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Yeast

A Newsletter for Persons Interested in Yeast

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Editorials

Henri Heslot 1921-2001

I regret to announce the passing of Professor Henri Heslot, on December 24 2001. One of the longest standing subscriber to the Yeast Newsletter, Prof. Heslot made life-long contributions to yeast genetics and its applications. I thank Dr. Claude Gaillardin for providing me with a detailed obituary. The text appearing in the YNL is my abridged translation.

N. J. W. Kreger -van Rij

The yeast research community is saddened by the recent passing of Dr. Nel Kreger-van Rij. A number of her closer colleagues are preparing an obituary for publication in FEMS Yeast Research in the spring. I hope to include a summary in the June issue of the Yeast Newsletter.

Van Uden International Advanced Course on Molecular Ecology, Taxonomy and Identification of Yeasts Caparica, Portugal, July 21 - August 1, 2003

I am delighted to learn that our colleagues Isabel Spencer-Martins, Álvaro Fonseca, and Zé Paulo Sampaio decided to organize an International Advanced Course in the van Uden tradition. Several such courses were held in the eighties at the Gulbenkian Institute in Oeiras. Many professionals that are active today in industry, academia, or culture collections learned their trade in one of these courses. The courses offer graduate students and other individuals interested in yeast taxonomy, the opportunity to learn directly from renowned practitioners in the field. The next instalment will be given in the modern facilities of the New University of Lisbon. All interested should consult the Brief News Item section for details.

I wish all our readers a happy and scientifically rewarding new year!

M.A. Lachance
Editor

I. Centraalbureau voor Schimmelcultures, Uppsalalaan 8, NL 3584 CT Utrecht, The Netherlands. Communicated by M. Th. Smith.

Upgrade of BioloMICS Web

As many of the readers of the Yeast Newsletter know, two years ago, the CBS started a new web-based service allowing online strains and species advanced searches and identifications. This search engine is called BioloMICS Web and is freely accessible at http://www.cbs.knaw.nl/yeast/webc.asp. Many researchers and people from the industry are now regularly visiting daily this web site. We've had recently some questions about the methods that we are using to generate our data, while others are asking for more functionalities and/or user friendliness. Since we constantly want to improve our services, we have decided to renew completely our web site to incorporate new information, features and software tools. We plan to release this new version for the first quarter of 2003. We would therefore be very happy to get criticisms and suggestions from our users in order to help the scientific community as best as we can. So please, feel free to contact us at:

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The following articles have appeared or are in press.

1. Naumova, E.S., Smith, M.Th., Boekhout, T., Hoog, G.S. de & Naumov, G.I. 2001. Molecular differentiation of sibling species in the *Galactomyces geotrichum* complex. Antonie van Leeuwenhoek **80**:263-273.

2. Petter, R., Kang, B.S., Boekhout, T., Davis, B.J. & Kwon-Chung, K.J. 2001. A survey of heterobasidiomycetous yeasts for the presence of the gene homologues to virulence factors of *Filobasidiella neoformans*, *CNLAC*1 and *CAP*59. Microbiology **147**:2029-2036.

3. Robert, V., Bonjean, B., Karutz, M., Paschold, H., Peeters, W. & Wubbolts, M.G. 2001. *Candida bituminophila*, a novel anamorphic species of yeast. Int. J. Syst. & Evol. Microbiol. **51**:2171-2176.

4. Theelen, B., Silvestri, M., Guého, E., van Belkum, A. & Boekhout, T. 2001. Identification and typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLP ™), random amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE). FEMS Yeast Research **1**:79-86.

5. Wolf, D.G., Falk, R., Hacham, M., Theelen, B., Boekhout, T., Scorzetti, G., Shapiro, C., Salkin, I.F. & Polacheck, I. 2001. Multidrug-resistant *Trichosporon asahii* infection of nongranulocytopenic patients in three intensive care units. J. Clin. Microbiol. **39**:4420-4425.

6. Averbuch, D., Boekhout, T., Falk, R., Engelhardt, D., Shapiro, M. & Polacheck, I. 2002. Fungemia in a cancer patient caused by fluconazole resistant *Cryptococcus laurentii*. Med. Mycol. **40**:479-484.

7. Bai, F.-Y., Zhao, J.-H., Takashima, M., Jia, J.-H., Boekhout, T. & Nakase, T. 2002. Reclassification of the *Sporobolomyces roseus* and the *Sporidiobolus pararoseus* complexes, with the description of *Sporobolomyces phaffii* sp. nov. Int. J. Syst. Evol. Microbiol. (in press).

8. Bauters, T.G.M., Swinne, D., Boekhout, T., Noens, L. & Nelis, H.J. 2002. Repeated isolation of *Cryptococcus laurentii* in the oropharynx despite treatment with fluconazole. Mycopathologia **153**:133-135).

9. Boekhout, T. & Guého, E. 2002. Basidiomycetous yeasts. In: Pathogenic fungi of humans and animals (Howard, D., ed.), Dekker, New York, pp. 535-564.

10. Boekhout, T. & Phaff, H.J. 2002. Yeast biodiversity and systematics. In: Yeasts and Food (Boekhout, T. & Robert, R., eds). Behr's Verlag Hamburg, Germany (in press).

11. Boekhout, T. & Robert, R. (eds). 2002. Yeasts and Food. Behr's Verlag Hamburg, Germany (in press).

12. Boekhout, T., Robert, V., Smith. M.Th., Stalpers, J., Yarrow, D., Boer, F., Gijswit, G., Kurtzman, C.P., Fell, J.W., Guého, E., Guillot, J. & Roberts, I.. 2002. Yeast species of the world - a CD-ROM, Expertise Center Taxonomic Identification (ETI), University of Amsterdam, Amsterdam.

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

14. De Leo, F., Urzì, C. & Hoog, G.S. de. A new meristematic fungus, *Pseudotaeniolina globosa*. Antonie van Leeuwenhoek (in press).

15. Gemmer, C.M., DeAngelis Y.M., Theelen, B., Boekhout, T. & Dawson, T.L. 2002. Fast, noninvasive method for molecular detection and differentiation of *Malassezia* yeast species on human skin and application of the method to dandruff microbiology. J. Clin. Microbiol. **40**:3350-3357.

16. Göttlich, E., Hoog, G.S. de, Genilloud, O., Jones, B.E., & Marinelli, F. MICROMAT: Culturable fungal diversity in microbial mats of Antarctic lakes. Bookchapter SCAR Symposium (in press).

17. Göttlich, E., Lubbe, W. van der, Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H.-C. & Hoog, G.S. de. 2002. Fungal flora in groundwater-derived public drinking water. Int. J. Hyg. Environm. Health **205**:269-279.

18. Kantarclu, A.S., Hatemi, G., Yücel, A., Hoog, G.S., Samson, R.A. & Mandel, N.M. 2002. *Paecilomyces variotii* central nervous system infection in a patient with cancer. Mycoses (in press).

19. Kurtzman, C.P., Fell, J.W., Robert, V., Yarrow, D., Deak, T. & Boekhout, T. 2002. Methods to identify yeast. In: Yeasts and Food (Boekhout, T. & Robert, R., eds). Behr's Verlag Hamburg, Germany (in press).

20. Matos, T., Haase, G. & Hoog, G.S. de. 2002. Ecology, molecular variation and taxonomy of oligotrophic and neurotropic members of the black yeast genus *Exophiala*. Antonie van Leeuwenhoek (in press).

21. Matos, T., Hoog, G.S. de, Boer, A.G. de, Crom, I. & Haase, G. 2002. High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities Mycoses (in press).

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

23. Trilles, L., Lazéra, M., Wanke, B., Theelen, B. & Boekhout, T. 2002. Genetic characterization of environmental isolates of the *Cryptococcus neoformans* species complex from Brazil. Med. Mycol. (accepted).

24. Vitale, R.G. & Hoog, G.S. de. 2002. Molecular diversity, new species and antifungal susceptibilities in the *Exophiala spinifera* clade. Med. Mycol. (in press).

25. Yurlova, N.A. & Hoog, G.S. de. 2002. Exopolysaccharides and capsules in human pathogenic *Exophiala* species. Mycoses (in press).

The following articles have been submitted.

26. Cadez, N., Poot, G.A., Raspor, P. & Smith, M.Th. Four new apiculate yeast species of the yeast genus *Hanseniaspora*: *H. meyeri, H. clermontiae, H. lachancei* and *H. opuntiae*. Int. J. Syst. Evol. Microbiol. (submitted).

27. Kerrigan, J.L., Smith, M.Th., Rogers, J.D., Poot, G.A. & Douhan, G.W. *Ascobotryozyma cognata*, a new species from nematodes in wood-boring beetle galleries. Mycological Research (submitted).

28. Kurzai, O., Keith, P., Hopp, H., Hoog, G.S. de, Abele-Horn, M. & Frosch, M. 2003. Post mortem isolation of *Pseudotaeniolina globosa* from a patient with aortic aneurysm. Mycoses (submitted).

29. Naumov, G.I., Naumova, E.S., Smith, M.Th & Hoog, G.S. de. Ribosomal DNA sequencing and reinstatement of the genus *Arthroascus* von Arx. J. Gen. Appl. Microbiol. (submitted).

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

31. Smith, M.Th & Poot, G.A. Genome comparisons in the genus *Dipodascus* de Lagerheim. FEMS Yeast Research (submitted).

32. Vitale, R.G., Hoog, G.S. de, Rijs, T.G. & Verweij, P.E. *In vitro* activity of amphotericin B and itraconazole in combination with fluorocytosine, sulphadiazine and quinolones against *Exophiala spinifera*. J. Clin. Microbiol. (submitted).

33. Vitale, R., Hoog, G.S. de & Verweij, P.E.. *In vitro* activity of antifungal drugs and evaluation of Post Antifungal Effect (PAFE) of *Exophiala spinifera* against amphotericin B, itraconazole, terbinafine and 5-fluorocytosine. Med. Mycol. (submitted).

II. Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yusong, Daejeon 305-600, Republic of Korea. Communicated by K.S. Bae <ksbae@mail.kribb.re.kr>.

The following papers have been published recently.

1. S.G. Hong, J. Chun, H.W. Oh, and K.S. Bae. 2002. *Metschnikowia koreensis* sp. nov., a novel yeast species isolated from flowers in Korea. Int. J. Syst. Evol. Microbiol. **51**:1927-1931.

A novel ascomycetous yeast was isolated from flowers of *Lilium* sp. and *Ipomoea* sp. in Korea. The name *Metschnikowia koreensis* sp. nov. (type strain SG99-34T=CBS 8854^T = KCTC 7998^T) is proposed for this novel species based on comparative sequence analyses of the D1/D2 domain of 26S rDNA and phenotypic characteristics.

2. S.G. Hong, K.S. Bae, M. Herzberg, A. Titze, and M.A. Lachance. In press. *Candida kunwiensis* sp. nov., a Yeast Associated with Flowers and Bumblebees. Int. J. Syst. Evol. Microbiol.

A novel ascomycetous yeast, *Candida kunwiensis* (type strain SG99-26^T = KCTC 17041), was isolated from sweet potato (*Ipomoea batatas*) flowers in Korea and from the body surface of pollinating bumblebees in Germany. Comparative analysis of the D1/D2 domain of 26S rDNA of all available sequences for ascomycetous yeasts showed that the new species is phylogenetically related to the genus *Metschnikowia*, but the sequence similarity was low. Morphologically and physiologically, *C. kunwiensis* resembles in many ways *Metschnikowia pulcherrima*, but can be distinguished by its ability to assimilate lactic acid and its inability to produce pulcherrimin.

3. Hong, S.G., Lee, K.H., and Bae, K.S. 2002. Diversity of yeasts associated with natural environments in Korea. J. Microbiol. (Korea) 40:55-62.

Biodiversity of yeasts in various natural environments including soils, swamps and plants was investigated. By molecular identification methods based on the partial sequences of 26S rDNA, 69 isolates were assigned to 44 taxa including 27 known species. The remaining 17 taxa could potentially form new species. All of them were classified into Ascomycota, Hymenomycetes, Urediniomycetes and Ustilaginomycetes. Ascomycetous and ustilaginomycetous yeasts were generally isolated from flower samples, and hymenomycetous and urediniomycetous yeasts were generally isolated from soil samples. Distribution of yeast groups exhibited geographical variation. Yeast biodiversity of root soil also varied according to the associated plant species.

III. Department of Biology, Carleton University, 587 Tory Building, 1125 Colonel By Drive, Ottawa, Ontario Canada K1S 5B6. Communicated by B.F. Johnson.

The following 6 chapters are in press in Lab Manual on Non-conventional Yeasts; Genetics, Biochemistry, Molecular Biology, and Biotechnology. Edited by K. Wolf, K. Breunig and G. Barth. Springer-Verlag, Berlin, 2002.

1. Calleja, G.B., Walker, T., Levy-Rick, S. and Johnson, B.F. Induction of amylases in *Schwanniomyces occidentalis*.

2. Calleja, G.B., Levy-Rick, S., Walker, T., and Johnson, B.F. Ethanol production from starch by *Schwanniomyces occidentalis*.

3. Calleja, G.B., Levy-Rick, S. and Johnson, B.F. Testing various yeasts for ethanol production from xylose.

4. Johnson, B.F., Calleja, G.B. and Walker, T. Subjective analysis of medium-induced fragility of *Schwanniomyces occidentalis*.

5. Johnson, B.F. Wahalawatta, D., Mukherjee, S., Ficker, C., Boroumandi, S., Clarkin, O., Ramsingh, B., Siddiqi, F., Vessal, M., Escorcia, J., Chock, E., Lopez, M., Khulbe, S., Privora, H., Booth, R., and Calleja, G. B. Objective analysis of medium-induced fragility of *Schwanniomyces occidentalis*.

6. Johnson, B.F., Calleja, G. B. , Yoo, B.-Y. and Liaquat, K. Analysis of structural discontinuities of the wall of *Schwanniomyces occidentalis*.

IV. Department of Plant Pathology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797. Communicated by J.W. Buck <jbuck@griffin.peachnet.edu>.

The following manuscripts were published this past year.

1. Buck, J.W., and Burpee, L.L. 2002. The effects of fungicides on the phylloplane yeast populations of creeping bentgrass. Can. J. Microbiol. 48:522-529.

2. Buck, J.W. 2002. In vitro antagonism of *Botrytis cinerea* by phylloplane yeasts. Can. J. Bot. **80**:885-891.

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

Current publications.

1. Barnett, J.A. & Robinow, C.F. 2002. A history of research on yeasts 4: cytology part II, 1950-1990. Yeast 19:745-772.

2. Barnett, J.A. 2003. A history of research on yeasts 5: the fermentation pathway. Yeast (in preparation).

3. Barnett, J.A. 2003. Beginnings of microbiology and biochemistry: the contribution of yeast research. Microbiology (in preparation).

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

Recent publications.

1. Caridi A. 2002. Protective agents used to reverse the metabolic changes induced in wine yeasts by concomitant osmotic and thermal stress. Lett. Appl. Microbiology **35**:98-101.

Aims: The reversion of the metabolic changes induced in wine yeasts by stressors. **Methods:** Six strains of *Saccharomyces* were inoculated in grape must containing over 400 g l^{-1} of sugar and incubated at 35°C, both with and without the addition of 100 mg l^{-1} of catechin, inositol or SO_2. **Results:** Significant correlations between addition of the stress-protectants and change in the metabolic behaviour of the wine yeasts were observed. Depending on strain and protectant, and expressing data as a percentage of increase or decrease compared to the control, fermentation vigour after 3 d increased by up to 10%, titratable acidity of the wines increased by up to 7%, ethanol content increased by up to 20%, unitary acetic acid production decreased by up to 35%, and unitary glycerol production decreased by up to 20%. **Impact of Study:** By using protective agents it is possible to minimize the abnormal fermentation performance that wine yeasts exhibit under thermal and osmotic stress.

2. Caridi A., Cufari A., Ramondino D. 2002. Winemaking from Gaglioppo Grapes with Hybrid Strains of *Saccharomyces*. Fol. Microbiol. **47**:407-408.

The fermentative behaviour of two hybrid wine yeast strains (first-generation hybrid-strain 12233x6167 – obtained by hybridization of the cryotolerant strain *S. bayanus* 12233 with the mesophilic strain *S. cerevisiae* 6167, and TT254x6392 arising by hybridization of the thermotolerant strain *S. cerevisiae* TT254 with the mesophilic strains *S. cerevisiae* 6392) was compared with that of a commercial wine yeast strain *S. cerevisiae* K1 in must from black grapes of the Calabrian variety Gaglioppo. The goal was to obtain wines with a high content of 'polyphenols' from a grape must with a limited phenolic content such as the Gaglioppo must. The progress of the winemaking was estimated according to residual sugars; at the end of fermentation, the wines were decanted, bottled and principal physico-chemical characteristics determined. Our results point to the possibility to select wine yeasts (significantly differing in the above parameters) by their ability to interact with phenolic compounds.

3. Caridi A., Cufari A., Ramondino D. 2002. Isolation and clonal pre-selection of oenological *Saccharomyces*. J. Gen. Appl. Microbiol. **48**: (in press).

The aim of the present study was to perform a fast pre-selection from a great number of wine yeasts using a simple phenotypic-based methodology that allows many different strains to be simultaneously tested. A total of 150 elliptic yeasts, isolated from must and wine from black grapes of a distinctive Italian variety, were studied. Yeasts were identified to genus level by assessing their ability to ferment glucose and their production of spores on acetate agar. The *Saccharomyces* strains were seeded on BiGGY agar to determine their H_2S production, on calcium carbonate agar to test their acetic acid production, and on grape-skin agar and on grape-seed agar to assess their interaction with phenolic compounds. The *Saccharomyces* strains were also examined for fermentative vigour after 2 d or 7 d both with and without the addition of 100 mg l^{-1} of SO_2 in must at 20°brix and pH 3.20. At the end of fermentation, the wines produced by the 18 best yeasts were analysed and the strains were studied for additional biochemical and technological characteristics. The resistance of the strains to simultaneous acid-stress and osmotic-stress was studied carrying out in duplicate winemaking tests in must at 30°brix and pH 2.60. A remarkable heterogeneity among the 150 autochthonous yeasts studied was demonstrated. The phenotypical biodiversity is particularly interesting for several technological characteristics useful in winemaking, such as fermentation vigour, acetic acid production and malic acid content of the wines. The vast majority of the elliptic wine yeasts isolated did not show suitable characteristics, so only 18 strains, 12% of the total, remained for the final tests. Many of the strains that had passed the preliminary screenings revealed some defects when they were studied for fermentation performance, both in standard winemaking and under stressors. Two strains exhibited particularly interesting performances: one strain for winemaking of normal musts and the other for winemaking of musts from dried grapes or under stressful conditions.

4. Caridi A., Micari P., Foti F., Ramondino D., Sarullo V. 2002. Ripening and seasonal changes in microbiological and chemical parameters of the artisanal cheese Caprino d'Aspromonte produced from raw or thermized goat's milk. Food Microbiol. **19**: (in press).

The aim of the present research was to monitor the microbiological and the chemical characteristics of Caprino d'Aspromonte, a cheese produced from raw or thermized goat's milk, to regulate, if necessary, the production process. In spring 2000, and again in summer 2000 and winter 2001, three independent batches of cheese were analysed after 0, 14 and 28 days of ripening. High logarithmic counts per gram of cheese for mesophilic coccal-shaped lactic acid bacteria (6.94-11.90), thermophilic coccal-shaped lactic acid bacteria (4.08-9.43), mesophilic lactobacilli (3.63-9.60), and thermophilic lactobacilli

(2.78-8.36) were found. Coliforms, *Escherichia coli*, enterococci, and yeasts were considerably lower. Physico-chemical parameters such as pH (5.09-6.89), dry matter (42.69-66.97%), ether extract (19.36-34.33%), crude protein (14.25-27.21%), and chloride content (0.92-2.60%) were also determined. Microorganisms indicating low level of hygiene were high and several cheeses failed the standards laid down by the Italian legislation. Lactic acid bacteria, cocci and rods, are the dominant microflora throughout ripening. Enterococci are two log units lower than in other goat's cheeses made from raw milk. The majority of the yeasts do not ferment the lactose with production of gas. To increase cheese quality, it is necessary to improve the hygiene conditions of milking, milk-storage, and cheese-making.

5. Micari P., Caridi A., Colacino T., Caparra P., Cufari A. 2002. Physicochemical, microbiological and coagulating properties of ewe's milk produced on the Calabrian Mount Poro plateau. Int. J. Dairy Technol. **55**: (in press).

Three hundred individual ewe's milks and six bulk milks were analysed over two seasons. The results show that in spring but not in winter the milks examined conform substantially to the standards laid down by Italian legislation. Nevertheless, the data acquired constitute a good starting point from which the quality of ewe's milk produced on the Calabrian Mount Poro plateau should be improved, by performing selection of sheep for mastitis resistance based on somatic cell count, improving the hygienic standards at the dairy, using selected autochthonous lactic starter cultures and, only if strictly necessary, thermal treatment of the milk.

6. Micari P., Caridi A., Colacino T., Foti F., Ramondino D. 2002. Characteristics of goat milk produced in the Aspromonte massif (Calabria, Italy). Ital. J. Food Sci. **14**: (in press).

Physico-chemical parameters - pH (mean 6.55-6.81), fat (mean 3.95-5.68%), protein (mean 3.57-4.30%), lactose (mean 4.63-4.95%), and dry matter (mean 14.02-15.28%) - micro-biological parameters - somatic cell (mean 5.1-5.7 Log mL^{-1}) and total microbial count (mean 4.0-5.0 Log CFU mL^{-1}) - and coagulation parameters - rennet coagulation time ® - mean 13'03"-17'22"), curd firming rate (k_{20} - mean 1'37"-2'32"), and curd firmness after 30 min (a_{30} - mean 33.13-53.67) - were determined in 300 individual native goat milk samples. Six bulk milk samples were also analysed. Most of the individual milk samples conformed to the European Community and Italian standards, but the bulk milk quality must be improved.

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

The following are abstracts of articles that were published recently.

1. Slaninová, I., Šesták, S., Svoboda, A., Farkaš,V. 2000. Cell wall and cytoskeleton reorganization as the response to hyperosmotic shock in Saccharomyces cerevisiae. Arch. Microbiol. **173**:245-252.

Transfer of exponentially growing yeast cells (*Saccharomyces cerevisiae*) to hypertonic growth medium containing 0.7-1.0 M KCl, 1 M mannitol and/or 1 M glycerol caused cessation of growth for about 2 h. Thereafter, the growth resumed at almost the original rate. During this time, formation of fluorescent patches on the inner surface of the cell walls stained with Primulin and/or Calcofluor white was observed. The fluorescent patches formed also when the cells were suspended in pure 1 M KCl , and also when the synthesis of proteins was blocked by cycloheximide. The patches gradually disappeared when the cells resumed growth and the newly formed buds had smooth cell walls. Electron microscopy of freeze-etched replicas of osmotically stressed cells revealed deep plasma membrane invaginations filled from the periplasmic side with amorphous cell-wall matrix material. No differences in cell wall composition between control and stressed cells were found. The hyperosmotic shock also caused changes in distribution of cytoskeletal elements as demonstrated by disappearance of microtubules and actin filaments. After 2-3 h in hyperosmotic medium, both microtubules and microfilaments regenerated and the actin patches resumed their positions at the apices of growing buds.

2. Šesták, S. and Farkaš,V. 2001. "In situ" assays of plasma membrane-bound enzymes in yeast and other fungal cells permeabilized by osmotic shock. Anal.Biochem. **292**:34-39.

Permeabilization of yeast and other fungal cells by osmotic shock enabled the "in situ" assays of intracellular plasma membrane-bound enzymes β-1,3-glucan synthase, chitin synthase and ATP-ase. The permeabilization was accomplished by rapid changes in osmolarity of the washing buffer at 0°C whereby 0.5-3 M glycerol, sorbitol, manitol and/or 1 M KCl could be used as the osmolytes. The described procedure enabled rapid assays of cytoplasm-facing plasma membrane-bound enzymes while avoiding treatments with organic solvents, detergents and other xenobiotics currently used for the permeabilization of microbial cells.

3. Košíková, B., Sláviková, E., Mikulášová, M. 2001. Novel biological method of wood-pretreatment before pulping. Drevársky výskum (Wood Research) **46**:21-28.

A significant increase of lignin reactivity in sprucewood and thermomechanical pulp treated with the yeast species *Sporobolomyces roseus* compared to that of untreated samples indicates that this strain might be considered as promising microorganism for pretreatment of wood prior to pulping. The characterization of lignin samples by ^{13}C NMR, FTIR, and GPC analyses shows that *Sp. roseus* exhibits ability to oxidize and degrade lignin wood component by ligninolytic enzymes Li-peroxidase and Mn(II)-peroxidase detected in the cultivation medium.

4. Khan, M.R., Saha, M.L., Anisuzzaman, M., Sláviková E. 2001. Yeasts isolated from the lakes of Dhanmondi and Ramna, Bangladesh. Czech Mycol. **53**:223-228.

The occurrence of yeasts in the water of two lakes located in Dhaka City over a period from September to December 1999 was investigated. The number of yeasts of lake Dhanmondi and Ramna ranged from 9.5 x 10^4 to 35 x 10^4 and 2.3 x 10^4 to 11 x 10^4 CFU/l, respectively. The isolated yeast strains belonged to 5 species: *Saccharomyces cerevisiae, Rhodotorula glutinis, Rhodotorula mucilaginosa, Debaryomyces hansenii* var. *fabryi* and *Candida suecica.* The maximum number of yeasts was found to be 3 times higher in the water samples of Dhanmondi lake than that of Ramna lake. The higher number of yeasts was correlated with the temperature of the water and with pH values.

5. Sláviková, E. and Košíková B. 2001. Modification of lignin by *Geotrichum klebahnii.* World J. Microbiol. & Biotechnol. **17**:1-3.

^{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_α - C_β side chain cleavage, C_α - oxidations, aromatic ring cleavage and reductive reactions. The obtained results outline prospective application of *G. klebahnii* for biotechnological pretreatment of lignocellulosic materials.

6. Sláviková,E., Košíková, B., Mikulášová, M. 2002. Biotransformation of waste lignin products by the soil-inhabiting yeast *Trichosporon pullulans.* Can. J. Microbiol., **48**:200-203.

In this study the biotransformation of lignin by-product of beechwood pulping with a soil-inhabiting yeast strain of *Trichosporon pullulans* was examined. The structural and molecular changes in the lignin during cultivation process determined by ^{13}C NMR spectroscopy and GPC analysis confirmed the ability of the yeast strain tested to biodegrade lignin. Enzymatic analysis showed the presence of lignin peroxidase and Mn (II) peroxidase in the culture supernatant. The ligninolytic activity of both enzymes increased under carbon-depleted conditions. This observation is particularly important in the biodegradation of lignin recalcitrants in soil.

7. Breierová, E., Vajcziková, I., Sasinková,V., Stratilová, E., Fišera, M., Gregor T., Šajbidor, J. 2002. Biosorption of cadmium ions by different yeast species. Z.Naturforch. **57**c:634-639.

Toxicity and accumulation of Cd^{2+} in yeasts were studied in eight different yeast species. The adaptation to toxic concentration of this metal was dependent on the production of extracellular yeast glycoproteins. The highest concentration of Cd^{2+} ions in the growth medium was tolerated by a *Hansenula anomala,* strain while the lowest tolerance was found by the strain of species *Saccharomyces cerevisiae.* Extracellular glycoproteins of *Hansenula anomala* absorbed nearly 90 % of the total content of Cd^{2+} ions bound by yeast cells, while extracellular glycoproteins of *Saccharomyces cerevisiae* bound only 6% of the total amount of cadmium. This difference is caused by the variable composition of the saccharide moiety in the extracellular glycoproteins. The composition of extracellular glycoproteins changed during the adaptation of the yeast cells to the presence of Cd^{2+} ions.

8. Fišera, M., Gregor, T., Breierová, E. 2002. Microorganisms and their products as bio-sorbents for detoxication in environment. Technology and processes for sustainable development and pollution reduction/prevention. Proceedings of ICS-UNIDO Workshop, 126-131, Brno.

Heavy metals (mostly a very low concentration) are frequently as contaminants of waters and soil in consequence contamination by industrial and communal waste. Some metals, like Cd^{2+}, Ni^{2+} ions are going for eukaryotic cells toxic already in low concentration. Entering metallic ions into cellular membrane presuming interaction with his wall by means of adsorption and precipitation. The toxic metals are able to accumulate too on surface dead cells by bio-sorption. The metallic ions can to the cell penetrate by passive process (endocytisis), or else one another transport process-actively by the help of electrochemical proton gradient. The transport process is inhibited by low temperature, however to total stopping not happen. Accumulation of metals is affected by also presence of other matters in media.

9. Omelková, J., Breierová, E., Márová I. 2002. The influence of oxidative and osmotic stress on the morphological features of the red yeasts. Technology and processes for sustainable development and pollution reduction/prevention. Proceedings of ICS-UNIDO Workshop, 286-288, Brno.

10. Omelková , J., Breierová, E., Švaňová, L., Stratilová, E. 2002. The response of yeasts and yeast-like microorganisms cultivated on pectin media under stress: their growth, morphology and production of polygalacturonases. Chemické listy (Symposia) **96**:S137-138.

11. Breierová, E. and Omelková, J. 2002. The osmotic extreme environments and yeast cultures. Chemické listy (Symposia) **96**:S149.

12. Èertík, M. and Breierová E. 2002. Adaptation responses of yeasts to environmental stress. Chemické listy (Symposia) **96**:S154.

13. Gregor, T., Breierová, E., Fišera, M. 2002. Biosorption of heavy metals by selected yeast strains. Chemické listy (Symposia) **96**:S155-156.

14. Bystrický, S., Machová, E., Bartek, P., Kolarova, N., Kogan, G. 2000. Conjugation of yeast mannans with protein employing cyanopyridinium agent (CDAP) – an effective route of antifungal vaccine preparation. Glycocon. J. **17**:677-680.

15. Kogan, G., Šandula, J., Korolenko, T.A., Falameeva, O.V., Poteryaeva, O.N., Zhanaeva, S.Ya., Levina, O.A., Filatova, T.G., Kaledin, V.I. 2002. Increased efficiency of Lewin lung carcinoma chemotherapy with a macrophage stimulator-yeast carboxymethyl glucan. International Immunopharmacology **2**:775-781.

VIII. 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>.

Recently publications.

1. Golubev W.I., Kulakovskaya T.V. and Golubeva E.W. 2001. *Pseudozyma fusiformata* VKM Y-2821 is a producer of antifungal glycolipid. Mikrobiologiya **70**:642-646.

The yeast *Ps. fusiformata* (Ustilaginales) secretes a low-molecular-weight protease-resistant thermostable fungicide which was active against more than 80% of 280 yeast and yeast-like species tested under acid conditions. This agent was extracted with mehanol and purified by column and thin-layer chromatography. It consists of glucose and saturated fatty acids.

2. Golubev W.I., Pfeiffer I. and Golubeva E. 2002. Mycocin production in *Trichosporon pullulans* populations colonizing tree exudates in the spring. FEMS Microbiology Ecology **40**:151-157.

Mycocin production was demonstrated in *Tr. pullulans* which is a dominant member of the yeast community in tree exudates released in the early spring (spring sap). Mycocin synthesis was associated with dsRNA-containing virus-like particles. Natural strains of *Tr. pullulans* free of dsRNA have a sensitive phenotype, and a mycocinogenic strain cured of small dsRNA becomes sensitive to its own mycocin. The mycocin studied was active against isolates from tree exudations only but not against *Tr. pullulans* strains isolated from other habitats. No activity was found against any other yeast species. The competitive advantage of mycocin production at the population level was exemplified by the predominance of mycocinogenic strains both in laboratory model communities and natural populations.

3. Golubev W.I. and Golubev N.W., 2002. Selenium tolerance of yeasts. Mikrobiologiya **71**(4) (in press).

Selenium tolerance of yeasts is varied over wide range: growth of some species is inhibited on the medium containing 0.0001 M selenate whereas other species can grow at concentration 0.1 M. Taxonomically heterogenous taxa are variable in level of tolerance to Se. As a whole ascomycetous yeasts are more tolerant than basidiomycetous ones. The species of the genera *Dekkera*, *Schizosaccharomyces*, *Bullera*, *Cryptococcus*, *Holltermannia* are the least tolerant. The species *Candida maltosa*, *Hanseniaspora valbyensis*, *Kluyveromyces marxianus*, *Yarrowia lipolytica*, *Cryptococcus curvatus*, *Cr. humicola* and the species of the genus *Trichosporon* are highly tolerant. Composition of medium, the presence of sulfate, sulfur-containing amino acids and glutamine, influences the level of tolerance to Se.

IX. Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Communicated by H. Lee <hlee@uoguelph.ca>.

The following is the abstract of a paper which appeared recently.

1. Jeong, E.Y., I.S. Kim and H. Lee. 2002. Identification of lysine-78 as an essential residue in the *Saccharomyces cerevisiae* xylose reductase. FEMS Microbiol. Lett **209**:223-228.

Yeast xylose reductases are hypothesized as hybrid enzymes as their primary sequences contain elements of both the aldo-keto reductases (AKR) and short chain dehydrogenase/reductase (SDR) enzyme families. During catalysis by members of both enzyme families, an essential Lys residue H-bonds to a Tyr residue that donates proton to the aldehyde substrate. In the *Saccharomyces cerevisiae* xylose reductase, Tyr49 has been identified as the proton donor. However, the primary sequence of the enzyme contains two Lys residues, Lys53 and Lys78, corresponding to the conserved motifs for SDR and AKR enzyme families, respectively, that may H-bond to Tyr49. We used site-directed mutagenesis to substitute each of these Lys residues with Met. Activity of the K53M variant was slightly decreased as compared to the wild-type, while that of the K78M variant was negligible. The results suggest that Lys78 is the essential residue that H-bonds to Tyr49 during catalysis and indicate that the active site residues of yeast xylose reductases match those of the AKR, rather than SDR, enzymes. Intrinsic enzyme fluorescence spectroscopic analysis suggests that Lys78 may also contribute to the efficient binding of NADPH to the enzyme.

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

Recent publications:

1. T. Wartmann, U.W. Stephan, I. Bube, E. Böer, M. Melzer, R. Manteuffel, R. Stoltenburg, L. Guengerich, G. Gellissen and G. Kunze. 2002. Post-translational modifications of the *AFET3* gene product - a component of the iron transport system in budding cells and mycelia of the yeast *Arxula adeninivorans*. Yeast **19**:849-862.

The yeast *Arxula adeninivorans* is characterized by a temperature-dependent dimorphism. *A. adeninivorans* grows as budding cells at temperatures up to 42°C, but forms mycelia at higher temperatures. A strong correlation exists between morphological status and iron uptake, achieved by two transport systems that differ in iron affinity. In the presence of high Fe(II) concentrations (> 2 µM), budding cells accumulate iron concentrations up to sevenfold those observed in mycelia, while at low Fe(II) concentrations (< 2 µM), both cell types accumulate similar amounts of iron. The copper-dependent Fe(II) oxidase Afet3p, composed of 615 amino acids, is a component of the high affinity iron transport system. This protein shares a high degree of homology with other yeast iron transport proteins, namely Fet3p of *Saccharomyces cerevisiae*, Cafet3p of *Candida albicans* and Pfet3p of *Pichia pastoris*. Expression of the *AFET3* gene is found to be strongly dependent on iron concentration but independent of the morphological stage; however, cell morphology was found to influence post-translational modifications of the gene product. *O*-glycosylation was observed in budding cells only, whereas *N*-glycosylation occurred in both cell types. The *N*-glycosylated 103 kDa glycoprotein matures into the 108.5 kDa form, further characterized by serine phosphorylation. Both N-glycosylation and phosphorylation occur at low iron concentrations (≤ 5 µM). The mature Afet3p of 108.5 kDa is uniformly distributed within the plasma membrane in cells of both morphological stages.

2. T. Wartmann, E. Böer, A. H. Pico, H. Sieber, O. Bartelsen, G. Gellissen and G. Kunze. 2002. High-level production and secretion of recombinant proteins by the dimorphic yeast *Arxula adeninivorans*. FEMS-Yeast Res. **2**:363-369.

The non-conventional dimorphic thermo- and salt-resistant yeast *Arxula adeninivorans* has been developed as a host for heterologous gene expression. For assessment of the system two model genes have been selected: the *GFP* gene encoding the intracellular green fluorescent protein, and the *HSA* gene encoding the secreted human serum albumin. The expression system includes two host strains, namely *A. adeninivorans* LS3, which forms budding cells at 30°C and mycelia at > 42°C, and the strain *A. adeninivorans* 135, which forms mycelia at temperatures as low as 30°C. For expression control the constitutive *A. adeninivorans*-derived *TEF1*-promoter and *S. cerevisiae*-derived *PHO5*-terminator were selected. The basic *A. adeninivorans* transformation/expression vector pAL-HPH1 is further equipped with the *E. coli*-derived *hph* gene conferring hygromycin B resistance and the 25S rDNA from *A. adeninivorans* for rDNA targeting. Transformants were obtained for both, budding cells and mycelia. In both cell types similar expression levels were achieved and the GFP was localised in the cytoplasm while more than 95% of the HSA accumulated in the culture media. In initial fermentation trials on a 200 ml shake flask scale maximal HSA product levels were observed after 96 h of cultivation.

3. T. Wartmann, R. Stoltenburg, E. Böer, H. Sieber, O. Bartelsen, G. Gellissen and G. Kunze. 2002. The *ALEU2* gene – a new component for an *Arxula adeninivorans*–based expression platform. FEMS Yeast Res. (in press).

The *ALEU2* gene encoding ß-isopropylmalate dehydrogenase was isolated from the non-conventional yeast *Arxula adeninivorans*. The isolated gene harbours an ORF of 1086 bp, encoding a putative protein of 362 amino acids. The derived protein sequence shares a high degree of homology with other fungal ß-isopropylmalate dehydrogenases thus confirming the identity of the gene. The isolated *ALEU2* gene was tested for suitability of complementing the auxotrophy of an *Arxula adeninivorans aleu2* host. For this purpose the plasmid pAL-ALEU2m which contains the *ALEU2* gene as selection marker and the 25S rDNA for targeting was employed in transformation experiments. Transformants harboured a single copy of the heterologous DNA and were found to be mitotically stable. For assessment of heterologous gene expression two model genes were incorporated into the vector: the *GFP* gene encoding intracellular green fluorescent protein, and the *HSA* gene encoding the secreted human serum albumin. For expression control both gene sequences were fused to the constitutive *A. adeninivorans*-derived *TEF1*-promoter and *S. cerevisiae*-derived *PHO5*-terminator. In respective recombinant strains the GFP was localised in the cytoplasm while more than 95% of the HSA accumulated in the culture media. In initial fermentation trials using a 200 ml shake flask scale maximal HSA product levels were observed after 96 h of cultivation.

XI. Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Room 705, Piscataway, New Jersey 08854-5635, U.S.A. Communicated by M.J. Leibowitz.

Recent publication.

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

The antimicrobial agent pentamidine inhibits the selfsplicing of the group I intron Ca.LSU from the transcripts of the 26S rRNA gene of *Candida albicans*, but the mechanism of pentamidine inhibition is not clear. We show that preincubation of the ribozyme with pentamidine enhances the inhibitory effect of the drug and alters the folding of the ribozyme in a pattern varying with drug concentration. Pentamidine at 25 μM prevents formation of the catalytically active F band conformation of the precursor RNA and alters the ribonuclease T1 cleavage pattern of Ca.LSU RNA. The effects on cleavage suggest that pentamidine mainly binds to specific sites in or near asymmetric loops of helices P2 and P2.1 on the ribozyme, as well as to the tetraloop of P9.2 and the loosely paired helix P9, resulting in an altered structure of helix P7, which contains the active site. Positively charged molecules antagonize pentamidine inhibition of catalysis and relieve the drug effect on ribozyme folding, suggesting that pentamidine binds to a magnesium binding site(s) of the ribozyme to exert its inhibitory effect.

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

The following papers appeared since June 2002.

1. Middelhoven, W.J. 2002. Yeasts and fungi in excrements of the sand hill snail, *Theba pisana*. Folia Microbiologica **47**:271-272.

Species found were: *Acremonium strictum, Aspergillus candidus, Aureobasidium pullulans, Galactomyces geotrichum, Mucor circinelloides* f. *lusitanicus, Rhodotorula glutinis, Rhodotorula minuta* and *Trichosporon asahii*. All but one species are strictly aerobic. Only *G. geotrichum* displays weak fermentative ability. Possibly, the fungi help to maintain anaerobic conditions in the gastrointestinal tract by consuming the oxygen leaking in.

2. Carreiro, S.C., Pagnocca, F.C., Bacci Jr., M, Bueno, O.C., Hebling, M.J.A and Middelhoven, W.J. 2002. Occurrence of killer yeasts in leaf-cutting ant nests. Folia Microbiologica **47**: 259-262.

This study was mainly carried out by investigators of the Rio Claro settlement of the University of São Paulo, Brazil.

3. Van Dijken, J.P., van Tuyl, A., Luttik, M.A.H., Middelhoven, W.J. and Pronk, J.T. 2002. Novel pathway for alcoholic fermentation of δ-gluconolactone in the yeast *Saccharomyces bulderi.* J. Bacteriol. **184**:672-678.

The major part of this study was carried out in the Kluyver Laboratory of Biotechnology, Delft University of Technology, Delft, The Netherlands.

4. Middelhoven, W.J. 2002. Identification of yeasts present in sour fermented foods and fodders. Molec. Biotechnol. **21**:279-292.

An identification key to fifty yeast species frequently isolated from foods and fodders that underwent a lactic acid fermentation is provided. However, several species present in fermented olive products were not included. The key is based on some morphological and physiological characteristics. These include growths tests using the API ID32C test strips, assimilation of methanol and starch, fermentation of glucose, growth on nitrate and ethylamine as sole nitrogen sources, vitamin requirement, maximum growth temperature and presence of urease. For morphology, slide cultures on maize meal agar are recommended. The tests are relatively simple and strains can be identified within a week also by those who are not experienced in more sophisticated procedures. Instead of the provided identification key, the BioloMICS of Vincent Robert of CBS Utrecht can be used. This is available via Internet, but was not yet when the manuscript was prepared. By applying the BioloMICS, the methods can be useful for identification of yeast strains isolated from other habitats.

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

The following papers were recently published.

1. E. Minárik. 2002. New opinions on yeast nutrition. Vinič a víno **2**(5):111-112 (in Slovak).

Recent wine research and practice confirmed that nine yeast revitalization should be preferentially carried out in water instead of grape must. Before yeast addition nutrients α-amino acids (*e.g.* Go-ferm) or ammonium salts (*e.g.* Fermaid K) should be added. Not only more rapid revitalization of the yeast cells occurs (appr. 30 min)/ but a more efficient fermentation without delay and without any residual sugar in the young wine may be achieved.

2. E. Minárik. 2002. What pure yeast starters and enzymes may accomplish. Vinič a víno **2(3)**: 65-66 (in Slovak).

The use of active dry wine yeast starters, in modern winemaking goes beyond the unimagination. A wide spectrum of strains available on the market enable a purposeful choice for the appropriate wine production. The choice of enzyme preparation is at this time much less. In the future it will probably be possible to use ß-glucosidase to raise aromatic properties and quality of varietal wines.

XIV. Microbial Culture Collection Laboratory, Central Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Phahonyothin Road, Pathumthani 12120, Thailand. Communicated by T. Nakase <Nakase@biotec.or.th>.

The following papers were read at the annual meetings of scientific societies in Thailand.

1. Two new anamorphic yeasts, *Candida khaoyaiensis* sp. nov. and *Candida thailandica* sp. nov., isolated from insect frasses in Thailand. by Sasitorn Jindamorakot, Wanchern Potacharoen, Hiroko Kawasaki and Takashi Nakase, 5th Annual Meeting, Biodiversity and Training Program (BRT), Nakornsritumarat, Oct. (2002).

2. *Candida flossculi* sp. nov., a novel anamorphic yeast species isolated from a flower in Thailand. by Sasitorn Jindamorakot, Savitree Limtong, Waken Yongmanitchai, Manee Tuntirungkit, Wanchern Potacharoen, Hiroko Kowasaki and Takashi Nakase. 15th Annual Meeting, Thai Soc. Biotechnol. Khon Kaen, Nov. (2002)

3. *Bullera panici* sp. nov. and *Bullera siamensis* sp. nov., two new yeasts in the *Bullera variabilis* cluster isolated in Thailand. by Bundit Fungsin, Masako Takashima, Suparp Artjariyasripong, Vullapa Arunpairojana and Takashi Nakase. 15th Annual Meeting, Thai Soc. Biotechnol. Khon Kaen, Nov. (2002)

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

We thank G.S. de Hoog and M.Th. Smith (CBS, Utrecht) for fruitful collaboration in yeast research in 2002.

The following are publications for 2002 or in press.

1. Naumov G.I., Naumova E.S. 2002. Five new combinations in the yeast genus *Zygofabospora* Kudriavzev emend. G. Naumov (pro parte *Kluyveromyces*) based on genetic data. FEMS Yeast Research **2**:39-46.

2. Naumov G.I., Naumova E.S., Korshunova I.V., Jakobsen M. 2002. Yeast comparative genetics: A new *MEL*15 α-galactosidase gene of *Saccharomyces cerevisiae.* Russian J. Genetics **38**:1127-1132.

3. Naumov G.I., Naumova E.S., Antunovics A., Sipiczki M. 2002. *Saccharomyces bayanus* var. *uvarum* in Tokaj wine-making of Slovakia and Hungary. Appl. Microbiol. Biotechnol. **59**:727-730.

Using genetic hybridisation analysis and molecular karyotyping we revealed an association of *S. bayanus* var. *uvarum* species with Tokaj wine-making. Along with identification of *Saccharomyces* strains isolated by E. Minárik in Slovakia, the composition of Tokaj populations in Hungary was studied. Twenty-eight Hungarian *Saccharomyces* strains were analysed in terms of karyotypes. The majority of strains belong to *S. bayanus* var. *uvarum*. Two non-identified *Saccharomyces* strains are found to be polyploid according to complex karyotype patterns.

4. Naumova E.S., Korshunova I.V., Jespersen L., Naumov G.I. 2002. Molecular genetic identification of *Saccharomyces* sensu stricto strains from African sorghum beer. FEMS Yeast Research (in press).

Genetic relationships of 24 phenotypically different strains isolated from sorghum beer in West Africa and the type cultures of the *Saccharomyces* sensu stricto species were investigated by UP-PCR analysis, microsatellite fingerprinting and PCR-RFLP of the ribosomal internal transcribed spacers. The results demonstrate that ITS-PCR RFLP analysis with the endonucleases *Hae*III, *Hpa*II, *Scr*FI and *Taq*I is useful for discriminating *S. cerevisiae, S. kudriavzevii, S. mikatae* from one another and from the *S. bayanus/ S. pastorianus* and *S. cariocanus/S. paradoxus* pairs. The sorghum beer strains exhibited the same restriction patterns as the type culture of *S. cerevisiae* CBS 1171. PCR profiles generated with the microsatellite primer $(GTG)_5$ and the universal primer N21 were almost identical for all isolates and strain CBS 1171. Despite phenotypic peculiarities, the strains involved in sorghum beer production in Ghana and Burkina Faso belong to *Saccharomyces cerevisiae*. Based on sequencing of the rDNA ITS1 region and Southern hybridisation analysis, these strains represent, however, a divergent population of *S. cerevisiae.*

5. Naumov G.I., Naumova E.S., Naumoff D.G., Korshunova I.V., Jakobsen M. 2002. Mobile α-galactosidase genes of melibiose fermentation in *Saccharomyces* yeasts. 1st Int. Congress. Biotechnol. – state of the art and prospects of development, 14-18 October 2002, Moscow, Russia, p. 186.

Fermentation of melibiose is of great interest for both basic and applied studies of yeasts. Regulation aspects of this fermentation are worthy of note of molecular zymologists, while the ability to utilize more completely molasses containing melibiose as a component of raffinose, is useful for industrial microbiologists. A lot of attention is focused on genetic polymorphism of melibiose fermentation in the light of ecological, evolutionary and applied genetics of yeasts (Tubb et al., 1986; Naumov, 1989,1996). The family of highly homologous α-galactosidase genes *MEL*1-*MEL*11 has been described in the *S. cerevisiae* (Naumov et al., 1990-1996). Three divergent genes *MEL*x, *MEL*p and *MEL*j were found in *S.pastorianus*, *S.paradoxus* and *S.mikatae*, respectively (Turakainen et al., 1991; Naumova et al., 1996). We identified three new divergent α-galactosidase genes, *MEL*12-*MEL*14, in South-African *S. cerevisiae* strain CBS 2888. Another divergent gene *MEL*15 was identified in strains from Ghana. Restriction analysis showed that the *MEL*12-*MEL*15 genes are physically very similar to each other, while different from the family of *MEL*1- *MEL*11 genes. We cloned and sequenced a 1300 bp PCR-amplified fragment of the *MEL*12 and *MEL*15 genes. The nucleotide sequences of the *MEL*12/*MEL*15 have over 90% identity with the *MEL*p (*S. paradoxus)* gene and from 77% to 80% with the other *MEL* genes. The *MEL*1-*MEL*11 genes show

similarity of 94-100%, whereas the homology of *MEL* genes from sibling species and hybrid taxon *S. pastorianus* varied from 76% to 81%. Thus, the relatedness between *MEL*12/*MEL*15 and *MEL*p genes is closer to the intraspecific level rather than interspecific one. The occurrence of foreign *MEL*12- *MEL*15 genes in African *S. cerevisiae* genome is probably the result of introgression between this yeast and its wild sibling species *S. paradoxus*. The divergent *MEL* genes are new genetic pool for yeast genetics and breeding.

XVI. Department of Microbiology, Institute of Applied Molecular Biology, University of the Saarland, Postfach 15 11 50, Building 2, D-66041 Saabrücken, Germany. Communicated by M.J. Schmitt <mjs@microbiol.uni-sb.de>.

The following are summaries of in press papers and recently submitted papers of the group.

1. Weiler, F. & M.J. Schmitt. 2002. Zygocin, a secreted antifungal toxin of the yeast *Zygosaccharomyces bailii*, and its effect on sensitive fungal cells. FEMS Yeast Res., in press.

Zygocin, a protein toxin produced and secreted by a killer virus-infected strain of the osmotolerant yeast *Zygosaccharomyces bailii*, kills a great variety of human and phytopathogenic yeast and filamentous fungi. Toxicity of the viral toxin is envisaged in a two-step receptor-mediated process in which the toxin interacts with cell surface receptors at the level of the cell wall and the plasma membrane. Isolated and partially purified Zygocin cell wall receptors were successfully used as biospecific ligand for efficient one-step purification of the 10 kDa protein toxin by receptor-mediated affinity chromatography. Evidence is presented that Zygocin-treated yeast cells are rapidly killed by the toxin, and intensive propidium iodide staining of Zygocin-treated cells indicated that the toxin is affecting cytoplasmic membrane function, most probably by lethal ion channel formation. The presented findings suggest that Zygocin has the potential as a novel antimycotic in combating yeast and fungal infections.

2. Weiler, F., Rehfeldt, K., Bautz, F. & M.J. Schmitt. 2002. The *Zygosaccharomyces bailii* antifungal virus toxin zygocin: cloning and expression in a heterologous fungal host. Mol. Microbiol. **46**: in press.

Zygocin, a monomeric protein toxin secreted by a virus-infected killer strain of the osmotolerant spoilage yeast *Zygosaccharomyces bailii* kills a broad spectrum of human and phytopathogenic yeasts and filamentous fungi by disrupting cytoplasmic membrane function. The toxin is encoded by a double-stranded (ds)RNA killer virus (ZbV-M, for *Z. bailii* virus M) that stably persists within the yeast cell cytosol. In this study, the protein toxin was purified, its N-terminal amino acid sequence was determined, and a full-length cDNA copy of the 2.1 kb viral dsRNA genome was cloned and successfully expressed in a heterologous fungal system. Sequence analysis as well as zygocin expression in *Schizosaccharomyces pombe* indicated that the toxin is *in vivo* expressed as a 238 amino acid preprotoxin precursor (pptox) consisting of a hydrophobic N-terminal secretion signal, followed by a potentially N-glycosylated pro-region and terminating in a classical Kex2p endopeptidase cleavage site that generates the N-terminus of the mature and biologically active protein toxin in a late Golgi compartment. Matrix-assisted laser desorption mass spectrometry further indicated that the secreted toxin is a monomeric 10.4 kDa protein lacking detectable posttranslational modifications. Furthermore, we present additional evidence that in contrast to other viral antifungal toxins, zygocin immunity is not mediated by the toxin precursor itself and, therefore, heterologous pptox expression in a zygocin-sensitive host results in a suicidal phenotype. Final sequence comparisons emphasize the conserved pattern of functional elements present in dsRNA killer viruses that naturally infect phylogenetically distant hosts (*S. cerevisiae* and *Z. bailii*) and reinforce models for the sequence elements that are *in vivo* required for viral RNA packaging and replication.

3. Breinig, F., Heintel, T., Schumacher, A., Meyerhans, A. & M.J. Schmitt, M.J. Cytomegalovirus pp65 expressed in *Schizosaccharomyces pombe* specifically activates CMV-primed human T lymphocytes, and indicates the potential of fission yeast in vaccine development, submitted.

Currently, threatening virus infections represent impressive examples illustrating the need for novel vaccine strategies. In this respect, the induction of efficient T cell-mediated immune responses is essential. Here, we describe a method to directly test yeast based vaccine candidates in human whole blood by using a system based on the expression of the model antigen HCMV tegument protein pp65 in fission yeast (*Schizosaccharomyces pombe*). Monocytes and neutrophils efficiently phagocytose intact yeast cells that *per se* did not activate a significant number of human T lymphocytes *ex vivo*. The ability of recombinant pp65 to specifically activate antigen-specific memory T cells in blood of HCMV seropositives is demonstrated. Therefore, fission yeast is capable to efficiently express antigens from mammalian viruses. Moreover, the immune response against recombinant pp65, in particular that of CD8 class I MHC restricted cytotoxic T cells, was similar to the response against the intact HCM-virus. The system described might represent a novel approach in vaccine development since it allows the rapid testing of the potential of heterologously expressed antigens in T lymphocyte activation, and the identification of vaccine candidates directly from human whole blood.

4. Heintel, T., Breinig, F., Schmitt, M.J. & A. Meyerhans. Extensive MHC class I-restricted CD8 T lymphocyte responses against various yeast genera in humans, submitted.

The human cellular immune response against 14 distantly related yeast species was analyzed by intracellular cytokine staining of lymphocytes after ex vivo stimulation of whole blood. While the CD4 T cell response was marginal, extensive MHC class I-restricted CD8 T cell responses were detected against a number of species including spoiling and environmental yeasts. The yeast-specific CD8 T cells expressed interferon-g but lacked expression of CD27 and CCR7, indicating that they were end-differentiated effector memory cells. Mainly intact yeast cells rather than spheroplasts were able to induce cytokine expression in T cells demonstrating that the dominant immunogens were located in the yeast cell wall. Together these data underline the importance of the cellular immune response in protecting humans against yeast and fungal infections. And, from another perspective, recombinant yeast suggests itself as a potential vaccine candidate to efficiently induce antigen-specific CD8 T cell responses.

XVII. Departamento de Microbiologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil. Communicated by Carlos A. Rosa <carlrosa@icb.ufmg.br>.

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

1. F.C.O. Gomes, C. Pataro, J.B. Guerra, M.J. Neves, S.R. Corrêa, E.S.A. Moreira and C.A. Rosa. 2002. Physiological diversity and trehalose accumulation in *Schizosaccharomyces pombe* strains isolated from spontaneous fermentations during the production of the artisanal Brazilian *cachaça*. Can. J. Microbiol. **48**:399-406.

2. R.C. Trindade, M.A. Resende, C.M. Silva, and C.A. Rosa. 2002. Yeasts associated with fresh and frozen pulps of brazilian tropical fruits. System. Appl. Microbiol. **25**: 294-300.

3. A.C.P. Teixeira, M.M. Marini, J.R. Nicoli, Y. Antonini, R.P. Martins, M.A. Lachance and C.A. Rosa. 2002. *Starmerella meliponinorum*, a novel ascomycetous yeast species associated with stingless bees. Int. J. Syst. Evol. Microbiol. (in press).

Thirty-two strains of the new species *Starmerella meliponinorum* were isolated from various substrates associated with three stingless bee (tribe Meliponini) species in Brazil and one in Costa Rica. The strains were found in garbage pellets, pollen provisions, adult bees, honey, and propolis of *Tetragonisca angustula*, from honey of *Melipona quadrifasciata*, and from adults of *Melipona rufiventris* and *Trigona fulviventris*. The sequence of the D1D2 domains of the large-subunit rDNA showed that the new species belongs to the *Starmerella* clade and is most closely related to *Candida etchellsii* although the two differ in their sequences by 7% base substitutions. *S. meliponinorum* is homothallic and assimilates few carbon sources. Nitrate is utilized as sole nitrogen source. The type strain of *S. meliponinorum* is strain UFMG-01-J26.1^T (= CBS 9117^T).

4. C. Pataro, J.B. Guerra, F.C.O. Gomes, M.J. Neves, P.F. Pimentel and C.A. Rosa. 2002. Trehalose accumulation, invertase activity and physiological characteristics of yeasts isolated from 24h fermentative cycles during the production of artisanal brazilian cachaça. Braz. J. Microbiol. **33** (in press).

Trehalose accumulation, invertase production and physiological characteristics of 86 yeast isolates from short fermentative cycles during the production of *cachaça* in three artisanal distilleries of the State of Minas Gerais were studied. Among these isolates, 70% were able to grow at temperatures between 40 and 42 ^{0}C. Only *Saccharomyces cerevisiae* isolates were able to grow over 40 ^{0}C. Lower temperatures (<40 ^{0}C) favoured the growth of yeasts such as *Candida parapsilosis*-like, *C. maltosa*-like, *Kloeckera japonica, S. exiguus* and *C. bombicola*-like. The isolates from all three distilleries were ethanol tolerant, produced invertase, and acumulated trehalose in the presence of glucose. The strains isolated from distillery A presented more resistance to ethanol (around 84.2 % of the strains were able to grow in the presence of 12% ethanol) when compared to the ones from distilleries C and B (9.5% and no strain, respectively). The strains of *S. cerevisiae* isolated from the three distilleries presented a higher capacity to produce invertase and accumulate trehalose in the presence of glucose. Based on the results of thermal and ethanol stress experiments, it was possible to identify a strong relationship between intracellular trehalose accumulation and cell viability. The increase in cell viability was even more pronounced when the strains were subjected to a pre-treatment at sublethal temperatures.

XVIII. Molecular Mycology Laboratory, Centre for Infectious Diseases and Microbiology, ICPMR, Level 3, Room 3114A, Darcy Road, Westmead Hospital, Westmead, NSW 2145, Australia. Communicated by W. Meyer <meyer@angis.usyd.edu.au>.

The following is the summary of a recently defended doctoral thesis.

1. H.M. Daniel. 2002. Molecular phylogeny applied to *Candida* species and related ascomycetous yeasts.

Yeast systematics has traditionally relied on phenotypic characters that are subject to convergent evolution and intraspecies variation. As a result, most genera are polyphyletic and should be considered as informal groupings to aid in communication. The artificial genus *Candida* comprises ascomycetous yeasts unified by the absence of sexual reproduction and other distinctive traits that would allow their natural classification. Its tremendous heterogeneity is shown by the affiliation of *Candida* species to at least 15 teleomorphic genera. The inference of their phylogeny relies almost exclusively on the phylogenies of genes and, to date, mostly on genes of the ribosomal gene cluster (rRNA).

This study compared gene phylogenies resulting from the actin gene and the small (SSU) and large (LSU) subunits of the rRNA of ascomycetous yeasts. As the gene phylogenies were only partially congruent, the evolutionary history of the genes was investigated and independent markers of high evolutionary stability were mapped onto the gene trees to confirm or reject phylogenetic hypotheses.

The phylogenetic groups and their interrelationships inferred by analyses of actin amino acid sequences, as the best marker for their relationships, were corroborated by coenzyme Q data, galactose presence and the non-universal usage of the CUG codon. A reliable resolution of species was only achieved by actin gene sequences in contrast to SSU and LSU gene sequences. As a result several anamorph-teleomorph and synonymous pairs of strains were separated. These strains were then confirmed by DNA relatedness to represent different species and the reinstatement of *C. bovina, C. rancoensis* and *C. nitrativorans* as well as the new combination *C. melibiosacea* were proposed.

Problems that occurred with the traditionally used LSU sequences were: 1) difficult homology assessment despite the use of secondary structure guided sequence alignment, 2) sibling species were not differentiated reliably, 3) higher order phylogenetic hypotheses were influenced by sequence length variation and guanine+cytosine (GC) content, both resulting in convergent grouping of unrelated taxa. No meaningful analysis of LSU data for widely divergent taxa was possible.

In contrast to the LSU, the GC content of SSU sequences was not biased by the nucleic GC content. The phylogenetic hypotheses obtained from SSU sequences showed more conformity to the actin-based trees than to LSU-based trees. The SSU gene was found to be a valuable phylogenetic marker.

Distance and parsimony methods were equally well suited for analysis of highly conserved data. Only the weighted parsimony analysis did accommodate the detected strong rate heterogeneity in the actin DNA dataset. In contrast, distance methods did not correct appropriately for rate heterogeneity. Parsimony was the most suitable method to analyse the data and taxa under investigation.

Combinability tests indicated homogeneity only for the most conserved parts of the actin gene with those of LSU or SSU genes, but also intramolecular conflicts in the LSU gene. The tree inferred from combined datasets according to the total evidence approach was misled by data heterogeneity. A conditional data combination is a better approach to utilise multiple data sets that might be biased by their evolutionary history.

This study improved the understanding of phylogenetic lineages among ascomycetous yeasts by multigene analysis under the integration of non-molecular characters. To reconstruct the tree of life for the entire diversity of ascomycetous yeasts specifically and fungi in general, global efforts need to coordinate the use of particular molecular and non-molecular markers.

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

The following papers have been accepted for publication.

1. Rodrigues, M.G. and Fonseca, A. 2002. Molecular systematics of the dimorphic ascomycete genus *Taphrina*. Int. J. Syst. Evol. Microbiol. Papers in press: http://dx.doi.org/10.1099/ijs.0.02437-0.

Members of the ascomycete genus *Taphrina* Fries comprise nearly 100 species recognised by their mycelial states parasitic on different vascular plants. Whereas the filamentous state is strictly phytoparasitic, the yeast state is saprobic and can be cultured on artificial media. *Taphrina* species are differentiated mainly on the basis of host range and geographic distribution, type and site of infection, and morphology of the sexual stage in the infected tissue. However, there has been little progress in the systematics of the genus in recent years mainly due to the scarcity of molecular studies and of available cultures. The main aim of the present study was the re-appraisal of species boundaries in *Taphrina* based on the genetic characterisation of cultures (yeast states) that represent ca. 1/3 of the currently recognised species. The molecular methods used comprised: (i) PCR-fingerprinting using single primers for microsatellite regions (MSP-PCR); and (ii) determination of nucleotide sequences of two ca. 600 bp long nuclear rDNA regions - the 5' end of the 26S rRNA (LSU) gene (D1/D2 domains) and the Internal Transcribed Spacer (ITS) region (which includes the 5.8S rRNA gene). The sequencing results confirmed the

monophyly of the genus (with the probable exclusion of *T. vestergrenii*) and the combined analysis of the two methods corroborated, in most cases, the separation of species defined on the basis of conventional criteria. However, genetic heterogeneity was found within some species and conspecifity was suggested for strains that were deemed to represent distinct species. Sequences from the ITS region displayed a higher degree of divergence than those of the D1/D2 between closely related species, but were relatively conserved within species (> 99% identity), and were thus more useful for the effective differentiation of *Taphrina* spp.. The results further allowed other topics to be addressed such as the correlation between the molecular phylogenetic clustering of certain species and the respective host plant family, and the significance of molecular methods in the accurate diagnosis of the different diseases caused by *Taphrina* species.

2. Inácio, J., Pereira, P., Carvalho, M., Fonseca, A., Amaral-Collaço, M.T. and Spencer-Martins, I. 2002. Estimation and diversity of phylloplane mycobiota on selected plants in a mediterranean-type ecosystem in Portugal. Microbial Ecology DOI: 10.1007/s00248-002-2022-z [epub ahead of print Oct 15].

Mediterranean ecosystems have not been consistently investigated as natural habitats for microbes in general, and fungi in particular. Here we present the results of a survey of epiphytic mycobiota (filamentous fungi and yeasts) on the phylloplane of selected plants in the Arrábida Natural Park, an ecosystem of Mediterranean characteristics in Portugal, using conventional culture-dependent isolation methods. Leaves from the species *Acer monspessulanum* and *Quercus faginea* (deciduous trees) and *Cistus albidus*, *Pistacia lentiscus*, and *Osyris quadripartita* (evergreen shrubs) were collected twice a year for two consecutive years, at two distinct locations of Serra da Arrábida: the more humid northern slope and the drier southern slope. A total of 1029 strains of filamentous fungi and 540 strains of yeasts were isolated, which represented at least 36 and 46 distinct species, respectively. Total counts were higher on the plants from the northern slope and there was a general increase from spring to autumn, notably on the deciduous trees for the yeasts. Plant species that had higher numbers of leaf colonists (*A. monspessulanum*, *C. albidus*, and *Q. faginea*) also yielded a wider range of species. Among the filamentous fungi there was a predominance of species of ascomycetous affinity, whereas basidiomycetous species dominated among yeast isolates. Some of the taxa recovered were common to other phylloplane studies (e.g., ubiquitous molds and yeasts such as *Cladosporium* spp. and *Cryptococcus* spp., respectively), but less common species were also found, some of which appeared to represent undescribed taxa. Interestingly, a few species seemed to be associated with a particular plant, notably in the case of the evergreen shrub *C. albidus*. However, for a considerable number of fungi and yeasts the same taxon was recovered throughout the year from more than one plant and at both sites, suggesting that such species might be genuine phylloplane inhabitants (or at least of aerial plant surfaces) even though they appeared not to display host specificity.

3. Gadanho, M., Almeida, J.M.F. and Sampaio, J.P. 2002. Assessment of yeast diversity in a marine environment in the South of Portugal by microsatellite-primed PCR. Antonie van Leeuwenhoek.

The occurrence and diversity of yeasts in seawater was investigated in a study site located 20 Km off Faro, Portugal, above the Álvares Cabral Trench. A total of 43 water samples from different layers (above the permanent thermocline, under the thermocline and near the bottom) and directly from the surface, originated 234 isolates. All the isolates were identified using a molecular approach that included, in a first stage, MSP-PCR fingerprinting. A total of 31 MSP-PCR classes were formed, 8 for the pigmented yeasts and 23 for the non-pigmented yeasts. The pink coloured isolates were identified by direct comparison of the new fingerprints with those obtained for representative strains of the various species. For identification of the non-pigmented yeasts, a representative isolate of each MSP-PCR class was selected for sequence analysis and compared with reference sequences. The five most abundant yeast species were *Sakaguchia dacryoidea, Pseudozyma aphidis, Rhodosporidium babjevae, R. diobovatum* and *Debaryomyces hansenii*. The distribution of isolates and species in the major taxonomic groups indicated that the number of basidiomycetous yeasts and their diversity are prevalent in relation to their ascomycetous counterpart. Diversity indices were determined and superficial water and water near the bottom had the highest diversity. The sampling effort effectiveness was estimated, and found to correspond to approximately 60% of the species present. MSP-PCR identification proved suitable for pigmented basidiomycetous yeasts and, when conjugated with sequence analysis, was effective for the characterization of non-pigmented populations. Our results indicate that the MSP-PCR fingerprinting method is appropriate for the characterization of large groups of isolates due to its simplicity and good reproducibility.

4. Kirschner, R., Sampaio, J.P., Begerow, D., Chen, Z.-C. and Oberwinkler, F. 2002. *Mycogloea nipponica* – the first known teleomorph in the heterobasidiomycetous yeast genus *Kurtzmanomyces*. Antonie van Leeuwenhoek.

A culture was obtained from a spore print of a basidiocarp of *Mycogloea nipponica* collected in Taiwan. A yeast stage and basidia identical to those of *M. nipponica* developed in laboratory media. The Taiwanese specimen of *M. nipponica* and its yeast anamorph were characterised in the present study. Comparative morphological, molecular, and ultrastructural studies indicated that the yeast stage could be assigned to the genus *Kurtzmanomyces*. The revealed connection between the sexual species *Mycogloea nipponica* and the asexual genus *Kurtzmanomyces* demonstrates the importance of anamorphic characteristics in the modern systematics of heterobasidiomycetous fungi.

5. Middelhoven, W.J., Fonseca, A., Carreiro, S.C., Pagnocca, F.C. and Bueno, O.C. 2002. *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. Antonie van Leeuwenhoek.

6. Scorzetti, G., Fell, J.W., Fonseca, A. and Statzell-Tallman, A. 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Research.

7. Golubev, W.I., Gadanho, M., Sampaio, J.P. and Golubev, N.W. 2002. *Cryptococcus nemorosus* and *Cryptococcus perniciosus*, two new species related to *Papiliotrema* Sampaio et al. (Tremellales). Int. J. Syst. Evol. Microbiol.

The following papers, whose abstracts were given in the last issue, have now been published.

8. Gadanho, M. and Sampaio, J.P. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodotorula*: *Rh. glutinis* sensu stricto and *Rh. dairenensis* comb. nov. FEMS Yeast Research **2**: 47-58.

9. Sampaio, J.P., Weiß, M., Gadanho, M. and Bauer, R. 2002. New taxa in the Tremellales: *Bulleribasidium oberjochense* gen. et sp. nov., *Papiliotrema bandonii* gen. et sp. nov. and *Fibulobasidium murrhardtense* sp. nov. Mycologia **94**: 873-887.

The following PhD thesis was recently defended.

10. J.M.F. Almeida. Ecological physiology of the yeast population of a solar saltern.

The presence of yeasts in coastal and estuarine waters is known for a long time, but their presence in adjacent solar salterns has not been reported. This work focused on the occurrence, distribution and characterisation of yeasts in a solar saltern (Salina do Brito) at Alcochete, on the south bank of the Tagus estuary.

Yeast isolates were obtained from the saltern brines and identified following two operative methodologies: a few yeasts (29) were fully identified up to the species level, whereas the other isolates (566) were just briefly characterised. Strains from the genera *Debaryomyces, Kluyveromyces, Pichia, Cryptococcus, Leucosporidium, Rhodotorula* and *Rhodosporidium* were found to occur in the brines. *Debaryomyces* prevailed among the ascomycetes and *Rhodotorula* and *Cryptococcus* were the basidiomycetous yeasts more often isolated. Other microbial populations were assessed throughout this work, namely bacteria, both halophilic and non-halophilic, filamentous fungi and planktonic photosyntethic microorganisms with chlorophyll *a* and *b*. The occurrence and distribution of the yeast population presented the expected correlations with salinity, but neither correlated with other environmental factors such as temperature.

A reduced biodiversity was observed among culturable yeasts obtained from the saltern ponds and both the composition and the density of the yeast population agreed with the extreme-like nature of the habitat and the adjoining estuary. Both the diversity and the concentration of isolates decreased during the operation of the saltern in summer (the *safra* season), the time when the salinity gradient became steeper and the hydraulic residence time was much shorter. The autochthony of yeasts in the brines during the *safra* season remains an open question.

Six strains (two strains of *Debaryomyces hansenii*, *Rhodotorula* and *Cryptococcus*) were selected among the isolates for subsequent physiological characterisation. The tests evaluated growth tolerance and viability studies as a function of relevant brine salts and temperature. The toxic effects observed followed the order: $MgCl_2$ >> NaCl >> KCl. The resistance/tolerance patterns found were complex and depended on the environmental factor under investigation. As expected from the occurrence profile, *Cryptococcus* strains were the least tolerant/resistant. Although *Debaryomyces hansenii* PYCC 5092 showed to be slightly more growth tolerant to the salts employed than *Rhodotorula mucilaginosa* PYCC 5242, the latter was clearly more resistant under stringent conditions involving higher salt concentrations and temperature. Results from the physiological studies agreed with the fieldwork and suggest an allochthonous character for the yeast population during the *safra* season in the saltern.

11. New web site: Dimorphic Basidiomycetes Web project:

http://www.crem.fct.unl.pt/dimorphic_basidiomycetes

This Internet site, coordinated by J.P. Sampaio and A. Fonseca, provides a series of web pages dedicated to the systematics, ecology and evolution of dimorphic basidiomycetes. The main goals of this project are to disseminate in-depth knowledge about this group of organisms and to present and discuss new ideas related to them. Since, at least in some cases, dimorphic basidiomycetes are studied by somewhat unrelated researchers (e.g., some phytoparasitic species are dealt with only by specialist mycologists, irrespective of the fact that their closest relatives are not plant parasites), we would like to contribute to bridge the gap between both the

organisms and the scientists who study them. We hope that, in the future, other authors will contribute to this project either by sending their comments and criticisms or by preparing contributions to be added to this site. Another important goal of this project is to provide a means to find clear and accessible taxon-specific information and therefore to help both specialists and non-specialists to understand the classification of dimorphic basidiomycetes.

XX. Dipartimento di Biologia Vegetale, Università degli Studi di Perugia, Borgo XX Giugno 74, 06100 Perugia, Italy. Communicated by A. Vaughan and A. Martini.

We are happy to announce that the website of the DBVPG Industial Yeasts Collection is now active. Please visit us at: http://www.agr.unipg.it/dbvpg/

Below are some recent publications by our group.

1. A. Vaughan-Martini, P. Angelini & G. Cardinali. 2000. Use of conventional taxonomy, electrophoretic karyotyping and DNA/DNA hybridization for the classification of fermentative apiculate yeasts. Int. J. Syst. Evol. Bacteriol. **50**:1665-1672.

2. W.J. Middelhoven, C.P. Kurtzman, and A. Vaughan-Martini. 2000. *Saccharomyces bulderi* sp. nov., a yeast that ferments gluconolactone. Antonie van Leeuwenhoek. **77**:223-228.

3. A. Vaughan-Martini, P. Angelini & L. Zacchi. 2000. The influence of human and animal visitation on the yeast ecology of three Italian caverns. Ann. Microbiol. **50**:133-140.

4. P. Buzzini, A. Martini. 2000. Biodiversity of killer activity in yeasts isolated from the Brazilian rain forest. Can. J. Microbiol. **46**:607-611.

5. P. Buzzini, A. Martini 2000. Utilisation of differential killer toxin sensitivity patterns for fingerprinting and clustering yeast strains belonging to different genera. Syst. Appl. Microbiol. **23**:450-457.

6. P. Buzzini, A. Martini. 2000. Differential growth inhibition as a tool to increase the discriminating power of killer toxini sensitivity in fingerprinting of yeasts. FEMS Microbiol. Lett. **193**:31-36.

7. P. Buzzini. 2001. Batch and fed-batch carotenoid production by *Rhodotorula glutinis – Debaryomyces castellii* co-cultures in corn syrup. J. Appl. Microbiol. **90**:843-847.

8. P. Buzzini, A. Martini. 2001. Large-scale screening of selected *Candida maltosa*, *Debaryomyces hansenii* and *Pichia anomala* killer toxin activity against pathogenic yeasts. Med. Mycol. **39**:479-482.

9. P. Buzzini, A. Vaughan-Martini, A. Martini. 2001. Fingerprinting of yeast cultures of industrial interest by the yeast killer system. Agro-Industry Hi-Tech, **12**:20-22.

10. P. Buzzini, A. Martini. 2001. Discrimination between *Candida albicans* and other pathogenic species of the genus *Candida* by their differential sensitivity to toxins of a panel of killer yeasts. J. Clin. Microbiol. **39**:3362-3364.

11. P. Buzzini, L. Rubinstein, A. Martini. 2001. Production of yeast carotenoids by using agro-industrial by-products. Agro-Industry Hi-Tech **12**:7-10.

12. A. Vaughan-Martini, A. Martini & P. Buzzini. 2001. Catalogue of cultures (4th edition) of the Industrial Yeasts Collection DBVPG Perugia, Centro Stampa dell'Ateneo dell'Università di Perugia.

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

I thank Dr. K.S. Bae and her colleagues at the Korean Research Institute for Bioscience and Biotechnology and Chungnam University, for their kind generosity during my recent visit in Korea.

The following paper, whose abstract was given in the previous issue, is now in print.

1. Lachance, M.A. J.M. Bowles. 2002. *Metschnikowia arizonensis* and *Metschnikowia dekortorum*, two new large-spored yeast species associated with floricolous beetles. FEMS Yeast Research **2**:81-86.

Recently accepted papers.

2. Duarte, E.R. M.A. Lachance, and J.S. Hamdan. 2002. Identification of atypical strains of *Malassezia* spp. from cattle and dog. Can. J. Microbiol. **48**:749–752 (2002)

Yeast species in the genus *Malassezia* are lipophilic with the exception of *Malassezia pachydermatis*. During a study of the occurrence of *Malassezia* species in the external ear of 964 cattle and 6 dogs in Minas Gerais, Brazil, six lipid-dependent isolates could not be identified to known species. Four isolates came from healthy cows, one from a cow with otitis, and one from a healthy dog. When tested with Tweens and Cremophor EL as single sources of lipids, the strains grew on all sources except Cremophor EL. None of the six strains hydrolyzed esculin, and all produced catalase. Pigment production from tryptophan was variable. Partial large subunit rRNA sequences were obtained for two isolates that remained viable in culture. The strain from the cow with otitis was identified as a lipid-dependent variant of *M. pachydermatis*, and the strain from the dog was an atypical variant of *Malassezia furfur*.

3. Teixeira, A.C.P., M.M. Marini, J.R. Nicoli, Y.Antonini, R.P. Martins, M.-A. Lachance & C.A. Rosa. (In press) *Starmerella meliponinorum*, a novel ascomycetous yeast species associated with stingless bees. Int. J. Syst. Evol. Microbiol. (See abstract under Dr. Rosa's entry).

4. Marinoni, G., J. Piskur, and M.A. Lachance. (In press) Ascospores of large-spored *Metschnikowia* species are genuine meiotic products of these yeasts. FEMS Yeast Res.

The asci of *Metschnikowia* species normally contain two ascospores (never more), raising the question of whether these spores are true meiotic products. We investigated this problem by crossing genetically-marked strains of the haploid, heterothallic taxa *Metschnikowia hawaiiensis*, *Metschnikowia continentalis* var. *continentalis*, and M. *continentalis* var. *borealis*. Asci were dissected and the segregation patterns for various phenotypes analyzed. In all cases (n = 47) both mating types (hþ and h3) were recovered in pairs of sister spores, casting further uncertainty as to whether normal meiosis takes place. However, the segregation patterns for cycloheximide resistance and several auxotrophic markers were random, suggesting that normal meiosis indeed occurs. To explain the lack of second-division segregation of mating types, we concluded that crossing-over does not occur between the mating-type locus and the centromere, and that meiosis I is tied to spore formation, which explains why the number of spores is limited to two. The latter assumption was also supported by fluorescence microscopy. The second meiotic division takes place inside the spores and is followed by the resorption of two nuclei, one in each spore.

5. Lachance, M.A. J.M. Bowles, and W.T. Starmer. (In press) *Metschnikowia santaceciliae, Candida hawaiiana*, and *Candida kipukae*, three new yeast species associated with insects of tropical morning glory. FEMS Yeast Res.

A new haplontic heterothallic species of *Metschnikowia* and two related asexual yeast species were discovered in morning glory flowers and associated insects. *Metschnikowia santaceciliae* came from *Conotelus* (Coleoptera: Nitidulidae) and other insect species associated with flowers of *Ipomoea indica* (purple morph) in Costa Rica. *Candida hawaiiana* and *Candida kipukae* were found in I. indica (syn. *I. acuminata*) and its insects in Hawai'i, and the former was also isolated in a specimen of *Conotelus* collected on *Merremia tuberosa* (Convolvulaceae) in Costa Rica. The three species have nearly identical physiological profiles, typical of the genus *Metschnikowia*. The sequences of the D1/D2 domains of their large subunit ribosomal DNA confirm that the species belong to the *Metschnikowia* clade, even though they share a very low degree of inter-relatedness. *M. santaceciliae* is a sister species to *Metschnikowia continentalis*. *C. kipukae* is a basal member of the large-spored *Metschnikowia* subclade, and *C. hawaiiana* has a weak affinity to *Metschnikowia* agaves. Two of the three species appear to be endemic. The type cultures are: *Metschnikowia santaceciliae*, strains UWO(PS)01-517a1 = CBS 9148 =NRRL Y-27475 (hþ, holotype) and UWO(PS)01-520a1 = CBS 9149 =NRRL Y-27476 (h3, isotype); *Candida hawaiiana*, strain UWO(PS)91-698.3 = CBS 9146 =NRRL Y-27473; *Candida kipukae*, strain UWO(PS)00-669.2= CBS 9147 =NRRL Y-27474.

6. Hong, S.G., Bae, K.S., Herzberg, M., Titze, A. and Lachance. M.A. (In press). *Candida kunwiensis* sp. nov., a yeast associated with flowers and bumblebees. Int. J. Syst. Evol. Microbiol. (accepted summer 2002). See abstract under Dr. Bae's entry.

The following lectures have been presented since the last issue.

7. Lachance, M.A. 2002. Biogeography of floricolous yeasts: is everything everywhere? Seventh International Mycology Congress, Oslo, Norway, August 2002.

The old dogma of microbial ecology, "Everything is everywhere, the environment selects," has had a profound influence on the study of yeast biodiversity. One outcome has been a widespread neglect of the habitat as a significant element of species descriptions. The traditional paradigm implies that a global "yeast seed bank" is available to fill any niche that is made available. Biogeographic theory, on the other hand, predicts that species diversity should be higher in the tropics, lower in isolated localities, and proportional to the size of contiguous landmasses. To test these opposing models, the yeast communities of ephemeral flowers were studied in various Pacific islands and several sites in Australia, Brazil, Costa Rica, the southern United States, and the eastern Nearctic region. These yeasts are vectored and maintained by insects such as bees and beetles. The yeast species composition is greatly but not exclusively affected by the nature of the vector insect species, and much less so by the plant species. Most biogeographic factors have a significant influence when not confounded by human interference. Different yeast species have different ranges on the global scale: some can be viewed as cosmopolitan and others as endemic. The emerging pattern is that indeed, the environment selects. However, geography plays a major role. The notion that "everything is everywhere," as least at it applies to floricolous ascomycetous yeasts, is misleading.

8. Lachance, M.A. 2002. Yeast Biodiversity, Progress and Challenges. Symposium on "Microbial Diversity and Evolution", Annual Meeting of the Federation of Korean Microbiological Societies, Millenum Town, Cheongju, Korea, October 2002.

Concluding paragraph. The early stages of exploration of yeast biodiversity were limited by our inability to identify species rapidly and correctly. Future success will hinge on the adoption of higher sampling intensities, better data management, and a multidisciplinary approach focused on natural history.

9. Lachance, M.A. 2002. Yeast biodiversity at the insect-flower interface. Seminars presented at the Korean Research Institute in Bioscience and Biotechnology, and in the Department of Microbiology, Chungnam University, Daejeon, Korea, October 2002.

Network: ***Yeasts in Food and beverages***

Publications on the theme "Molecular characterization of yeasts from food"

Communicated by P. Romano <pot2930@iperbole.bologna.it>.

California: Department of Viticulture and Enology, University of California, One Shield Ave., Davis, CA, 95616-8749, USA and Dipartimento di Scienze degli Alimenti, Università di Udine, via Marangoni 97, 33100 Udine, Italy. Communicated by David A. Mills <damills@ucdavis.edu>.

1. Cocolin L., Heisey A., Mills D.A. 2001. Direct identification of the indigenous yeasts in commercial wine fermentations. Am. J. Enol. Vitic. **52**:49-53.

A method is presented to directly characterize the yeast diversity in wine fermentations using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified ribosomal RNA genes. PCR-DGGE analysis of a commercial sweet wine fermentation clearly profiled the shifts in microbial diversity that occurred throughout the fermentation. *Botrytis* populations identified in press pan samples were absent from the settling tank and ensuing fermentation samples. Indigenous yeasts including *Candida, Metschnikowia,* and *Pichia* species were distinguished in the early stages of the fermentation prior to emergence of a *Saccharomyces* population. Surprisingly, the PCR-DGGE signature of *Candida* species persisted well into the fermentation long after the development of a dominant *Saccharomyces* population. By direct identification yeast populations, PCR-DGGE can provide a rapid and comprehensive view of the microbial diversity present in wine fermentations without the necessity for enrichment plating.

2. Mills D.A., Johannsen, E.A., Cocolin L. 2002. Yeast diversity and persistence in botrytis-affected wine fermentations. Appl. Environ. Microbiol., **68**:4884-4893.

Culture dependent and independent methods were used to examine the yeast diversity present in botytis –affected (botrytized) wine fermentations carried out at high (~80ºC) and ambient (~ 56ºC) temperatures. Fermentations at both temperatures possessed similar populations of *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Metschnikowia*, *Kluyveromyces*, and *Candida* species. However, higher populations of non-*Saccharomyces* yeasts persisted in ambient fermentations with *Candida*, and to a lesser extent *Kluyveromyces,* species remaining long after the fermentation was dominated by *Saccharomyces*. In general, denaturing gradient gel electrophoresis profiles of yeast rDNA or rRNA amplified from the fermentation samples correlated well with the plating data. These direct methods also revealed a *Hanseniaspora osmophila* population not identified in the plating analysis. rRNA analysis also indicated large population (>10^6 cells per mL) of viable-but-not-culturable *Candida* sp. in the high temperature fermentation. Monoculture analysis of *Candida* isolates indicated an extreme fructophilic phenotype and correlated with an increased glucose/fructose ratio in fermentations with higher populations of *Candida*. Analysis of wine fermentation microbial ecology using both culture-dependant and independent methods reveals the complexity of yeast interactions enriched during spontaneous fermentations.

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1. Pallmann C.L., Brown, J.A., Olineka T.L., Cocolin L., Mills D.A., Bisson L.F. 2001. Use of WL medium to profile native flora fermentations. Am. J. Enol. Vitic. **52**:198-203.

Vineyard, winery, barrel, and controlled temperature fermentation samples from a single commercial winery (Luna Vineyards) conducting fermentations with indigenous organisms were plated onto Wallerstein Laboratory Nutrient Agar (WL) to evaluate colony diversity. Seventeen unique colony morphologies were identified. Sequence analysis of the DNA encoding portion of the large ribosomal 26S rRNA indicated that colony types defined members of six genera: *Hanseniaspora uvarum* (*Kloeckera apiculata*), *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Pichia kluyveri*, *Candida oleophila*, and *Metschnikowia*. Distinct colony subtypes were identified within the pulcherrimin producers traditionally classified as a single species, *Metschnikowia pulcherrima*. Sequence analysis of the D1/D2 region of the 26S rDNA of these biotypes showed a high degree of divergence, suggesting that these organisms might define separate species. Analysis of fermentations revealed that colony type as indicated on this medium could be used to monitor the yeast population dynamics.

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I. Middelhoven, W.J. 2002. Identification of yeasts present in sour fermented foods and fodders. Molec. Biotechnol. **21**:279-292.

An identification key to fifty yeast species frequently isolated from foods and fodders that underwent a lactic acid fermentation is provided. However, several species present in fermented olive products were not included. The key is based on some morphological and physiological characteristics. These include growths tests using the API ID32C test strips, assimilation of methanol and starch, fermentation of glucose, growth on nitrate and ethylamine as sole nitrogen sources, vitamin requirement, maximum growth temperature and presence of urease. For morphology, slide cultures on maize meal agar are recommended. The tests are relatively simple and strains can be identified within a week also by those who are not experienced in more sophisticated procedures. Instead of the provided identification key, the BioloMICS of Vincent Robert of CBS Utrecht can be used. This is available via Internet, but was not yet when the manuscript was prepared. By applying the BioloMICS, the methods can be useful for identification of yeast strains isolated from other habitats.

Italy (Firenze): Dipartimento di Biotecnologie Agrarie, Università degli Studi di Firenze. P.le delle Cascine 24, 50144 Firenze, Italy.Communicated by L. Granchi <lisa.granchi@unifi.it>.

1. Granchi L., Ganucci D., Viti C., Vincenzini M. 2002. Assimilable-nitrogen content of grape must and *Saccharomyces cerevisiae* biodiversity in natural wine fermentations. Proceedings of 22nd ISSY, 25-28 March 2002, Pilanesberg National Park, South Africa, p. 84.

2. Granchi L., D. Ganucci, C. Viti, L. Giovannetti, M. Vincenzini. 2002. *Saccharomyces cerevisiae* biodiversity in spontaneous commercial fermentations of grape musts with "adequate" and "inadequate" assimilable-nitrogen content. Lett. Appl. Microbiol., (in press)

Spontaneous wine fermentations are generally carried out by different strains of *Saccharomyces cerevisiae* with possible differences in organoleptic wine properties. Since these strains may differ in their nitrogen demand and initial assimilable-nitrogen amounts in musts largely vary, nitrogen-limited conditions could select strains with low-nitrogen requirements and, possibly, affect *S. cerevisiae* biodiversity. With the aim to assess whether the initial nitrogen content in musts may affect intraspecific diversity of *S. cerevisiae*, 52 isolates from two spontaneous commercial wine fermentations with adequate and inadequate initial nitrogen amounts, were characterized at strain level by mitochondrial DNA restriction analysis using *Rsa*I and *Hinf*I endonucleases. Several strains occurred in each fermentation, two strains, but not the same ones, being predominant at frequencies of about 30%. No significant differences were detected by comparing the biodiversity indices of the two fermentations. Cluster analysis demonstrated that the strain distribution was independent of nitrogen content, the two pairs of closely related dominant strains grouping into clusters at low similarity. According to the findings, the nitrogen availability in musts did not affect the genetic diversity of *S. cerevisiae* but may have induced a "selection effect" on strains dominating wine fermentations, with possible consequences on wine properties.

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1. Foschino R., Gallina S., Picozzi C., Galli A. 2002. Characterization of yeast strains isolated from sour dough for sweet baked products. Poster presented to International Conference "Quality and risk assessment of agricultural food in the Mediterranean area" September 24-27 2002, Foggia, Italy.

Sour dough is a spontaneous or constantly renewed fermented mixture of flour and water in which lactic acid bacteria and yeasts are predominant microorganisms, often in mutualistic relationship between them. This traditional practice is still used in food technology to make some Italian sweet products. In this work 22 yeast strains isolated from sourdoughs for Panettone, Pandoro and Cornetto in 7 different industrial bakeries located in northern Italy were characterized. Phenotypic analyses was carried out according to the SIM method of Deak and Beuchat, the method of Kurtzman and Fell and the API ID 32C system. Sequences of 18S-28S rDNA spacer regions were investigated in order to verify the identification. Yeast counts in sourdough samples ranged from 1.4×10^6 to 6.2×10^7 CFU/g with a mean value of 1.2×10^7 CFU/g. Comparison of results obtained by different methods of classification reveals that misidentification can easy occurs. *S. exiguus*, *S. cerevisiae, S. bayanus* and *S. pastorianus* were the species frequently detected with one or more methods based on physiological characteristics, that were partially confirmed by the sequence analysis of ITS1 and ITS2. Some isolates showed good similarity (>80%) of sequence with *S. cerevisiae sensu stricto* group whereas the most of strains clustered only at 57% of similarity with *S. exiguus* and *S. barnetti*.

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1. Pulvirenti A., Caggia C., Restuccia C., Gullo M., Giudici P. 2001. DNA fingerprinting methods used for identification of yeasts isolated from Sicilian sourdoughs. Ann. Microbiol., **51:**107-120.

The nature of sourdough yeast microflora was investigated. Samples were collected from different bakeries that do not use starter cultures, but rely on the traditional art of bread making. Pulsed field gel electrophoresis (PFGE), PCR/RFLP of the Non-Transcribed-Spacer 2 (NTS2), the interdelta regions and the Internal Transcribed Spacers (ITS) of the ribosomal DNA (rDNA) and partial sequencing of the 26S rDNA were used to identify and differentiate the yeast culture isolated. In particular, PCR/RFLP of the ITS region, with restriction enzyme *Hae*III made it possible to differentiate *Candida milleri* from *Candida humilis*. A high degree of polymorphism was observed in the interdelta regions of the strains belonging to the *S. cerevisiae* species; contrary to what has been mentioned in literature, strains with the same profile are non necessary identical. *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* old type *anamensis, Issatchenkia orientalis, Candida mille*ri and *Candida humilis* were found to be the predominant species in Sicilian sourdough.

2. Giudici P., Pulvirenti A., Zambonelli C., Todaro A. 2002. Horizontal transfer of genetic materials in *Saccharomyces* promoted by animals. Bulletin de L'O.I.V., **75**(857-858):484-500.

The objective of this study was to verify whether animals promoted the formation of new yeast strains by increasing the probability of encounters between otherwise separated spores. This study was carried out on three invertebrates, marine fresh water and territorial ecosystems were nourished with two different foods composed of one or several spore yeast strains and digested matter was analysed as an index. The result revealed that the asci cell wall (as do enzymes) produced by species belonging to the *Saccharomyces* genus without affecting the enclosed spore vitality. Under favourable conditions, the spores evacuated in faecal matter have a high agglutination capacity. They can germinate directly or can combine to form hybrids. Hybrids between strains of the same species give rise to cultures for witch the parent characteristic present a new combination. Interspecific hybrids *Saccharomyces cerevisiae* x *Saccharomyces uvarum* obtained by the same procedure and although they are sterile can propagate by asexual reproduction. These results support the hypothesis of horizontal transfer of genetic materials between yeast species and the probability of forming hybrids in natural environment and provides doubts on the great diversity of yeasts already observed in nature.

3. Gullo M., Romano A.D., Pulvirenti A., Giudici P. 2002. *Candida humilis* dominant species in sourdoughs for the production of durum wheat bran flour bread. Int. J. Microbiol, in press.

Yeasts present in the sourdough that is generally used for the production of durum wheat bran-flour bread were isolated and identified. Samples were taken during the rebuilding phase and at different intervals of time in order to monitor the population dynamics. The results obtained from the phenotypic studies were further confirmed by the molecular studies and enabled us to affirm that most of the strains, more than 95%, belong to the species *Candida humilis.* As a matter of fact, not only is *Candida humilis* the dominant species in the sourdough studied, but it is also steady in time. The isolations were carried out at sufficiently long intervals so that it was possible to ascertain that the conditions in which the sourdough is kept are fundamental to the microbiological stability of the dough.

4. Pulvirenti A., Zambonelli C., Todaro A., Giudici P. 2002. Interspecific hybridisation by digestive tract of invertebrates as a source of environmental biodiversity within the *Saccharomyces cerevisiae* species. Ann. Microbiol., **52:**49-59.

In order to verify whether animals can promote the formation of new yeast strains by increasing the chance of encounters between otherwise segregate spores within persistent asci, three invertebrate species representative of the marine, freshwater and terrestrial ecosystems were fed with two different diets composed of one or a combination of two strains of sporulated yeast and the egested material was analyzed for evidence. The digestive apparatus of the tested species is able to break open the asci wall (most probably by enzymatic action) produced in the species belonging to the *Saccharomyces sensu stricto* group without affecting the viability of the spores contained therein. The spores ejected with the fecal material have a high capacity for agglutination and, in the presence of favorable conditions, can germinate directly or conjugate to form hybrids. Hybrids between strains of the same species give rise to cultures in which the parent characteristics have new combinations. The interspecific hybrids *S. cerevisiae* x *S. uvarum*, although sterile, can propagate by asexual reproduction. These results support the hypothesis of horizontal transfer of genetic material between yeast species and the likelihood of hybrid formation in natural settings, and possibly shed light on the high biodiversity of yeast observed in nature.

5. Pulvirenti A., Gullo M., De Vero L., Giudici P. 2002. Uniparental Mitochondrial Inheritance in *Saccharomyces* spore conjugation. 9th Intern. Symp. "Genetics of Industrial Microorganisms" (GIM). July 1-5 2002, Gyeongju, Corea.

Spore conjugation between *Saccharomyces cerevisiae* and *S. uvarum* strains yields hybrids, which have mtDNA profile identical to only one parental type: either of *S. cerevisiae* or *S. uvarum*. The peculiarity of examined hybrids is due to the homogeneity of clone populations that are consisting of cells characterised by the same mitochondrial type. For this reason participation of mitotic processes in the parental mitochondria segregation can be excluded. It seems to be more probable to suppose an incompatibility of parental mitochondrial DNA (mtDNA). Parental inheritability can involve more complex mechanisms and remarkable information on mitochondrial behaviour can be obtained through the study of these hybrids. On this evidence, we have considered interesting to examine the way of mitochondrial transmission to intraspecific hybrids *S. cerevisiae* x *S. uvarum*.

6. Giudici P., Pulvirenti A. 2002. Molecular Methods for identification of wine yeasts. In "Biodiversity and Biotechnology of wine yeast". Managing Editor Research – India, in press.

Conventional taxonomy is based on the method of sexual reproduction, where it is present, on the morphology of cells, and on physiological and biochemical features, the tests are often long and laborious and can even require up to three weeks for complete results. In the last few years, the times required for identification of yeast has been drastically reduced, thanks to the introduction of molecular techniques applied to the study of entire chromosomes or analysis of specific domains or parts of these. This study takes into consideration the use of electrophoresis in a pulsed field for karyotype analysis, PCR/RFLP of the NTS2 and ITS regions of the rDNA, sequencing the D1/D2 domain or rDNA and, for identification at the strain level, PCR of the inter-δ regions, and the RFLP of mitochondrial DNA.

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1. Caruso M., Capece A., Salzano G., Romano P. 2002. Typing of *Kloeckera apiculata* and *Saccharomyces cerevisiae* strains from Aglianico wine. Lett. Appl. Microbiol. **34**:323-328.

Kloeckera apiculata and *Saccharomyces cerevisiae* yeast species are dominant, respectively, at the early and at the following stages of wine fermentation. With the aim to monitoring yeast population performing Aglianico of Vulture grape must fermentation, 30 *S.cerevisiae* and 30 *K.apiculata* strains were typed by PCR fingerprinting with $(GAC)_5$ and $(GTG)_5$ primers and by complete NTS region amplification followed by restriction with *Hae*III and *Msp*I enzymes. The results showed that the techniques used are suitable to discriminate at the strain level *S.cerevisiae* that perform the fermentative process and to recognize and follow the presence of apiculate yeasts during the fermentation.

2. Capece A., Salzano G., Romano P. 2002. Molecular typing techniques as a tool to differentiate non-*Saccharomyces* wine species. Int. J. Food Microbiol., in press.

A total of thirty-two yeast strains belonging to four non-*Saccharomyces* species associated with wine making were characterized by different molecular techniques. The PCR amplification of 18S rRNA-coding DNA and nontranscribed spacer, followed by restriction analysis with the endonucleases Hae III and Msp I, and PCR fingerprinting with microsatellite primers $(GAC)_5$ and $(GTG)_5$ were used. The methods used provided species-specific profiles and proved to be fast and reliable for monitoring the evolution of the four non-*Saccharomyces* yeast populations throughout wine fermentation.

The following conference abstracts have been published.

3. Capece A., Salzano G., Lipani G., Paraggio M., Romano P. 2001. Screening of non-*Saccharomyces* wine yeasts by molecular methods. Proc. 21st ISSY "Biochemistry, Genetics, Biotechnology and Ecology of non-conventional Yeasts (NCY), Lviv (Ukraine), 21-25 Agosto.

4. Romano P., Sipiczki M., Capece A., Paraggio M., Lipani G., Salzano G. 2002. Analysis of *Saccharomyces cerevisiae* strains derived from spontaneous fermentation of Aglianico wine. Proc. 22nd ISSY "Yeast Fermentations and other Yeast Bioprocesses", Pilanesberg National Park (South Africa), 25-28 March.

5. Capece A. 2002. Molecular methods for the control of wine fermentation. Proc. Int. Conf. "Quality and risk assessment of agricultural food in the Mediterranean area", Foggia (Italy), 24-27 September.

Italy (Udine): Dipartimento di Scienze degli Alimenti, Università di Udine, via Marangoni 97, 33100 Udine, Italy. Communicated by L. Cocolin, E-mail: luca.cocolin@dsa.uniud.it.

1. Cocolin L., Aggio D., Manzano M., Cantoni C., Comi G. 2002. An application of PCR-DGGE analysis to profile the yeast populations in raw milk. Int. Dairy J. **12**:407-411.

Four different zones from the Friuli Venezia Giulia region, North East of Italy, were sampled for the study of the yeast bio-diversity in raw milk. Samples were analysed by traditional methods to isolate different yeast strains that were subjected to identification by sequencing the D1-D2 domains of the 26S rRNA gene. Twelve different species of yeast were identified, six of them belonging to the genera *Candida* and two to the genera *Kluyveromyces*. The identified strains were then used for the optimization of a method based on PCR and denaturing gradient gel electrophoresis that was used for a direct monitoring of the populations in the samples. Applying the method to the DNA extracted directly from the raw milk samples, new bands appeared in the gel underlining a different bio-diversity in respect to the traditional method. The approach described is a powerful and reliable tool to monitor directly yeast ecology in milk and milk products without the need of traditional isolation and it could be used to follow specific populations to prevent spoilage or to control contamination.

2. Cocolin L., Manzano M., Rebecca S., Comi G. 2002..Monitoring of the yeast population changes during a continuous wine fermentation by molecular methods. Am. J. Enol. Vitic. **53**:24-27.

A continuous wine fermentation was followed using molecular methods to monitor the changes in the yeast population and to assess the capability of the *Saccharomyces cerevisiae* used as a starter culture, to persist throughout the period considered. Plating analysis was performed to isolate yeasts present in the must and molecular analysis was used for the characterization of the strains. Restriction fragment length polymorphism (RFLP) of the 18S rRNA gene and ITS1 region and mitochondrial DNA (mtDNA) restriction endonuclease analysis (REA) were exploited. Moreover denaturing gradient gel electrophoresis (DGGE) of the 5' end 26S rRNA gene, from DNA extracted directly from must, was used to profile the yeast population avoiding the potential biases of traditional methods. Considering the results obtained from each analysis, it was possible to determine the predominance of *S. cerevisiae* population during the fermentation, which remained stable throughout the period. From mtDNA-REA analysis, identical patterns were obtained from all the *S. cerevisiae* strains isolated, demonstrating the capability of the starter culture to perform the continuous fermentation studied.

Obituary

In Memoriam - Henri Heslot 1921-2001

A native of Paris, Henri Heslot obtained his degree in agricultural engineering at the Institut National Agronomique (INA) in 1945. This was the start of a lifetime career at that institute. In 1948, he became one of the first graduates in the new genetics program at the Sorbonne. He then obtained a British Council fellowship to study with the famous statistician R. Fisher at Queen's College, Cambridge. To Fisher's interest for mouse genetics, Heslot preferred to explore the new science of genetics of bacteria, collaborating with the now renowned human geneticist, L. Cavalli-Sforza.

At his return to France, he headed the genetics laboratory of INA working on his doctorate on the cytology and genetics of Ascomycetes in the Sordariaceae, which he obtained in 1957. His studies involved the isolation of mutants altered in centrosome function. This controversial discovery earned him a prize from the Academy of Science in 1961. He then turned his interest to applied plant genetics and eventually became a member of the editorial board of journals such as Mutation Research.

Wishing to work with a more efficient model organism, Heslot turned to *Schizosaccharomyces pombe*, and gradually moved to the new field of molecular yeast genetics. The industrial sector (ELF-ERAP) approached him to develop lysine or citric acid-producing strains of *Candida lipolytica* and *Candida tropicalis*, which caused him to assemble a research team in the genetics of "non-conventional" yeasts, laying the foundations of his research in microbial biotechnology. He then obtained the Chair in Molecular and Cellular Genetics and lobbied the government and its agencies to create technology transfer centres, in order to spread the benefits of genetical research to society. One of these was built at the Grignon campus under his personal supervision.

During his most active period (1970-1990) Heslot was a pioneer of the applications of protoplast fusion to yeast transformation with the 2 micron plasmid in *Saccharomyces cerevisiae*, for which he obtained a patent. In the early 80s, he was involved in the production of heterologous proteins by yeast. Then, he turned to bacteria, successfully cloning the thermostable amylase of *Bacillus licheniformis*. This work eventually led to the new field of enzyme modification, a topic for which he developed a lifetime passion. This culminated in 1996 with the publication of a treatise on Protein Engineering and its Applications, earning him the Roberval prize. He was still working on an English translation during summer 2001.

Heslot was a passionate of innovation and never stopped thinking like an engineer. His advice was sought after by many research agencies, including CNRS, INRA, the Academy of Science, the Academy of Technology and the Academy of Agriculture.

Henri Heslot was a charismatic teacher, appreciated for his clarity and his sense of synthesis. Many students of the molecular biology of microorganisms, plants, and animals including humans benefitted from his guidance. He was also an avid traveler with a special interest in Middle-Eastern and Asian culture. He will be remembered for his joy, his openness, his rigor, his passion, and above all, his honesty.

Original text by Claude Gaillardin — Translated from the French and abridged by the Editor

International Commission on Yeasts

During the IUMS Meeting in Paris, July 2002, Professor Graham H. Fleet has been elected as Vice Chairman of the Mycology Division of the International Union of Microbiological Societies (IUMS).

Lex Scheffers, Chair, ICY

Recent Meeting

30th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts Smolenice, Slovakia, May 29-31, 2002

The 30th 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 the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, during May 29-31, 2002. This conference differed from previous ones in several aspects. First of all, it was a real jubilee conference. The number 30 points to a great tradition of this scientific event in Slovakia. The long series was occasionally interrupted by international specialized yeast symposia (ISSY) which took place also in the Smolenice Castle. Then, the organizers invited to the 30th conference older and retired scientists who participated in the activities of the Czech and Slovak Commission for Yeasts in previous years. All of them were defrayed from part of the conference fees. In addition, the meeting had several prominent guests. The meeting was honoured by the presence of the General Secretary of FEMS, Prof. Peter Raspor from Ljubljana, Slovenia, who not only gave a plenary lecture but also, on behalf of the FEMS, presented a letter of appreciation to the Czech and Slovak Commission for Yeasts for extraordinary long-term activities within the Czechoslovak Society for Microbiology. Another distinguished participant of the meeting was Prof. Pjotr P. Slonimski from CNRS in Gif-sur-Yvette, France, one of the founders of yeast genetics, who arrived in Smolenice after being awarded Honorary Doctorate at the Komensky University in Bratislava. For the first time in last twenty years the accommodation capacity (90) of the Smolenice Castle was fully used during an annual yeast conference.

The meeting was attended by 43 participants from the Czech Republic, 39 participants from Slovakia, and 9 participants from abroad. The program consisted of four lecture sessions devoted Apoptosis of Yeast, Molecular Biology and Genetics, Molecular Biology of Yeast Cytoskeleton, and Yeasts and Problems of Current Medicine. The lectures of first two sessions were presented in English, while the last two sessions in Czech and Slovak, the two closely related languages of recently separated nations. The lectures were complemented with 59 posters. Program and abstract of lectures and posters were printed in English, so the foreign participants could follow the program easily. The titles of all contributions are listed below:

Plenary lectures in the session Apoptosis of Yeasts

1. Madeo F.: Regulators and physiology of yeast apoptosis.
2. Breitenbach M., Laun P., Pichová A., Madeo F., Fuchs J., Ellinger A., Kohlwein S.,Fröhlich K. U., Dawes I.: Mother cell-specific ageing in *S. cerevisiae*.
3. Laun P., Heeren G., Jarolim S., Madeo F., Hauser N., Hoheisel J., Pichová A.,Breitenbach M.: Genome-wide transcript analysis of aged yeast mother cells.
4. Heeren G., Laun P., Jarolim S., Pichová A., Dawes I., Breitenbach M.: Role of CUP1 and yeast flavohemoglobin in oxidative stress and ageing.
5. Jarolim S., Laun P., Heeren G., Pichová A., Breitenbach M.: Is there a way to select for ageing mutants?
6. Aguilaniu H., Gustafsson L., Rigoulet M., Nyström T.:Oxidatively damaged proteins and senescence in *S. cerevisiae*.
7. Hlavatá L., Aguilaniu H., Pichová A., Nyström T.: Another role of RAS2 gene in aging of *S. cerevisiae* - regulation of oxidative stress level.
8. Kolarov J.: Mitochondria and cell death - introduction.
9. Kiššová I., Trancíková A., Gromová P., Šabová L., Kolarov J.: Search for mitochondrial determinats of yeast apoptosis.
10. Poliaková D., Sokolíková B., Kolarov J., Šabová L.: Bcl-XL prevents the cytotoxic effect of Bax though it does not prevent Bax induced formation of reactive oxygen species in *Kluyveromyces lactis*.

Plenary lectures in the session Molecular Biology of the Yeast Cytoskeleton

1. Necas O.: New findings on molecular motors.
2. Kopecká M., Gabriel M., Svoboda A. Takeo K., Yamaguchi M., Ohkusu M., Hata K.: Microtubules and actin cytoskeleton in growth and conidiogenesis in *Aureobasidium pullulans*.
3. Slaninová I., Holubárová A., Svoboda A.: Spectrin-like protein in yeasts.
4. Farkaš V., Kishida E., Takeo K.: Regulation of capsule formation in *Cryptococcus neoformans*.

Plenary lectures in the session Molecular Biology and Genetics of Yeasts

1. Janatová I., Koubek Z., Malínská K., Raková R., Hašek J.: Overproduction of *Schizosaccharomyces pombe* Rpg1/Tif32p causes aberrant morphology of *Saccharomyces cerevisiae* cells.
2. Valachovic M., Klobucníková V., Hronská L., Griac P., Hapala I.: Heme-regulated expression of ARE1 and ARE2 genes is involved in the adaptation of yeast to hypoxia.
3. Zeman I., Schwimmer C., Trézéguet V., Lauquin G. J.-M.: Modified yeast mitochondrial ADP/ATP carrier can operate despite presence of specific inhibitor: identification and characterization of mutants resistant to bongkrekic acid.
4. Kovác L.: Pjotr P. Slonimski and his impact on yeast mitochondriology.
5. Slonimski P. P.: Analysis of genomes by a cryptoanalytic approach.

Plenary lectures in session Yeasts and Problems of Current Medicine

1. Hanzen J., Hamal P., Volleková A., Horn F., Raclavský V., Pavlícek J.: *Candida lusitaniae*, with defect productivity of cellobiose, causing a shunt infection in a cancer child.
2. Hrušková-Heidingsfeldová O., Hamal P., Dostál J., Pichová I.: Clinical isolates of *Candida*: screening of extracellular proteolysis.
3. Lisalová M., Sládeková M., Hanzen J., Milošovic P.: The representation of the species of the genus *Candida* in the gastrointestinal tract with the oncological patients.
4. Nosek J., Tomáška L., Rycovská A.: Mitochondrial telomeres as a diagnostic marker for the pathogenic yeast *Candida parapsilosis*.
5. Tomšíková A.: *Saccharomyces cerevisiae* - antibody in the diagnosis of some diseases of intestinal tract.
6. Kucej M., Foury F.:Using *Saccharomyces cerevisiae* in study of Friedreich ataxia.
7. Šajbidor J., Breierová E.: Fenpropimorph derivatives as effective antimycotics.
8. Kogan G., Williams D. L.: Yeast polysaccharides as pathogen-associated molecular patterns (PAMPs) and their role in innate immunity.

Evening Lecture

Raspor P., Povhe-Jemec K., Cadez N., Cus F., Miklic D.: Current views on microbiology of grape/must/wine system in Slovenian vine-growing regions.

List of Posters

1. Hlavatá L., Aguilaniu H., Pichová A., Nyström T.: Does Ras2 protein regulate replicative aging through cAMP/PKA pathway in *Saccharomyces cerevisiae*?
2. Fiala J., Neumajer T., Pichová A.: Controlling cell proliferation and cell death (apoptosis) by flow cytometry.
3. Yoshida S., Farkaš V., Takeo K.: Biochemical basis of acid-induced suicide death in *Cryptococcus neoformans*.
4. Havelková M., Unger E.: The role of calmodulin in DNA segregation during mitosis.
5. Raclavský V., Riháková P., Godava M., Novotná J., Ohkusu M., Takeo K.: New method for cell cycle analysis in *Cryptococcus neoformans* based on image analysis of stained cells.
6. Raclavský V., Hamal P.: Evaluation of RAPD typing for epidemiological analysis of Candida spp. isolates.
7. Gabriel M., Kopecká M., Svoboda A., Takeo K., Yamaguchi M., Ohkusu M., Hata K.: Cytoskeleton in human fungal pathogens *Cryptococcus neoformans* and *Aureobasidium pullulans* and the effect of cytoskeletal inhibitors.
8. 8. David M., Gabriel M., Kopecká M.: The study of potentially pathogenic lipophilic yeast *Malassezia pachydermatis*.
9. Dorosh A., Hašek J., Janatová I.: Searching for a suitable dominant selection marker in *Schwanniomyces occidentalis*.
10. Mrkvicková P., Hašek J., Janatová I.: Expression of the Cd5 antigen, surface glycoprotein of thymocytes, in *Pichia pastoris*.
11. Dlasková A., Dorosh A., Frýdlová I., Trachtulcová P., Hašek J., Janatová I.: Distribution of Isw2p in *Saccharomyces cerevisiae*.
12. Vlcková V., Miadoková E.: Comparison of antimutagenic effect of two natural anitmutagens on *S. cerevisiae* and *S. typhimurium*.
13. Dudáš A., Brozmanová J., Gellon L., Boiteux S.: The disruption of Ntg1 and Ntg2 genes in pso3 mutant of *Saccharomyces cerevisiae* leads to increased sensitivity to hydrogen peroxide.
14. Lampartová Z., Marková E., Dudáš A., Vlcková V., Brozmanová J.: Is recombination involved in the DNA repair of oxidative damage in *Saccharomyces cerevisiae*?
15. Takácová M., Sklenár P., Izsáková K., Gbelská Y., Šubík J.: Cycloheximide resistance in the yeast *Kluyveromyces lactis*.
16. Kucejová B., Foury F.: Search for the synthetic lethal mutations in a yeast strain deleted for the *pif1* gene.
17. Sadovská J., Tomáška L., Nosek J.: Searching for a mitochondrial telomeric double-stranded DNA-binding protein of the yeast *Candida parapsilosis*.
18. Tomáška L., Rohác P., Nosek J., Steensma H. Y.: Regulation of pyruvate dehydrogenase of *Saccharomyces cerevisiae* by phosphorylation: known phenomenon, unknown protein kinase(s).
19. Špírek M., Polákova S., Malikova D., Slamka T., Sulo P.: Are mitochondrial introns the motive power of yeast speciation?
20. Fekete V., Mäsiarová E., Sulo P.: Absence of mitochondrial ADP/ATP translocator protein is compatible with mit- mutation.
21. Malikova D., Polákova S., Mäsiarová E., Špírek M., Sulo P.: Living in the bad environment.

22. Gromová P., Polcic P., Kolarov J.: Mammalian BAX protein and some mitochondrial inhibitors rapidly induce ROS formation and it is partially reduced by *bcl-xl*.
23. Trancíková A., Kiššová I., Gavurníková G., Kolarov J.: The benefits of bcl-xl expression in cells with defective mitochondria.
24. Adamec T., Palková Z., Forstová J.: Subcellular localization of polyomavirus capsid protein VP1 and its non-assembling variant in *Saccharomyces cerevisiae*.
25. Synek L., Palková Z., Forstová J.: Interaction of polyomavirus capsid protein vp1 with yeast mitotic spindle.
26. Kuthan M., Palková Z.: Different strategies of *Saccharomyces cerevisiae* colony development: laboratory versus nature.
27. Mináriková L., Palková, Z.: "Acid-to-alkali" transition of s. cerevisiae colonies: distribution of amino acids between cytoplasm and vacuole.
28. Palková Z., Devaux F., Ricicová M., Crom S., Claude J.: "Acid-to-alkali" transition of Saccharomyces cerevisiae colonies I: microarray analyses.
29. Ricicová M., Devaux F., Palková Z.: "Acid-to-alkali" transition of *Saccharomyces cerevisiae* colonies II: mutant analysis.
30. Slaninová I., Ilkovics L., Kuthan M., Palková Z.: SEM and AQUASEM studies of *S. cerevisiae* and *C. moggii* colonies.
31. Horová I., Janderová B., Palková Z.: Exploitation of cytological methods for analysis of yeast colonies in situ.
32. Novotná D., Flegelová H., Janderová B.: Do *S. cerevisiae* killer toxins K1 and K2 really exhibit the same mechanism of action?
33. Kinclová O., Potier S., Sychrová H.: Functional characterization of the family of yeast plasma membrane Na^+/H^+-antiporters.
34. Marešová L., Sychrová H.: Physiological properties of osmotolerant yeast *Pichia sorbitophila*.
35. Pribylová L., Sychrová H.: Osmotic stress resistant yeast *Zygosaccharomyces rouxii* - isolation of auxotrophic mutants and efficient transformation.
36. Gášková D., Chaloupka R., Cadek R., Chládková K., Sigler K.: Kinetic characterization of ABC pumps in *S. cerevisiae* by a fluorescence assay.
37. Váchová L., Kucerová H., Sigler K.: Coping with problems when measuring stress-induced changes in yeast cell and membrane proteins.
38. Holoubek A., Vecer J., Sigler K.: Measurement and calibration of H+-ATPase-generated ?? in reconstituted yeast plasma membrane vesicles.
39. Klobucníková V., Kohút P., Fuchsbichler S., Turnowsky F., Hapala I.: Phenotypic characterization of a pleiotropic yeast mutant resistant to the antimycotic terbinafine.
40. Pichová I., Pavlícková L., Dostál J., Dolejší E., Hrušková-Heidingsfeldová O., Ruml T., Soucek M.: Inhibition of aspartic proteinases secreted by different pathogenic *Candida* species.
41. Márová I., Záhejský J., Kanková K.: Influence of antioxidative status on progression of yeast skin infections in diabetics.
42. Kriková L., Šandula J., Krajcovic J., Sasinková V.: Fungal ß-D-glucan derivatives inhibit chemically induced mutagenesis in plastids of the flagellate *Euglena gracilis*.
43. Šandula J., Machová E., Kogan G., Hríbalová V.: Preparation of biological active polysaccharides from yeasts and fungi using ultrasonication.
44. Machová E., Bystrický S., Gáliková A., Kogan G.: ß-Glucan from *Saccharomyces cerevisiae* as a matrix for protein-polysaccharide immunoconjugate with Vibrio cholerae 01 antigen.
45. Stajkovic B., Bulajic N., Vujcic Z.: Preparation of *Candida albicans* antigens for sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblot.
46. Vadkertiová R., Sláviková E.: Killer activity of yeasts from natural habitats against the pathogenic yeasts.
47. Sláviková E., Košíková B., Mikulášová M.: Application of *Sporobolomyces roseus* for biological pretreatment of wood before pulping.
48. Pokorná J., Slovák B., Stávek P., Vcelná P., Márová I., Drdák M.: Induction of molecular changes in some strains of carotenogenic yeast by exogenous stress factors.
49. Kocí R., Slovák B., Vcelná P., Stávek P., Márová I., Fišera M.: Molecular changes in *Rhodotorula glutinis* cells stressed by hydrogen peroxide.
50. Gregor T., Breierová E., Koubeková A., Fišera M.: Influence of osmotic stress on the yeast cultures in occurrence heavy metals.
51. Breierová E., Certík M., Strhanová K., Omelková J.: The study of adaptation characters of the carotenoid yeasts in environment of heavy metals.
52. Breierová E., Certík M., Koubeková A.: The role of extracellular yeast glycoproteins in the osmotic extreme environments.
53. Certík M., Breierová E., Koubeková A., Sláviková L., Šajbidor J.: Effect of salt stress on lipid composition of yeasts.
54. Certík M., Breierová E., Koubeková A., Magdolen P., Šajbidor J.: Modulation of yeast lipid composition in extreme conditions induced by sorbitol.
55. Dostálek P., Patzak M., Matejka P.: Influence of specific growth limitation on biosorption of heavy metals by *Saccharomyces cerevisiae*.
56. Lauková D., Valík L., Schmidt Š., Görner F.: Effect of lactic acid and temperature on growth dynamics of *Candida maltosa*.
57. Švanová L., Hesová J., Breierová E., Augustín J., Omelková J., Stratilová E.: Effect of pH on the production of multiple forms of polygalacturonase by three strains of *Aureobasidium pullulans*.
58. Vajcziková I., Breierová E., Vojteková G., Sláviková E.: Yeast contaminations in vine industry.
59. Dudíková J., Kolarova N., Capek P.: The extracellular glycoproteins from acapsular yeast *Cryptococcus laurentii*.

During the 30th Annual Conference on Yeasts, meeting of the Committee of the Czech and Slovak Yeast Commission took place. The most important news of this meeting are: the 31st Annual Conference on Yeast will also be held in Smolenice in May 2003; the leadership of the Czech and Slovak Commission for Yeasts has changed at the Committee meeting. After more than 20 years working in the function of Secretary and Chairman of the Commission, Dr. Peter Biely has resigned from the position of the Chairman, and Dr. Maria Vrsanska has resigned from the position of the Secretary. Dr. Vladimir Farkas and Dr. Emila Breierova were voted in as the new Chairman and

Secretary of the Czech and Slovak Commission for yeasts, respectively. Similarly as previous Commission dignitaries, the new ones are professionally associated with the Institute of Chemistry, Slovak Academy of Sciences, which houses the Czechoslovak Collection of Yeast and Yeast-like Microorganisms. We wish them much success in their work for the Commission in the years to come.

Peter Biely, past Chairman of the Czech and Slovak Commission for Yeasts

Forthcoming Meetings

XXI International Conference on Yeast Genetics and Molecular Biology

Goteborg, Sweden, July 7-12, 2003

We would like to send to you the second announcement, which contains all information on the conference and instructions for registration and abstract submission. Please use the hyperlink below to enter your address into our database. Your data will be used strictly only for the purpose of sending information on the International Yeast Conference.

The conference "yeast2003" will be a lively meeting with an exciting program. The website "www.yeast2003.se" will constantly be updated with relevant information and will be site for registration and abstract submission. Welcome to Sweden in summer 2003.

http://130.241.156.175/collegues/update.aspx?Email=____**your email**____

For the Organising Committee, Stefan Hohmann and Kerstin Straby

ISSY23 Conference, Budapest, August 26-29 2003

We are pleased to inform you that we have refreshed the web site of the 23rd International Specialised Symposium on Yeasts to be held in Budapest, Hungary from 26 to 29 August, 2003. Meanwhile the online database for registration and abstract submission is also opened. Please note that the abstract submission deadline is 1 February, 2003.

After you have pre-registered in the database, you will be able to ●submit your abstract(s) , ●arrange registration, ● reserve accommodation, and ● register accompanying persons and optional programmes. Please visit http://www.diamond-congress.hu/issy/. Looking forward to meeting you in Budapest.

Varga Attila <diamond@diamond-congress.hu>

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

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

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

Secretariat:

Departamento de Biología Celular y Molecular
Facultad de Ciencias
Universidad de A Coruña <secrebcm@udc.es>

Eleventh International Symposium on Yeasts, ISY 11
Rio de Janeiro, August 15-20 2004

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

The Conference venue is Hotel Gloria Convention Center, a traditional five star hotel, located in the south zone of Rio, close to the city center and with a panoramic view over Guanabara Bay. The first announcement information is available at the homepage http://www.icy2004.com.br. Further information can be obtained by e-mail:
<congress@icy2004.com.br> or <leda@icy2004.com.br>.

The theme of the symposium will be “Yeasts in Science and

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

Leda Mendonça-Hagler, ICY2004 Chair

Brief News Items

Research Position - Microbial Physiologist / Fermentation / Yeast

Lallemand Inc., in Montréal, Québec, Canada is searching for an individual who will report to the Vice-President R&D/QA, initiate and carry forward yeast research programs based on yeast microbial physiology, and coordinate technology transfer to the plants. Requires a PhD with 3-5 years experience in yeast microbial physiology / fermentation OR other appropriate scientific experience (Food Engineering) with a significant record of achievement in this area. Knowledge of fermentation processes and bilingualism (French and English) a plus. Supervisory experience managing staff, student trainees, and post doctoral fellows is preferred.

Contact:

info@lallemand.com

Van Uden International Advanced Course on Molecular Ecology, Taxonomy and Identification of Yeasts CREM - Centro de Recursos Microbiológicos Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa FCT/UNL, Caparica, Portugal July 21 - August 1, 2003

An intensive two-week course for graduate students and researchers with a background in microbiology and with specific interests in the fields of yeast ecology, systematics and/or identification. Molecular techniques are revolutionizing the way microbes are detected, identified and classified. This course will focus on both theoretical and practical aspects of the molecular methods that are currently used for yeast detection, identification and classification, as well as on the informatic tools available for data analysis. Topics to be covered include the most recent developments in yeast classification and phylogeny, and modern approaches to the assessment of yeast populations and their habitats.

Lecturers. C. P. Kurtzman (NCAUR, Peoria/Illinois, USA); J. W. Fell (Univ. Miami, Key Biscayne/Florida, USA); M.-A. Lachance (Univ. Western Ontario, London/Ontario, Canada); T. Boekhout (CBS, Utrecht, The Netherlands); M. Weiss (Univ. Tübingen, Germany); A. Fonseca & J. P. Sampaio (CREM, Univ. Nova Lisboa, Portugal).

Course Organizers. I. Spencer-Martins, A. Fonseca, J.P. Sampaio (CREM, FCT/UNL).

Participants. The course is primarily of interest to graduate students and researchers in yeast systematics, yeast ecology, yeast science related to food and fermentation technologies, medical and veterinary mycology, culture collections and related fields. The number of participants will be limited to 24, to ensure an efficient interaction between students and lecturers.

Registration. Details for registration will appear on the course web site: http://www.crem.fct.unl.pt/yeastcourse. Applicants will be requested to complete a form and to send an abbreviated CV (max. 2 pages). The deadline for online registration will be March 28, 2003. Letters of acceptance will be mailed to all participants by e-mail before April 24, 2003.

Registration Fee. 750 EUR, includes accommodation at the venue, lunches from Monday to Friday, and the course documentation.

Course Secretariat:

CREM
FCT/UNL
Quinta da Torre, Monte de Caparica
2829-516 Caparica, Portugal

e-mail: ism@fct.unl.pt
Tel/Fax: +351 21 2948530

Publication of Interest

Yeasts of the World - Morphology, physiology, sequences and identification

by Boekhout, T., Robert, V., Smith, M.Th., Stalpers, J., Yarrow, D., Boer, P., Gijswijt, G., Kurtzman, C.P., Fell, J.W., Guého, E., Guillot, J., Roberts, I.

Version: 2.0. Platforms: Win CD only, Mac CD not planned. Retail Price (without VAT and postage) in EURO: 95.00; in STERLING £59.95. ISBN: Win: 90-75000-47-2.

This CD-ROM offers a multi-disciplinary approach to the identification of all known species of this fascinating group of micro-organisms and has been produced by leading experts in the field. It not only contains descriptions and photographs of the morphology, but also a complete set of physiological data as well as ribosomal DNA sequences. All these data can be used for an interactive identification of unknown yeast isolates. In addition there are hyperlinked introductory and explanatory texts, an illustrated glossary and a selection of important literature. Moreover, the system contains the identification software for ALLEV, at this moment the most flexible identification system based on automated reading of microtitre plates.

Order by e-mail from: <orders@eti.uva.nl>

Yeasts of the World CD ROM - A review

This is one of many CD-ROMs published in the World Biodiversity Database Series by the Biodiversity Center of ETI, Multimedia Interactive Software (UNESCO). The interactive database is the result of a collaboration involving recognized yeast taxonomists from nine institutions worldwide.

I examined the Windows Version 2.0. Installation of the program on my PC, which runs on Windows 2000 Professional, was straightforward. A 29 MB folder is added to the 'Program Files' folder of the main drive. Once installed, the program runs on its own, although it is necessary to insert the CD ROM to have access to photographs. Each time the program is started, the loading of all available databases takes about 40 sec with a moderately fast (1.3 GHz) processor.

The program is divided into six sections (introduction; glossary; species; type strains; literature; unidentified strains). Users familiar with the BioloMICS, the Web database maintained by the Centraalbureau voor Schimmelcultures, will feel at home. Unlike the Web version, the CD-ROM has species listings (based on several strains) and individual type strains, but not other strains. The database spans 761 species.

The introductory text and its hyperlinks to a glossary are excellent. I checked the glossary for how it deals with frequently misused terms and was delighted to see that the authors had agreed on correct, albeit succinct definitions.

A grand classification scheme is presented that appears very similar to the one used in the "Taxonomy" section of the NCBI database (GenBank). Some of the generic assignments are a bit strange. For example, one will always wonder why the genera *Kodamaea*, *Sporopachydermia*, *Starmerella*, and *Wickerhamiella* were placed in the Dipodascaceae.

For each species, the program provides links to morphology, physiology (CBS data), taxonomically useful gene sequences, photographs, and the literature. Many of the photos (light or EM) are spectacular (see for example *Metschnikowia continentalis*). I would purchase the disk for the photos alone. Although the images are also available on the Web (CBS Biolomics), the ease and speed of access are far better with the disk.

The extensive list of references is up-to-date to 2000 and could be invaluable for those wishing to look up the taxonomic literature. A very strong point of the program is the ease with which one can move across various sections of the database. Unfortunately, this does not include the ability to search species entries for all specific literature citations (only the reverse is possible). The weaker point may be the cumbersome nature of data entry for new species or for identification, and the possibility that the microtitre plate method does not always give results that are compatible with methods using liquid media or replica plates. Having said this, casual taxonomists in search for a method of storing yeast strain information will find the relevant section of the program useful.

One also notes, as in the vast majority of similar publications, a very minimal treatment of ecological relevant aspects of yeast biology.

To what audience is *Yeasts of the World* addressed? Clearly it is not meant to be a substitute for the standard reference, "The Yeasts, a Taxonomic Study" (Kurtzman and Fell, eds., 1998). The non-specialist will certainly benefit from the general concepts and enjoy the photographs, but will probably not find it sufficient as an introductory text or a stand-alone identification tool. Laboratories involved strictly in identification are rapidly moving away from the so-called conventional method based on microscopy and growth tests and towards the more efficient and accurate DNA sequence comparison approach. Although DNA sequences are present on the disk, the Web databases (*e.g.*, GenBank) benefit from being updated almost daily and does not restrict the search to any particular gene or group of organisms. Of course, on-line searches can be very slow, and the BLASTn algorithm has its idiosyncrasies.

I expect that all yeast professionals will enjoy the disk for its easily accessible, high quality photographs, the species lists, and the references. I cannot see anyone with a serious interest in yeast taxonomy not benefitting from *Yeasts of the World*. It is a compact, high quality, and affordable alternative to Barnett, Yarrow, and Payne's tome.

M.A. Lachance