of cytosolic Cu-Zn superoxide dismutase enzyme in the marine yeast *Debaryomyces hansenii.*

This paper reports the presence of two forms of cytosolic CuZn superoxide dismutase in the marine yeast *Debaryomyces hansenii*. From the physicochemical characterization of the proteins after purification, and based on differences in N-terminal, internal peptide sequences, and amino acid content, we found two cytosolic forms. Trying to determine the origin of this two proteins (genic or allelic), two induction experiments were done (by use of 8 mM of $CuSO_4$ and 3.5 µg/mL of $ClO_2$), finding a different activity pattern under nondenaturing PAGE; from a three isoform pattern (two homodimers and one heterodimer) found under normal conditions or after $Cu^{++}$ induction, after treatment with $ClO_2$ there was only one homodimer. The possibility of direct effect of the $ClO_2$ on the activity of the enzyme was eliminated by an experiment *in vitro* determining the residual activity after treatment with different concentrations (1.4 mg/mL to 0.14 µg/mL). Considering all these results together, we conclude the presence of three forms of CuZn-SOD enzyme in the marine yeast *D. hansenii* is the result of two different encoding sequences in the yeast, which do not correspond to allelic forms. More detailed research must be done to try to understand the regulation mechanisms of these enzymes and physiological meaning on cell survival.

5. Hernández-Saavedra, N.Y. Universal primers to amplify the encoding sequence of the enzyme copper-zinc superoxide dismutase of marine yeast and yeast-like organisms.

Degenerate oligonucleotides were tested for their ability to amplify the complete encoding region of the copper-zinc superoxide dismutase (CuZn-SOD or SODC) from several yeast and yeast-like organisms, including marine and terrestrial forms. Primer pairs were capable of amplifying products of the *SODC* gene encoding region from genomic DNA of several terrestrial and marine yeast species, and yeast-like organisms. In addition, we found a strong correlation between the presence of protein and amplification products (around 500 bp) for all unpigmented species (strains of *Pichia*, *Debaryomyces* and *Saccharomyces*), and for *Udeniomyces puniceus* and the yeast-like *Aureobasidium pullulans*. For other pigmented yeasts (*Rhodotorula mucilaginosa* and *Rh. graminis*), protein could not be found by activity on gel, using specific antibodies, nor after copper induction. However, from genomic DNA we may amplify specific fragments (~500 bp), that by sequencing analyses were identified as a CuZn-SOD encoding sequences. Although taxonomic differences exist among species of marine yeast studied, the success of amplification of specific genomic DNA fragments makes the degenerated primers useful to amplify the SODC encoding sequences from a wide range of yeast (including marine and terrestrial forms) and yeast-like organisms.

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

Recent publications.

1. K. Tag, A.W.K. Kwong, M. Lehmann, C. Chan, R. Renneberg, K. Riedel, G. Kunze. 2000. Fast detection of high molecular weight substances in wastewater based on an enzymatic hydrolysis combined with the *Arxula* BOD sensor system. J. Chem. Technol. Biotechnol. **75**:1080-1082.

A microbial amperometric sensor based on the yeast *Arxula adeninivorans* LS3 was tested for its suitability to measure the biochemical oxygen demand incurred by the use of high molecular weight substances, like starch, cellulose and milk powder. Analogous to other sensors, the untreated samples varied strongly to the corresponding $BOD_5$. Digestion of macromolecules with α-amylase, cellulase and protease for 30 min at 37°C resulted in sensor BOD values, which correlated better with $BOD_5$ values, when corrected for the enzyme blank signal.

2. T. Wartmann, G. Kunze. 2000. Genetic transformation and biotechnological application of the yeast *Arxula adeninivorans*. Appl. Microbiol. Biotechnol. (in press).

The relatively unknown non-pathogenic, dimorphic, haploid, ascomycetous yeast *Arxula adeninivorans* exhibits some unusual properties which are of biotechnological interest. The yeast is able to assimilate and ferment many compounds as sole source of carbon and/or nitrogen, it utilises n-alkanes and degrades starch efficiently. Features like thermo- and haloresistance as well as its uncommon growth and secretion behaviour should be especially emphasised. *Arxula adeninivorans* is able to grow up to a cultivation temperature of 48°C in media containing up to 20% NaCl. Additionally, the dimorphism of the yeast is unusual. *Arxula* grows at up to 42°C as budding cells, which turn into mycelia at higher temperatures. This environmentally conditioned dimorphism is reversible and budding is reestablished when the cultivation temperature is decreased below 42°C. Alteration of morphology correlate to changes of secretion behaviour. Mycelium cultures accumulate 2 fold higher protein concentrations and contain 2-5 fold higher glucoamylase and invertase activities in the medium than budding cells. Based on this unusual properties *Arxula adeninivorans* is used for the heterologous gene expression and as a gene donor to construct more suitable yeasts for biotechnology. For example the *Arxula* glucoamylase gene was successfully expressed in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. Both transformed yeasts are able to assimilate and ferment starch as carbon source. A transformation system is used for heterologous gene expression which is based on integration of linearised DNA fragments in 2-10 copies e.g. into the 25S rDNA of *A. adeninivorans* by homologous recombination. The obtained transformants are mitotically stable. The expression of the *lacZ* gene from *E. coli* as well as the *XylE* gene from *Pseudomonas putida* indicates the suitability of *Arxula adeninivorans* as host for heterologous gene expression.

## XXVI. Collection de Levures d'Intérêt Biotechnologique (CLIB), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.-V. <clib@grignon.inra.fr>.

Within *S. bayanus*, the type strain of the species (CBS380) and the former type strain of *S. uvarum* (CBS 395) were until now considered as mere synonyms. We have found them to be dissimilar in electrokaryotypes and NTS2 profiles (3, 4). In contrast we have analysed more than one hundred strains, most of them recently isolated from wine, cider, and various fermentation habitats. These strains share the same molecular characteristics with the type strain of the former species, *S. uvarum*. They have common physiological characteristics, such as gal$^+$, mel$^+$, and a maximum growth temperature of 35°C or less. Molecular typing of the nuclear and mitochondrial genomes as well as D1/D2 sequence comparisons (2) confirmed the homogeneity of *S. uvarum* and demonstrated that the type strain of *S. bayanus* (CBS380) was actually a hybrid between *S. uvarum* and *S. cerevisiae* (6) because its genome is a composite of the two. Compared to the type of *S. uvarum* (CBS 395), the type of *S. bayanus* has an identical mitochondrial genome and a karyotype with 13 isomorphic chromosomes, at least six of which hybridise strongly with *S. uvarum* chromosomes or with an *S. uvarum*-specific sequence. Four of its chromosome bands bear the *S. cerevisiae* Y' sequence as well as several other *S. cerevisiae* sequences (Piškur, personal communication). Furthermore, *S. bayanus* CBS380$^T$ behaves physiologically as an intermediate between *S. uvarum* and *S. cerevisiae* (5) and cannot sporulate. Because of the hybrid nature of *S. bayanus* CBS380$^T$, which was isolated from beer in the past, and in view of the scarcity of similar hybrids among present days isolates, *S. uvarum* must be considered again as a species as already proposed by van der Walt. These recent developments suggest that in practice the *Saccharomyces sensu* Vaughan-Martini & Kurtzman complex is composed of *S. cerevisiae*, *S. uvarum*, *S. paradoxus*, and hybrids including *S. bayanus* CBS380, *S. pastorianus*, and others. We believe that this new delimitation provides a clear differentiation of species and facilitates their identification by conventional methods.

The following have been recently published.

1. Nguyen H-V, Pulvirenti A., Gaillardin C. 2000. Rapid differentiation of the closely related *Kluyveromyces lactis* var. *lactis* and *K. marxianus* strains isolated from dairy products using selective media and PCR/RFLP of the rDNA non transcribed spacer 2. Can J. Microbiol. **46**:1115-1122.

PCR/RFLP of the NTS2 (IGS2) of rDNA was applied to differentiate two closely related species *Kluyveromyces lactis* var. *lactis* (referred to as *K. lactis*) and *K. marxianus*. Using specific primers, the NTS2 region was amplified from DNA of both *K. lactis* and *K. marxianus* type and collection strains. *Alu*I restriction of amplified fragments generated patterns characteristic for each species. The NTS2 region from *K. lactis* var. *drosophilarum* and related species *K. aestuarii*, *K. africanus*, *K. dobzhanskii* and *K. wickerhamii* could also be amplified with the same primers, but *Alu*I patterns generated were clearly different. PCR/RFLP of the NTS2 appears thus to be a convenient method for rapid identification of *K. lactis* and *K. marxianus*, frequently found in dairy products. This test was validated therefore on *K. lactis* and *K. marxianus* from natural habitats. We showed actually that all yeast strains collected from whey samples and scoring blue on X-gal glucose plates were either *K. lactis* or *K. marxianus*. For application purposes, we propose here an approach for quickly screening for *K. lactis/marxianus* and *Saccharomyces cerevisiae* in dairy products using X-gal coloured and lysine growth media.

2. Pulvirenti A., Nguyen H-V, Caggia C., Giudici P., Rainieri S., Zambonelli C. 2000. *Saccharomyces uvarum*, a proper species within *Saccharomyces sensu stricto*. FEMS Microbiol. Lett. **192**:191-196.

*Saccharomyces uvarum* is proposed as a proper species within the complex *Saccharomyces sensu stricto*. Molecular characteristics including the similarity of the restriction profile of the NTS2 (IGS2) and of the D1/D2 sequence of the rDNA, as well as other genotypic and phenotypic characteristics confirm that this group of strains is highly homogeneous and distinguishable from other species of the *Saccharomyces sensu stricto* group.

Previous publications by the same authors.


3. Nguyen, H.-V., Gaillardin, C. 1997. Two Subgroups within the *Saccharomyces bayanus* species evidenced by PCR amplification and restriction polymorphism of the non-transcribed spacer 2 in the ribosomal DNA unit. Syst. Appl. Microbiol. **20**:286-294.

4. Giudici, P., Caggia, C., Pulvirenti, A., Rainieri, S. 1998. Karyotyping of *Saccharomyces* strains with different temperature profiles. J. Appl. Microbiol. **84**:811-819.

5. Rainieri, S., Zambonelli, C., Hallsworth, J.E., Pulvirenti, A., Giudici, P. 1999. *Saccharomyces uvarum*: a distinct group within *Saccharomyces sensu stricto*. FEMS Microbiol. Lett. **177**:177-185.

6. Nguyen, H.-V., Lépingle, A., Gaillardin, C. 2000. Molecular typing demonstrates Homogeneity of *Saccharomyces uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae*, including the *S. bayanus* type strain CBS380. Syst. Appl. Microbiol. **23**:71-85.

## XXVII. Center for Process Biotechnology, Department of Biotechnology, Building 223, The Technical University of Denmark, DK-2800 Lyngby, Denmark. Communicated by L.Olsson <LO@ibt.dtu.dk>.

The research activities on yeast at the Center for Process Biotechnogy combines physiological studies with advanced analytical techniques and mathematical modelling with the objective of increasing our understanding of yeast. The following topics are studied. (1) Fermentation of complex substrates (metabolic engineering of the galactose and the xylose metabolism, mixed sugar utilisation, glucose repression and fermentation inhibitors). (2) Yeast physiology (pyruvate metabolism in *Saccharomyces kluyveri,* modelling of the pyruvate node, transcriptome analysis of *Saccharomyces cerevisiae,* redox metabolism). (3) Metabolic network analysis (futile cycles, functional genomics). (4) Analytical biotechnology (measurement of intracellular metabolites, multiwave-length flourescence, CE and combination sensors). The following were published recently.

1. Nissen T. L.; C. W. Hamann; M. C. Kielland-Brandt; J. Nielsen; J. Villadsen. 2000. Anaerobic and batch cultivations of *Saccharomyces cerevisiae* mutants impaired in glycerol synthesis. Yeast **16**:463-474.

2. Nissen T. L.; M. C. Kielland-Brandt; J. Nielsen; J. Villadsen. 2000. Optimisation of ethanol production in *Sacchaoromyces cerevisiae* by metabolic engineering of the ammonia assimilation. Metabol. Eng. **2**:69-77.

3. Olsson, L. and J. Nielsen. 2000. The role of metabolic engineering in the improvement of *Saccharomyces cerevisiae*: utilization of industrial media. Enz. Microbial Technol. **26**:785-792.

4. Ostergaard S., Olsson L., Johnston M., J. Nielsen. 2000. Increasing galactose consumption by *Saccharomyces cerevisiae* through metabolic engineering of the *GAL* gene regulatory network. *Nature Biotechnol.* in press.

6. Ostergaard, S., Olsson, L., and J. Nielsen. 2000. Metabolic engineering of *Saccharomyces cerevisiae*. Microbiology and Molecular Biology Reviews. **64**:34-50.

7. Ostergaard, S., Roca, C., Rønnow, B., Nielsen J., L. Olsson. 2000. Physiological studies in aerobic batch cultivations of *Saccharomyces cerevisiae* genetically engineered with respect to the *MEL1* gene. **68**:252-259.

8. Smits, H. P.; Hauf, J.; Müller S.; Hobley T. J.; Zimmermann, F. K.; Hahn-Hägerdal, B.; J. Nielsen; L. Olsson. 2000. Simultaneous over-expression of enzymes of the lower part of glycolysis can enhance the fermentative capacity of *Saccharomyces cerevisiae*. Yeast **16**:1325-1334.

9. van Dijken J. P. *et al.* 2000. An inter-laboratory comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains. Enz. Microb. Technol. **26**:706-714.

## XXVIII. Division of Industrial Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Communicated by J.C. Verdoes. <jan verdoes@imb.ftns.wau.nl> www.ftns.wau.nl/imb

The following papers have recently been published.

1. Verdoes J.C., Krubasik P., Sandmann G. and van Ooyen A.J.J. 1999. Isolation and functional characterisation of a novel type of carotenoid biosynthetic gene from *Xanthophyllomyces dendrorhous*. Mol. Gen. Genet. **262**:453-461.

The red heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous* (perfect state of *Phaffia rhodozyma)* contains a novel type of carotenoid biosynthetic enzyme. The gene, denominated *crtYB*, was isolated by functional complementation in a genetically modified, carotenogenic *Escherichia coli* strain. By expression studies in different carotenogenic *E. coli* strains it was demonstrated that the *crtYB* gene encodes a bifunctional protein involved in both phytoene synthesis from geranylgeranyl diphosphate and cyclisation of lycopene into β-carotene. By sequence comparison of the polypeptide with other phytoene synthases and genetic complementation in *E. coli* with various deletion constructs of the *crtYB* gene, the regions responsible for phytoene synthesis and lycopene cyclisation within the protein were localised.

2. Verdoes J.C. and van Ooyen A.J.J. 2000. Codon usage in *Xanthophyllomyces dendrorhous*. Biotechnol. Lett. **22**:9-13.

By sequence analysis of 96 randomly selected clones in a cDNA library of *Xanthophyllomyces dendrorhous*, ten novel full length clones encoding cytoplasmic ribosomal proteins (rp) were found. The deduced amino acid sequences showed significant homology to their counterparts from eukaryotic origin including mammals, fungi and plants. Some ribosmal protein encoding cDNAs appeared several times, but by Southern blot analysis it was shown they are encoded by a single copy gene. The nucleotide sequences of ten full length cDNAs were used to investigate the codon usage in *X. dendrorhous.*

3. Visser H., Vreugdenhil S., de Bont J.A.M. and Verdoes J.C. 2000. Cloning and characterization of an epoxide hydrolase encoding gene from *Rhodotorula glutinis***.** Appl. Microbiol. Biotechnol **53**:415-419

We cloned and characterized the epoxide hydrolase gene, *EPH1*, from *Rhodotorula glutinis*. The *EPH1* open reading frame of 1230 bp was interrupted by 9 introns and encoded a polypeptide of 409 amino acids with a calculated molecular mass of 46.3-kDa. The amino acid sequence was similar to that of microsomal epoxide hydrolase which suggests that the epoxide hydrolase of *R. glutinis* also belongs to the α/β hydrolase fold family. *EPH1* cDNA was expressed in *Escherichia coli* and resting cells showed a specific activity of 200 nmol $min^{-1}$ (mg $protein)^{-1}$ towards 1,2-epoxyhexane.

4. Choi W.J., Choi C.Y., de Bont J.A.M.and Weijers C.A.G.M. 2000. Continuous production of enantiopure 1,2-epoxyhexane by yeast epoxide hydrolase in a two-phase membrane bioreactor. Appl. Microbiol. Biotechnol. **54**:641-646.

A two-phase membrane bioreactor was developed to perform continuous production of enantiopure epoxides with the epoxide hydrolase activity of *Rhodotorula glutinis*. An aqueous/organic cascade hydrophilic hollow-fiber membrane bioreactor was used (1) to carry out large-scale resolution of epoxides, (2) to continuously extract residual enantiopure epoxides from the aqueous phase, and (3) to separate inhibitory formed diol from the yeast cell contained in the aqueous phase. Dodecane was employed to dissolve feed epoxide as well as to extract residual epoxide. 1,2-Epoxyhexane was used as a model substrate. By use of this membrane bioreactor, enantiopure (S)-1,2-epoxyhexane (>98%ee) was obtained with a volumetric productivity of 3.8 g $l^{-1}$ $h^{-1}$. The continuous production system was operated for 12 days and resulted in 38 g enantiopure (S)-1,2-epoxyhexane.

5. Kronenburg N.A.E. and de Bont J.A.M. Effects of detergents on specific activity and enantioselectivity of the epoxide hydrolase from Rhodotorula glutinis. Enz. Microb. Technol., in press.

The yeast *Rhodotorula glutinis* contains an enantioselective epoxide hydrolase. Previous work showed that the enzyme is a membrane-associated enzyme that can be solubilised from the membranes by a detergent treatment. Now, the effect of detergents on reaction rate and particularly enantioselectivity was investigated. Three types of detergents were tested: non-ionic, anionic and zwitterionic. Non-ionic detergents stimulated the specific activity of the enzyme. Enantioselectivity of the enzyme was strongly affected by several detergents. Thesit and sucrosemonolaurate had the most pronounced effects and enantiomeric ratios were strongly enhanced. The effects are most likely due to the ability of detergents to stabilise membrane-proteins by forming micelles and thus mimicking the membrane structure.

## XXIX. Institute for Wine Technology, University of Stellenbosch, Victoria Street ZA-7600 Stellenbosch, South Africa. Communicated by I.S. Pretorius.

The following review papers have appeared as a special issue of the South African Journal of Enology.

1. M A Vivier and I S Pretorius. 2000. Genetic improvement of grapevine: tailoring grape varieties for the third millennium - a review. S.Afr. J. Enol. **21** (special issue):5.

2. F F Bauer and I S Pretorius. 2000. Yeast stress response and fermentation efficiency: how to survive the making of wine -a review. S.Afr. J. Enol. **21** (special issue):27.

3. P van Rensburg and I S Pretorius. 2000. Enzymes in winemaking: harnessing natural catalysts for efficient biotransformations - a review. S.Afr. J. Enol. **21** (special issue):52.

4. M du Toit and I S Pretorius. 2000. Microbial spoilage and preservation of wine: using weapons from nature's own arsenal - a review. S.Afr. J. Enol. **21** (special issue):74.

5. M G Lambrechts and I S Pretorius. 2000. Yeast and its importance to wine aroma - a review. S.Afr. J. Enol. **21** (special issue):97.

The following review has appeared recently.

6. I. S. Pretorius. 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast: **16**:675-729.

Yeasts are predominant in the ancient and complex process of winemaking. In spontaneous fermentations, there is a progressive growth pattern of indigenous yeasts, with the final stages invariably being dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae*. This species is universally known as the 'wine yeast' and is widely preferred for initiating wine fermentations. The primary role of wine yeast is to catalyze the rapid, complete and efficient conversion of grape sugars to ethanol, carbon dioxide and other minor, but important, metabolites without the development of off-flavours. However, due to the demanding nature of modern winemaking practices and sophisticated wine markets, there is an ever-growing quest for specialized wine yeast strains possessing a wide range of optimized, improved or novel oenological properties. This review highlights the wealth of untapped indigenous yeasts with oenological potential, the complexity of wine yeasts' genetic features and the genetic techniques often used in strain development. The current status of genetically improved wine yeasts and potential targets for further strain development are outlined. In light of the limited knowledge of industrial wine yeasts' complex genomes and the daunting challenges to comply with strict statutory regulations and consumer demands regarding the future use of genetically modified strains, this review cautions against unrealistic expectations over the short term. However, the staggering potential advantages of improved wine yeasts to both the winemaker and consumer in the third millennium are pointed out.

**Toulouse Levure Club. Communicated by J.M. François, Centre de Bioingenierie Gilbert Durand, UMR-CNRS 5504, UMR- INRA 792, Département de Génie Biochimique et Alimentaire, Institut National des Sciences Appliquées, Avenue de Rangeuil 135, 31077 Toulouse Cedex 04, France <fran_jm@insa-tlse.fr>.**

Several yeast groups in the Toulouse area participate in a yearly meeting and exchange results, techniques, tips, abstracts books from International Meetings, etc.

## Cell-cycle. B. Ducommun, Laboratoire de Biologie cellulaire et Moleculaire du contrôle de la proliferation, 118 rte de Narbonne, 31062 Toulouse <ducommun@cict.fr>.

1. Bonnet, C., Perret, E., Bonnin, O., Picard, A., Caput, D. and Lenaers, G 2000. Identification of *rpaP1-5* and *rpaP2-6* genes encoding two additionnal variants of the 60S acidic ribosomal proteins of *Schizosaccharomyces pombe*. Genome **4**:205-207.

2. Bonnet, C., Perret, E., Dumont, X., Picard, A., Caput, D. and Lenaers, G. 2000. Identification and transcription control of fission yeast genes repressed by an ammonium starvation growth arrest. Yeast **16**:23-33.

## Protein Engineering. J.M. Masson, Institut de Pharmacologie et de Biologie Structurale, CNRS 205 Route de Narbonne, 31077 Toulouse cédex France <masson @ipbs.fr>.

1. Lagane, B., Gaibelet, G., Meilhoc, E., Masson, J.M., Cézanne, L. & Lopez, A. In press. Role of sterols in modulating the human beta-opioid receptor function in *Saccharomyces cerevisiae*. J. Biol. Chem.

2. Janatova, I., Gourdon, P., Meilhoc, E., Klein, R. D. & Masson, J.M. 2000. ARS sequences in homologous and heterologous *ADE2* loci are capable of promoting autonomous replication of plasmids in *Schwanniomyces occidentalis.* Curr. Genetics **37**:298-303.

## Molecular Microbial Physiology. J.M. François, Centre de Bioingenierie Gilbert Durand, Département de Genie Biochimique et Alimentaire, 135 Avenue de Rangueil, 31077 Toulouse <fran_jm@insa-tlse.fr>.

1. Groussac, E., Ortiz, M. and François, J. 2000. Improved methods for quantitative determination of intra and extracellular metabolites by high pressure ionic exchange chromatography coupled to conductimetry or pulse amperometry detection. Enz. Microb. Technol. **26**:715-723.

2. Parrou, J.L., Enjalbert, B. Blazquez, M.A. and François, J. 1999. Stre- and cAMP-independent induction of *Saccharomyces cerevisiae GSY2* encoding glycogen synthase during the diauxic growth of on glucose. Yeast **15**:1471-1484.

3. Plourde-Owobi, L., Durnez, S. and François, J. 2000. Trehalose reserve in *Saccharomyces cerevisiae*: Phenomenon of transport and accumulation, and its role in cell viability. Int. J. Food Microbiol.. **55**:33-40.

4. François, J., Bergaud, C. & Escudier J.M. 2000. Micro-technologie de l'ADN et macro-conséquences sur la connaissance du vivant. Bulletin spécial de l'ASEDIS-SO.

5. François, J. and Parrou, J.L 2000. Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*: a review. FEMS Microbiol Rev. (in press).

6. Teste M.A., Enjalbert, B., Parrou, J.L., and François, J. 2000. The *YPR184*w encodes the glycogen debranching enzyme in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. (in press).

7. Enjalbert, B., Parrou, J.L., Vincent, O. and François, J. 2000. Mitochondrial respiratory mutants of *Saccharomyces cerevisiae* accumulate glycogen, and readily mobilise it upon glucose depletion. Microbiology **146**:2685-2694.

8. François J. 2000. Puces à AND: état de l'art, nouveaux concepts et activités de la plateforme génomique de Toulouse. Bull. Soc. Fr. Microbiol. **15**:200-206.

9. Huet, C., Menendez, J. Gancedo, C. and François, J. 2000. Transcriptional control of *PYC1* encoding pyruvate carboxylase isoforms I by nitrogen sources. Eur. J. Biochem. (in press).

## A debate on the description of new yeast species based on single strains

The following arose out of email exchanges between Jack Fell, Clete Kurtzman, and André Lachance. Readers who are interested in contributing to this discussion are welcome to do so.

**(Fell)** We need to establish a consensus regarding the description of new species based on single strains. In some cases a single strain is quite interesting and deserves naming. However, many single strains of large anamorphic genera (*e.g.*, *Cryptococcus, Rhodotorula*, etc.) could potentially be described as new species. One wonders if that is appropriate. The single-strain species could instead be included in a published tree, the sequencing data in GenBank, and the physiological data in the CBS database. The information would be available for researchers interested in finding more strains and describing the species. The creation of the new journal, *FEMS Yeast Research*, could serve as an opportunity to establish a sensible practice in this area.

**(Kurtzman)** The appropriateness of describing single-strain *Candida* species, or teleomorphic species for that matter, is often brought up by some reviewers. In conversations, Lachance has questioned the description of new species from single strains. In some ways the criticism is valid in that when species are based on single strains, it is not possible to assess the genetic and physiological variability within the species. On the other hand, if our molecular analyses are correct and we can recognize individual species, especially if we avoid naming species based on too little genetic divergence, then the description of new single strain species tells us quite a lot about biodiversity.

To test this idea, I searched through the 4th edition (Kurtzman and Fell 1998) and tallied up those species descriptions based on a single strain, with the assumption that most authors would have examined more than one strain if more had been available. I excluded taxa for which there were no molecular or genetic comparisons that indicated that the taxa were unique species. Of the 696 apparently distinct species considered in this exercise, 205 were described from a single strain. This represents 29.5% of known yeast species. Thus, nearly one-third of yeast biodiversity would be unknown if we did not accept species based on a single strain. This is certainly a strong argument in favour of describing species from single strains, if only to increase our understanding of biodiversity.

Further, our molecular comparisons often show that strains assigned to certain species are misidentified, so that only one strain (the type strain) may actually represent the species correctly. This suggests that the one-third figure is probably conservative. Based on these considerations, I feel that every single strain that represents a different species should be properly described, and not filed away until more strains are available. Many single-strain species were described decades ago and additional strains have not been found since. Furthermore, some of these single-strain species are phylogenetically quite divergent and are important for understanding yeast relationships.

**(Lachance)** There is no simple answer. I share with Phaff and Starmer the view that species are populations, and that single strains may not be properly representative of species. Further, I think that in many cases, single-strain descriptions trivialize yeast taxonomy. Biodiversity, if it is to be more than a buzzword, must amount to more than just a long list of names. Species must be set in the context of their habitats, which, for single strain species, is more or less undefined. I therefore tend to lean more towards Fell's view. The availability of international databases such as GenBank addresses Kurtzman's concern that so many species would remain unknown to science. Of course, to deposit a sequence that is not associated with a valid description poses the risk that someone might describe new species on the basis of strains discovered by others. I believe this is unlikely, and expect that researchers who find, in the database, a sequence that matches one of their own, will normally contact the depositor and propose collaborative work.

I disagree that the large proportion of single-strain species described in the past justifies doing it now. We now have powerful tools that were not available in the past,

including rDNA sequencing and internationally available databases. Similar databases exist that contain physiological data and could be expanded to include photographs of morphologically unusual specimens. My first *FEMS Yeast Research* paper will introduce a large number of new species that will not be described in the very near future, because due diligence has not yet been exercised in documenting out their distribution in nature. Nonetheless, the existence of those species will be known, at least from sequences.

I am one of those reviewers who criticizes single-strain descriptions, but I have not yet recommended rejection of a paper on that basis. Whereas I frown on the practice, I believe that individual taxonomists (and journals) should be free to deal with this matter in the way they please. But I shall keep commenting, in my reviews, on the fact that this fails to provide the ecological background in which to describe a species properly.

Kurtzman and others have recently convinced me that in special cases like that of *"Tortispora"*, a remarkable new genus represented by one single-strain species, should be given a formal description. In this case, due diligence (numerous collections by Phaff, Starmer, Ganter, Rosa, and others) has not resulted in the isolation of new strains.

**(Fell)** I favour a middle-of-the road approach. As per Lachance's example (*"Tortispora"*), a phenotypically and/or genetically distinct strain should be described. *Reniforma* is a good example of a genus based on a single strain whose description was well justified from its distinctive morphology. In both cases, the unique morphology is complemented by distinctive gene sequences. However, this was not the situation for *Wingea*, a genus described on the basis of its unique lentiform ascospores. Molecular comparisons showed *Wingea robertsiae* to be a member of *Debaryomyces* and quite closely related to *D. hansenii*.

As outlined by Kurtzman, the degree of genetic divergence may be a key factor, particularly among the anamorphs. We have several single strains that differ from described species at only a few D1/D2 and ITS positions. Our experience with heterothallic mating type compatibility indicates that these strains are indeed distinct species. As we continue to look, will we find strains that occupy intermediate positions? Probably so. What does this all mean? If we start naming multitudes of cryptic anamorphs purely on the basis of small sequence differences, what are we creating? The solution would be, when appropriate, to include the GenBank accession numbers as well as the CBS numbers in published trees. The culture collection availability of the strains might prompt colleagues to search their collections, perform mating studies, and as a result, accumulate sufficient biological information to make a better case for or against describing the strains as new species.

I would probably take a different view on teleomorphic species. If one can demonstrate reproductive isolation between a single homothallic or homokaryotic isolate and closely related species, then a new species could be described. Among the basidiomycetes we are only beginning to understand the diversity of sexual cycles. The availability of many new species defined on the basis on reproductive isolation will provide a better evaluation of sequence divergence as a criterion for species recognition.

Dissemination of biodiversity data is important, whether or not we describe our isolates as new species. As Lachance points out, GenBank is a prime vehicle for sharing those data. In reply to Lachance's comments on the use on information taken from public databases, authors should be reminded to give proper credit to the original collectors and sequence providers. A few recent manuscripts that I reviewed have often failed to include appropriate credits. In most cases, the authors provided trees with GenBank accession numbers and details of their sequencing methods, but did not include a statement indicating which sequences originated from their own work or from someone else's research. At the extreme, a tree might contain 50 species, only one of which was sequenced in the authors' laboratory. Massive amounts of work performed by others goes unacknowledged. Similarly, some descriptions list strains by their CBS or other collection numbers, without a cross-reference to the original worker who collected the species. Clearly these oversights are unintentional, but it is important to realize that public databases and strains do not appear out of thin air.

**(Lachance)** A comment on Fell's remarks. If one can positively demonstrate that a single strain is the heterothallic mating type of a sexual species, but one cannot demonstrate the whole sexual cycle, is one looking at the anamorph or the teleomorph? Given the powerful tools that are now available, will a new nomenclature, based on a new concept of the holomorph, be acceptable? Perhaps that is a question for a later debate.

**(Kurtzman)** I will argue that publication of a full species description will have greater impact in making unique species known to the scientific community than will reference to the sequence of a unique unnamed strain in GenBank, despite the ease of searching this database.

Another issue is that of scientific ethics or convention. If a strain is cited in a publication, it is expected that it will be made available with no strings attached. To do otherwise impacts on the freedom of scientific inquiry that we have all come to expect. Therefore, I think it unfair to imply that another investigator must "collaborate" with the person depositing a sequence should they find another strain of the same species and wish to describe it. The original culture should be freely available without obligation if the sequence is published. Whether a collaboration develops should be the decision of the later investigator.

# International Commission on Yeasts (ICY)

## Minutes of the Meeting of the Commissioners held at ISY10
## Papendal, Arnhem, The Netherlands, 29 August 2000

**In attendance.** Argentina: L. de Figueroa. Australia: G.H. Fleet. Austria: H.-J. Prillinger. Brazil: L. Mendonca-Hagler. Canada: M.A. Lachance. Finland: M. Korhola, M. Penttilä. Hungary: T. Deák, A. Maraz. Italy: P. Romano, A. Vaughan Martini. Latvia: A. Rapoport. Mexico: P. Lappe, R. Vazquez-Juarez. Netherlands: J.P. van Dijken, W.A. Scheffers, M.Th. Smith. Portugal: C. Leão, I. Spencer Martins. Russia: G. Naumov. Slovenia: P. Raspor. South Africa: J.C. du Preez, I.S. Pretorius. Sweden: B. Hahn-Hägerdal. Ukraine: A.A. Sibirny. United Kingdom: G. Stewart. United States of America: C.P. Kurtzman, S.A. Meyer, H.J. Phaff.

**Apologies.** Argentina: F. Spencer. Belgium: J. Thevelein. Brazil: C. Schenberg. Canada: M. Ingledew, B. Johnson. Croatia: U. Johanides. Czech Republic: M. Kopecka. Denmark: T.B. Nielsen, J. Stenderup. Egypt: M. Gibriel. Italy: A. Martini. Latvia: M. Bekers. Portugal: M. Dias. Russia: I. Bab'eva. Slovakia: P. Biely, E. Minarik. South Africa: B. Prior, J. Van der Walt. Spain: J. Peinado, R. Sentandreu. Sweden: H. Neujahr. United Kingdom: D. Berry, R. Moore.

**Report of Chairperson - Graham H Fleet.** Graham reported that minutes of Commissioner meetings held at ISSY20 (May 1999, Smolenice) and IUMS (August 1999, Sydney) had been posted to all Commissioners, and had been published in the December 1999 issue of the Yeast Newsletter. He thanked the commissioners for their attendance and reported that the vast majority of the commissioners were now very active in correspondence and involvement with ICY. However, there were some countries (e.g. Germany, France, Switzerland, China, Korea, Japan, S.E. Asia, etc) where further work was needed to find suitable commissioners. The following were proposed and accepted as new commissioners: Vladimir Mrša (Croatia); Eric Johnson (USA, to replace F. Sherman). The meeting welcomed P. Lappe and R. Vazquez-Juarez of Mexico who were accepted as new commissioners.

Graham had received correspondence (9 June 2000) from Peter Biely (Slovakia) proposing Dr Hana Sychrová as a second commissioner for the Czech Republic. Dr Sychrová was to attend the meeting with Professor Julius Subík, as Dr Biely's nominee, but neither were present. It is proposed to include Dr Sychrová in the list of commissioners, with formal acceptance to occur at the next meeting.

By correspondence, Dave Berry (UK) had indicated that he had now retired and would resign from his position as one of the commissioners for the UK. This would leave a vacant position for that country. The matter was briefly discussed and Graham Stewart agreed to initiate the process for proposing a new commissioner for the UK.

**Yeast Newsletter.** André Lachance (editor) reported the continuing strong interest in the Newsletter and the introduction of the "Essay" or "Mini-Review" section. He encouraged contributions to this section. He welcomed the support of Patrizia Romano as an Associate Editor for stimulating interest on yeasts in foods and beverages.

**Symposium Reports.**

**IUMS Sydney, Australia, August 1999**. Graham noted that ICY contributed a successful symposium on "Yeast Biodiversity" to this Congress (see minutes of meeting 17 August 1999). The symposium was attended by about 75 people. At the IUMS Congress, Inge Russell (ICY commissioner for Canada) was elected as Chair of the Mycology Division of IUMS.

**ISY 10/ISY2000.** Hans van Dijken and Lex Scheffers briefly reported on their experiences in organising this symposium and noted the strong registration (around 400 delegates) and submission of poster papers (about 200). Lex has asked Graham Fleet to write a formal report on (ISY10/ISY2000) for publication in the new journal, FEMS Yeast Research.

**ISY21 (2001). "Biochemistry, Genetics, Biotechnology and Ecology of Non-conventional Yeasts, NCY" 21 August - 25 August, Lviv, Ukraine.** Andrei Sibirny is chairperson of the organising committee and reported good progress with planning of the program. A local planning committee and an international scientific advisory committee have been established (e-mail: ISSY2001@biochem.Lviv.ua).

**ISSY22 (2002). "Yeast Fermentations and other Bioprocesses" March-April, Pilanesberg National Park, Northern Province, South Africa.** James du Preez is chairperson of the organising committee and reported on the planning to date. ISSY22 is planned as a back-to-back meeting with the South African Society for Microbiology Congress. The venue is a lodge within a wild animal park and can accommodate 150 registrants (maximum). Local Planning and International Advisory Committees are being formed.

**ISSY23 (2003).** Tentative proposals from T. Deák (Hungary) on "Saprophytic Yeasts" and R. Sentandreu (Spain) "Signal Transduction and Cell Wall Morphogenesis", as noted in the Minutes of the meeting held at ISSY20, were placed on the agenda for discussion. Graham Fleet noted that he had received further correspondence from Professor Sentandreu indicating his willingness to host ISSY23. In addition, he had received correspondence from Dr Maria van Broock (Argentina) offering to host ISSY23 at the Patagonian city of Bariloche - but only in December due to weather patterns. The various options were discussed and the meeting decided to support the proposal offered by Tibor Deák, who will provide more detailed information for the next meeting.

**ISY11 (2004).** Leda Mendonca-Hagler offered a proposal to organise ISY11 in Brazil, probably Rio de Janeiro. This was unanimously accepted by the meeting.

**Chair of ICY.** The statute of ICY indicates that the chair of the organising committee for the ISY should become the next chairperson of ICY. With this protocol, Hans van Dijken would become the next chair of ICY. In earlier correspondence to Graham Fleet, Hans had indicated that a combination of professional and personal commitments would prevent him from taking on the chairmanship of ICY and he proposed that Lex Scheffers take on this position since he had contributed substantially to the organisation of ISY10 and had been a strong participant in ICY for many years. The commissioners were in unanimous agreement with this proposal, and Lex Scheffers was accepted as the next chair of ICY (2000-2004). Graham thanked Hans for his consideration of this matter and the excellent work he had put into ISY10, and then welcomed Lex as the new

chairperson for ICY. In accordance with the ICY statute, Graham will support Lex as the vice-chair.

FEMS Yeast Research

Lex Scheffers informed the meeting that the Federation of European Microbiological Societies had commissioned a new journal "FEMS Yeast Research" that would be of interest to all yeast researchers. Lex is the Chief Editor of the Journal and Teun Boekhout is the Deputy Chief Editor. Many ICY commissioners are on the editorial board.

**Other business.** No other items of business were raised. Graham thanked all the commissioners, for their excellent cooperation and support during the last 4 years and also, the Vice-Chair, Sally Meyer for her support. Finally, on behalf of all the commissioners, he thanked Hans, Lex and their colleagues for their great work in staging ISY10.

Graham H Fleet

# Recent meetings

## European Brewery Convention - November 1999
## EBC Monograph - Yeast Physiology: a New Era of Opportunity

Dramatic changes have occurred since the last EBC Symposium on yeast physiology was held in Finland only 13 years ago. The European Brewing Industry has changed markedly with consolidation resulting in many brewery closures and the rise of international beer brands, many of which are completely new products. The business drivers pushing these changes forward are continually demanding new technological solutions which brewery research and development staff must provide. Undoubtedly the biggest changes since 1986 have been the sequencing of the yeast genome and the development of approaches to allow the integrated study of genetics and physiology.

In this symposium book the reader will find presentations produced by leading scientists in their respective fields alongside a number of articles from brewery representatives
who give the science an industrial perspective. Among the topics covered are flocculation, carbon and nitrogen metabolism, flavour production, fermentation performance, yeast stress responses and the application of the "new genetics" to brewing. The net result is a distillation of the status of yeast physiology at the beginning of the twenty-first century together with thoughts on the research priorities of the brewing industry in both the short and long terms.

Contents: 15 oral presentations (with ensuing discussion) and 7 poster presentations as presented at the 28th Symposium of the European Brewery Convention held in November 1999; 288 pages, numerous figures and tables. Published in spring 2000.

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## 28th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts, Smolenice, Slovakia, May 15-17, 2000

The 28th Annual Conference on Yeasts organized regularly by the Czech and Slovak Commission for Yeasts and the Institute of Chemistry, Slovak Academy of Sciences, took place in Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, May 15-17, 2000. The previous conference of this series (the 27th) was held in 1998 because in1999 the Commission organized the 20th ISSY on Yeast Cell Surfaces and Membrane Phenomena. The interruption of the series could be the main reason why the Smolenice Castle was completely full for the 28th Annual Conference. The Conference was attended by 37 participants from the Czech Republic, 38 from Slovakia, 3 from Hungary and four distinguished guests from other countries who gave plenary lectures in English. The program consisted of a block of plenary lectures in English and three plenary sessions in Czech and Slovak, the two closely related languages of recently separated nations, and a poster session. The meeting covered the following areas of yeast research: Biotechnology, Molecular Biology and Genetics, Cytology and Immunology and Pathogenesis. The lectures were complemented by 50 posters. The titles of lectures are listed below.

**Plenary lectures of foreign guests**

C. Jacq (France): Yeasts transcriptomes and the multi drug resistence phenomenon.

G. Daum (Austria): Acyltransferases and friends.

J. Piškur (Denmark): Studies of different *Saccharomyces* yeasts provide insight into the origin of the *Saccharomyces cerevisiae* genome and its metabolic pathways

**Plenary lectures in the session Biotechnology**

A. Maraz (Hungary): Role of indegeneous yeast biota and selected strains in Tokaj wine fermentation.

M. Pesti (Hungary): Metal ion resistance in yeast and fungi: Chromium sensitivity and tolerance of fission yeast.

J. Sajbidor: The effect of cadmium on lipid content and composition of fatty acids in selected yeast species.

M. Rychtera, J. Cermak, J. Votruba: Optimalization of the ergosterol production during growth of *Saccharomyces cerevisiae.*

I. Hollerova, P. Kubizniakova: Yeast contamination in breweries.

J. Patkova, D. Smogrovicova: Stress factor during high concentration fermentations.

S. Sestak, V. Farkas, K. Sigler, D. Gaskova, B. Brodska: Effect of the osmotic stress on permeability and integrity of plasma membrane in *Saccharomyces cerevisiae.*

**Plenary lectures in the session Molecular biology and Genetics**

A. Kotyk, G. Lapathitis: G-Proteins in the activation of $H^+$-ATP-ase from the plasma membrane of *Saccharomyces cerevisiae.*

J. Subik, D. Papajova, M. Obernauerova, T. Simonics, Z. Kozovska, Y. Gbelska: Multiple mechanisms of resistance of *Saccharomyces cerevisiae* to mucidine.

Y. Gbelska, J. Subik: Biogenesis of mitochondrial respiration chain in the yeast *Kluyveromyces lactis*

M. Obernauerova, V. Dzugasova. K. Horvathova, J. Subik: Essencial role of phospholipids in mitochondrial functions of *petite* mutants of *Saccharomyces cerevisiae.*

P. Polcic, M. Sabova, P. Kempna, I. Kissova, J. Kolarov: Yeast mitochondrial ADP/ATP translocator - regulation, biogenesis and its role in the Bax-induced lethal effect.

L. Tomaska, J. Nosek, B. Kutejova: Molecular anatomy of yeast mitochondrial telomeres.

M. Spirek, A. Soltesova, J. Piškur, A. Horvath, P. Sulo: Yeast organelle engineering. What happens when natural mitochondria are replaced with foreign ones?

I. Janatova: Molecular biology of the yeast *Schwaniomyces occidentalis.*

E. Farkasova, M. Chovanec, J. Brozmanova: Estimation of DNA double strand breaks after induction and repair in *Saccharomyces cerevisiae* using a pulsed field gel electrophoresis analysis.

**Plenary lectures in the session Yeast Cytology**

M. Kopecká, M. Gabriel, A. Svoboda, K. Takeo, M. Yamaguchi, M. Ohkusu, K. Hata, S. Yoshida: Cytoskeleton and ultrastructure in some human fungal pathogens

M. Gabriel, J. Ishiguro , M. Kopecká A. Svoboda: Study of c*ps* - mutants of *Schizosaccharomyces pombe.*

Z. Palkova: Yeast colonies as organized multicellular structures.

**Plenary lectures in the session Immunology and Pathogenity**

T.Korolenko (Russia), E. Filushina, I. Buzueva, I. Svechnikova, J. Šandula: Yeast (1→3)-β-D-glucans as macrophage stimulators.

A. Tomsikova: Sepses caused by yeasts.

J. Sandula, E. Machova, G. Kogan: Structure, biological activity and application potential of yeast (1→→3)-β-D-glucans.

G. Kogan: Surface polysaccharides and glycoproteins – factors determining immunological specificity and virulence of pathogenic yeasts.

E. Machova, D. Chorvatovicova, G. Kogan, J. Sandula: Antimutagenic properties of *Saccharomyces cerevisiae* β-glucan, *Candida utilis* glucomannan and *Aspergillus niger* chitin-glucan.

L. Slovakova, J. Sandula: The modulation of defense response of plants against viruses by glucomannan derived from yeast cell wall.

S. Bystrický, E. Machová, G. Kogan: Preparation and characterization of reactive yeast glucan matrix for immunoconjugates.

E. Breierová, J. Sajbidor, I. Vajcziková: The changes of lipid contents some pathogen yeast strains induced with effect of fenpropidine derivates.

On the meeting of the Committee of the Czech and Slovak Commission for Yeasts held during the Conference it was decided that the 29th Annual Conference on Yeasts will be organized in Smolenice Castle in May 2001. Due to the fact that in the last years the Annual Conferences became more open for foreign scientists, particularly from neighboring countries like Hungary, it was recommended that in the next years the abstracts should be submitted and posters prepared in English rather than in Czech or Slovak languages. The Committee also suggested to try to organize every third Annual Conference on Yeasts as an international event, particularly for yeast researchers from Central European countries. The Committee agreed upon that the 20th ISSY, Yeast Cell Surfaces and Membrane Phenomena, organized by the Commission in 1999, was a great success both scientifically and socially.

Communicated by Peter Biely

## Tenth International Symposium on Yeasts, ISY 2000
## "The Rising Power of Yeasts in Science and Industry"

Over 400 participants enjoyed the meeting held at Papendal, Arnhem, The Netherlands, from 27 August to 1 September 2000. The Symposium Book, containing the programme, 429 pages of abstracts and the list of participants, is still available and can be ordered at a price of NLG 75,- from:

ISY 2000 c/o Lex Scheffers
Kluyver Institute for Biotechnology
Julianalaan 67
NL-2628 BC Delft, The Netherlands

E-mail: lex.scheffers@tnw.tudelft.nl

# Forthcoming Meetings

## Third International Alcohol Production Workshop, Matanzas University, Cuba
## "Plaza América" Convention Centre, Varadero, Cuba, April 16 to 19, 2001

**Aims.** 1. To promote the scientific exchange dealing with the current advances in the field of ethanol production and Yeast Biotechnology. 2. To foment the development of alternative biotechnological methods in ethanol production and wastewater treatment systems. 3. To contribute to take reliable actions in order to improve the international co-operation in alcohol production and research.

**Topics for Discussion.** 1.Ethanol production. 2. Biochemistry of ethanolic fermentation. 3. Production of rum and other alcoholic beverages. 4. Fuel ethanol. 5. Selection of yeasts for the production of ethanol and alcoholic beverages. 6. Lignocellulosic materials as feedstocks for ethanol production. 7. Molecular Biology of yeasts. 8. Application of electricity and magnetism in yeast and fermentation studies. 9. Treatment and reutilization of alcohol industrial wastes and wastewaters. 10. Alcohol and health problems. Alcoholism.

**Official Languages.** Spanish and English. Translation services will be offered on request during the sessions and activities of the meeting.

**Venue.** The venue for the workshop is the International Convention Centre "Plaza América", a modern istalation with conference facilities for up to 1000 people. It is located only 5 minutes walk to the sands of Varadero, the largest Cuban beach. Many restaurants and hotels are situated very close.

For more information, contact:

Ing. Rolando Madruga Rodríguez,
Organizador Profesional de Eventos
Fax: (53-5) 66 8181, 66 7895
Tel.:(53-5) 66 8163
E-mail: eventos@plamer.var.cyt.cu

**Registration.** The registration fees will be $100 USD. The registration must be paid in cash at Matanzas University.

**Deadline For Abstract Submission Receipt.** February 1st, 2001. Abstracts should preferably be submitted by E-mail.

**Accommodation**

MERCADU travel agency offers full lodging facilities at the University Motel in the campus of the University of Matanzas, as well as at five- and four-stars hotels in Varadero beach, near to the International Convention Centre.

For more information, contact:

Lic. Humberto Suárez
Gerente Turismo Especializado.
Fax: (53)(52) 253101, Phone: (53)(52) 261934
E-mail: merca@cdict.umtz.edu.cu

**Social Activities.** During the free time, a group of social activities will be held for attendants and companions. Also a special activity will be organized in which different alcoholic beverages will be presented. (Equipment, instruments and new technologies could also be presented). This presentation will be performed by national or international producers or by official firms or companies established in Cuba.

**Matanzas and the University.** The University of Matanzas was founded in 1972. It is located in the outskirts of Matanzas, the capital of the province with the same name. The city of Matanzas is situated in the Northwest part of Cuba, 100 kilometres away from Havana and about 25 kilometres from the world-wide known Varadero beach, the most relevant tourist resort in Cuba. The foundation of the city dates back to October 12, l693 with the name of "Villa de San Carlos y San Severino de Matanzas". Known by many as "The City of Many Bridges and Rivers", Matanzas cradles a rich cultural tradition and beautiful landscapes. Its heights and plains, the fertility of it's soils, the famous Yumuri slope mouth and its exclusive flora and fauna, turn it to be one of the most attractive places in Cuba. Recreated by painters and sang by poets and musicians, Matanzas possesses many museums and places of extraordinary importance for their antique values and cultural richness, among them: Gener y Del Monte Library, the French Pharmacy founded in the XIX Century, San Pedro Apostol Church, the Ermita de los

Catalanes de Monserrat, as well as many historical and unique landscapes in Playa Girón and Ciénega de Zapata. Many sugar mills are located in Matanzas province. Sugarcane molasses are the main feedstock

For further information and abstract submission, please contact:

Prof. Marcelo Marcet, PhD.
Chairman of the Organizing Committee
Department of Chemistry and Chemical Engineering
Matanzas University.
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Phone: 53 52 261432
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for alcohol production. There are in Matanzas two alcohol distilleries which produce the ingredients for the famous Havana Club Rum.

## European Brewery Convention
## 28th International EBC Congress, Budapest, Hungary, 12-17 May 2001

**Scientific Programme.** The scientific programme will comprise invited lectures, submitted lectures, poster presentations and poster debates. The presentations will cover different aspects of brewing science and technology and relevant topics will include scientific development in the supply chain from raw materials to finished packaged products. In addition there will be 4 special interest areas: (1) Beer & Health, (2) Environmental Issues, (3) Risk Management, and (4) Packaging Strategies. In particular also technological topics will be given a high priority.

**Technical Visits.** The Hungarian brewers will arrange a number of technical visits to malting plants and breweries in the country. The total Hungarian beer production, serving a population of 10 million, is some 7 million hectolitres annually. Thanks to the complete privatisation of the formerly state-owned breweries and maltings, Hungary now meets international standards for production and beer quality. The Technical Visits will take place on Thursday 17 May.

**Social Programme.** Social events for delegates and their partners will include the Opening Ceremony and Welcome Reception on Sunday 13 May and a Closing Session and Farewell Cocktail Party on Wednesday 16 May. Tours for partners will also be arranged. More details will be given in the Provisional Programme.

**Congress Venue.** The recently built Congress Centre of Budapest, designed by leading Hungarian architects, serves the express purpose of housing large international meetings like the EBC Congress. Surrounded by beautiful gardens and hotel Novotel alongside, it contains everything to cater for the needs of an international audience. Hotel Accommodation Rooms have been reserved in hotels of various price categories. Details will be announced in the Provisional Programme.

**Registration.** The Registration Form and detailed information about the scientific and social programmes and hotel accommodation will be included in the Provisional Programme, scheduled to become available in ]anuary 2001. To receive this information please contact your national Brewers Association if residing in an EBC member country or contact the EBC Secretariat.

Secretariat General, EBC
P.O. Box 510
2380 BB Zoeterwoude
The Netherlands

## IMC7 - 7" International Mycological Congress

The 7th International Mycological Congress will take place at the University of Oslo, Oslo, Norway, August 11-17, 2002.

Oslo, Norway's capital, located at the bottom of the Oslo Fjord, lies in the heart of Scandinavia. The city was founded at the turn of the 10'h century, surrounded by forested hills with lakes and paths. The university campus, where the congress will take place, is 5-10 minutes away by subway from the city center.

Oslo with its bristling boating and swimming activity in the summer, offers a multitude of sport and recreational opportunities, an opera and several concert halls and more than 50 unique museums including the Munch Museum, Kon-Tiki/Ra Museum, Fram and the Viking Ship Museum, Nonvegian Folk Museum, Vigeland Sculpture Park and Vigeland Museum, and Holmenkollen skijump, featuring the world's oldest Ski Museum. The city enjoys an outgoing atmosphere with hundreds of restaurants covering almost any ethnic tastes.

We warmly invite you to participate at the Conference, in the company of colleagues from all over the world. When in

Norway in summer, why not go (with your family?) to the spectacular fjords of Western Norway or the North Cape plateau in the far north?

The Organizing Committee and the Plus Convention Norway AS will make every effort to ensure a scientifically interesting congress and a memorable stay.

Leif Ryvarden, chairman

Trend Sehumneher, vice-chairman

Major themes of the congress are: Fungal cell biology. Fungal populations, dynamics and ecology. Fungal pathogens. Fungus-host interactions. Phylogeny and Evolution. Biodiversity and Conservation.

To receive the Second Circular for the Congress, contact

IMC7 Congress Secretariat
P.O. Box 24 Blindern
N-0314 Oslo, Norway

IMC-7@bio.uio.no
www.uio.no/conferences/imc7

## Yeasts of the Third Millenium
## 21st International Specialized Symposium on Yeasts - 21th ISSY 2001
## Biochemistry, Genetics, Biotechnology and Ecology of Non-conventional Yeasts (NCY)
## Tuesday, 21 August - Saturday, 25 August, 2001, Lviv, Ukraine

The symposium will be organized by Lviv Institute of Cell Biology (formerly: Lviv Division of Institute of Biochemistry) and Lviv National University (Ukraine) together with Rzeszów Pedagogical University (Poland) under auspices of the Presidium of National Academy of Sciences of Ukraine and Ministry of Education and Science of Ukraine.

List of Sessions (preliminary): Systematics and Ecology of NCY. Genome Organization and Gene Expression. Genome Sequencing in NCY. Metabolic Regulation and Engineering . Organelles Cell Surface: Structure and Functions . Stress Response. Heterologous Gene Expression. Industrial Applications. Medically Important Yeasts. *Saccharomyces versus* Non-*Saccharomyces*: Similarities and Differences.

Leading scientists from different fields of investigation of non-conventional yeasts will be invited to present lectures. Symposium program will include 4 plenary lectures . Each session will have 6 oral presentations by invited speakers. Some of these oral presentations will be selected from submitted poster abstracts. In addition, each session will include a poster presentation. Each session will be convened by two Go-Chairpersons.

At present, the following leading scientists have agreed to speak at the meeting: J.M. Cregg (USA), F. Sherman (USA), D. Klionsky (USA), C.P. Kurtzman (USA), P. Slonimski (France), C. Gaillardin (France) , C. Gancedo (Spain) , A. Dominguez (Spain) , S. Oliver (UK) , P. Sudbery (UK) , M. Veenhuis (The Netherlands), J. Thevelein (Belgium), G. Earth (Germany), C.P. Hollenberg (Germany) , P. Rasper (Slovenia) and others . Arrangement of the scientific program of the Symposium by the members of the International Scientific Committee is in progress.

**International Scientific Committee:** Gerold Earth, Dresden Technical University, Germany ( *Yarrowia lipolytica*) . James M. Cregg, Keek Graduate Institute, Claremont, USA (heterologous gene expression, organelles). Graham H. Fleet, University of New South Wales, Sydney, Australia (ecology) . Laura Frontali, Rome University "La Sapienza", Italy (Kluyveromyces) . Sergei G. Inge-vechtomov, St. Petersburg University, Russia (genetics) . Cornelis P. Hollenberg, Düsseldorf University, Germany (heterologous gene expression) Cletus P. Kurtzman, Center of Agricultural Research, Peoria, USA (systematics). Jesus Pla, Madrid University, Spain (*Candida albicans*). Peter Raspor, University of Ljubljana, Slovenia (yeast diversity). Andrei A. Sibirny, Institute of Cell Biology, Lviv, Ukraine (metabolic regulation). Suresh Subramani, University of California at San Diego, La Jolla, USA (organelles). Masamichi Takagi, The University of Tokyo, Japan (*Candida maltosa*). Yuri A. Trotsenko, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia (biochemistry). Marten Veenhuis, Groningen University, The Netherlands (cell biology, organelles).

**Local Organizing Committee:** Andrei A. Sibirny, Inst. Cell Biol., Lviv, Chairman. Mykhailo V. Gonchar, Inst. Cell Biol., Lviv, Treasurer. Daria V. Fedorovych, Inst. Cell Biol., Lviv. Stepan P. Gudz, Lviv National University. Zbigniew Kotylak, Rzeszów Pedagogical University. Aleksandr R. Kulachkovsky, Lviv National University. Valentyn S. Pidgorsky, Inst. Microbiol. Virol., Kiev. Oleh V. Stasyk, Inst. Cell Biol., Lviv. Vira M. Ubyivovk, Inst. Cell Biol., Lviv.

**Secretariat:** Mykela M. Maidan, Inst. Cell Biol., Lviv (Head). Yuri R. Boretsky, Inst. Cell Biol., Lviv. Andrii Y. Voronovsky, Inst. Cell Biol., Lviv. Taras Y. Nazarko, Inst. Cell Biol., Lviv Oksana M. Moroz, Lviv National University.

**Venue.** Conference hall of the main building of Lviv National University, Lviv, Ukraine. This building is located in the historical centre of the city. The best hotels, museums, art exhibitions, and restaurants are located nearby. Lviv (also known as: Lvov, Lwow, Lwów, Lemberg, Leopoli) is the largest scientific, cultural and industrial city in the Western Ukraine with a population of nearly 1 million located in the geographical centre of Europe. It was founded as a fort in the mid-13th century by the Duke Danylo Galitsky of Galicia and was a former principality of Kyivan Rus.

**Symposium Language.** English will be the working language.

**Registration Fee.** Tentative registration fees

(including Book of Abstracts, a bag, 8 coffee breaks, 4 lunches, get-together party and concert in Lviv opera house): Regular: 350 USD; Students: 200 USD; Accompanying persons : 100 USD. Registration is limited to 250 participants.

**Accompanying person programme.** Lunches, get-together party, concert in Lviv opera house.

**Transportation.** Lviv has daily flight connections from Kiev, Moscow and Warsaw, twice weekly (Tuesday, Friday) flights from Frankfurt /Ma in ( Germany ) and once weekly from Toronto (Canada). During summers, Lviv also has direct flights from New York. Lviv has direct train connections with major cities of the former Soviet Union including the Baltic republics. There are also direct train connections from Warsaw, Prague, Bratislava, Budapest, Bucharest, Varna as well as several western European cities including Berlin, Vienna and Venice. Lviv also has well developed bus connections with many cities of Europe, including Eastern Europe and some Western European cities (London, Manchester, Brussels, Amsterdam, Paris, Berlin, Frankfurt and others).

**Hotel Accommodations.** The average price per person per night is from 50-100 USD (Grand Hotel, Dnister, George) to 2540 USD (Lviv, Independence). All these hotels are located in downt own within walking distance (5-15 min) from the Lviv National University, where the symposium will be held. Less expensive accommodation (10-15 USD per person per night) will be available for students.

**Social Programme.** A get-together party will be arranged on the opening day of the Conference. The symposium dinner will be held at the Palace of Arts in the centre of old city. Two excursions will be organized by Organizing Committee: a walking tour around the historical centre of Lviv and a bus tour to Olesko castle (70 km from Lviv). Additional sightseeing tours will be available on an individual basis.

**Information and Correspondence**

Organizing Committee ISSY2001
Lviv Institute of Cell Biology
Drahomanov Street 14/16
Lviv 79005
Ukraine

Phone: +380-322/740363
Fax: +380-322/721648
E-mail: ISSY2001@biochem.Lviv.ua

## XXth International Conference on Yeast Genetics and Molecular Biology
## Prague, Czech Republic, August 26 to 31, 2001

The XXth International Conference on Yeast Genetics and Molecular Biology will be held in Prague, Czech Republic, from August 26 to 31, 2001. More information is available from the website: http://www.biomed.cas.cz/yeast2001

The main aim of international meetings organized by the yeast community at intervals of two years is to provide a forum to present and discuss recent advances in rapidly expanding fields of yeast genetics and molecular biology and to bring into focus novel themes for future investigations.

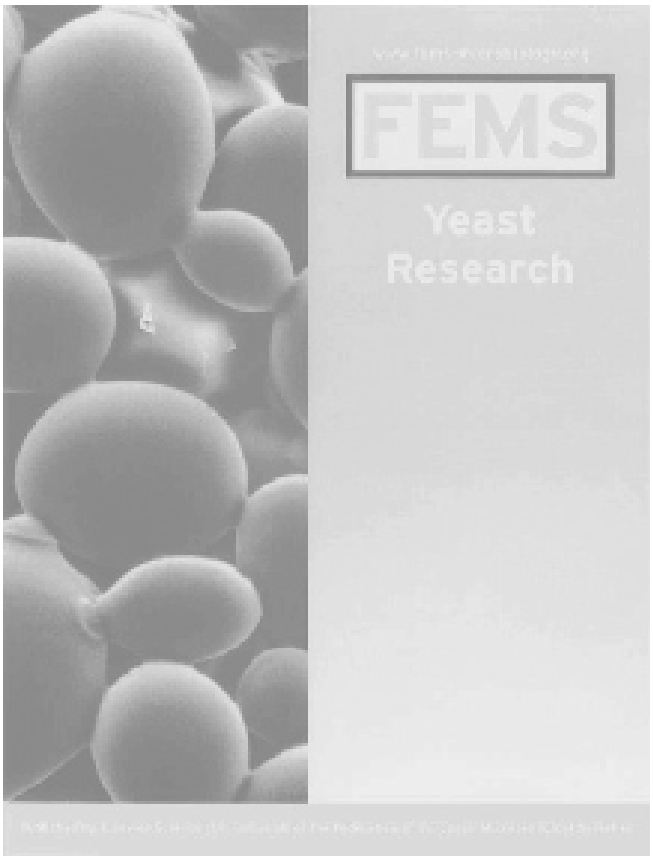
The scientific programme has been already set up and includes top speakers covering the major areas in the field: J. Berman (USA), M. Breitenbach (A), A. Bretscher (USA), M. Carlson (USA), T. G. Cooper (USA), B. S. Cox (UK), B. Dujon (F), D. G. Drubin (USA), E. A. Elion (USA), S. D. Emr (USA), D. Finley (USA), A. Goffeau (F), N. A. Gow (UK), M. Grunstein (USA), L. Gustaffson (SE), L. Hicke (USA), M. Johnston (USA), M. C. Kielland-Brandt (DK), D. Klionsky (USA), S. L. Lindquist (USA), F. Madeo (D), A. Murray (USA), K. Nasmyth (A), A. Nicolas (F), P. Novick (USA), P. Nurse (UK), S. G. Oliver (UK), Z. Palkova (CZ), D. Pellman (USA), M. Peter (CH), M. A. Romanos (UK), E. Schiebel (UK), P. P. Slonimski (F), K. Struhl (USA), M. F. Tuite (UK), M. Werner-Washburne (USA), H. V. Westerhoff (NL), E. A. Winzeler (USA), D. H. Wolf (D), M. Yamamoto (J).

The Second Announcement with Call for Abstracts and a Registration Form will be distributed in November 2000. You are encouraged to register on the website but if you are interested in receiving a paper version of the Second Announcement send your mailing address to: yeast@biomed.cas.cz.

We look forward to your contribution and participation in the XXth ICYGMB Prague 2001.

Local Organizing Committee.

## New Journal - *FEMS Yeast Research* - Call for papers

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Deputy Chief Editor: Teun Boekhout, CBS - Centraal Bureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands <t.boekhout@tnw.tudelft.nl>.

The journal will publish high-quality original research papers and mini-reviews that cover both yeast and yeast-like organisms. The editors aim to cover the entire field of yeast research in its broadest sense. The audience of the journal will include yeast researchers in academic institutions, but also those that use yeast as a model organism or work in industry. The following list of disciplines is not exclusive but these main topics are explicitly mentioned here to illustrate the wide spectrum of topics covered by the journal: physiology, taxonomy, phylogenetics, evolution, biodiversity, ecology, genetics, molecular biology, metabolic engineering, biotechnology, food microbiology, heterologous protein production and secretion, pathogenic yeasts, typing and diagnostics. In the field of physiology, the journal encourages submission of papers in rapidly developing fields like: cell cycle, morphogenesis, cell wall, organelle biosynthesis, cell ageing, metabolic regulation, transport, energetics, stress response, and signal transduction. Submissions dealing with genome sequencing and functional genomics are welcomed and greatly encouraged, as are papers in the important new area of comparative genomics.

Publication will commence with Volume 1, starting in 2001, with 4 issues. ISSN: 1567-1356. Visit the yeast website for more information and instructions to authors: www.elsevier.com/locate/femsyr