

Y E A S T

A Newsletter for Persons Interested in Yeast

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Editorials

Change in the Editorial Board

After 15 years, Dr. Tadashi Hirano has decided to step down as Associate Editor of the Yeast Newsletter. Dr. Hirano served in that capacity since 1983, enabling our Japanese subscribers to pay their subscription in Japanese currency, with considerable convenience for both the subscribers and the publication staff. I thank him warmly for his dedication. I am pleased to welcome Dr. Yasuji Oshima, of Kansai University, Osaka, who has agreed to join the Editorial Board and continue to assist in the collection of subscription dues in Japan.

The Yeasts, a Taxonomic Study, Fourth Edition

The long awaited publication of the fourth edition of the taxonomic treatise, THE YEASTS, A TAXONOMIC STUDY is now reality. The Editors and the Publisher are to be congratulated for having put together such an attractive volume. Introductory chapters give an broad overview of the state of the field. The new phylogenetic organization of anamorphic genera conveys an insightful image of the yeasts as they are now understood. An ecological approach is also espoused, with the inclusion of a chapter on *Prototheca*, which, although it is not a fungus, often is a member of natural yeast communities and shares similar niches. The treatment of many basidiomycetous taxa not included previously is no doubt an offshoot of the Amersfoort meeting which set the first steps to an enlarged view of the yeast concept, and the gradual disappearance of the term "yeastlike organisms". A series of general keys and tables of yeast properties are also a welcome feature. Although THE YEASTS is over a thousand pages long, the Publisher managed to select a paper that combines lightness and opacity, maintaining the size of the treatise within reasonable limits without sacrificing legibility. Readers who have not yet purchased their own copy of THE YEASTS are invited to read the information at the end of this issue of the Yeast Newsletter.

ISSY-19, Braga, Portugal

Dr. Cecília Leão, Chair of the organizing committee of the forthcoming International Specialized Symposium on Yeasts, informs us that the last preparations are on schedule. To be held from August 30 to September 3, 1998, the 19th ISSY sponsored by the International Commission on Yeasts will deal with all aspects of yeast in the production and spoilage of foods and beverages. The venue is the campus of the University of Minho, in Braga, Portugal. Interested readers should be aware that late registrations will be accepted right till the start of the symposium. A summary of the second announcement appears in this issue of the Yeast Newsletter. The preliminary program permits us to anticipate an excellent meeting.

M.A. Lachance
Editor

I. All-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>.

The following are our recent publications.

1. Golubev, W., Ikeda, R., Shinoda T., and Nakase, T. 1997. Antifungal activity of *Bullera alba* (Hanna) Derx. *Mycoscience* **38**:25-29.

A strain of *B. alba* that secretes a killer toxin inhibitory (at pH from 3 to 7) to many ascomycetous and basidiomycetous yeast-like fungi was discovered. Its killer phenotype was incurable. The toxin was relatively thermostable and resistant to

proteases, and it was specified as a microcin. It inhibited the growth of some pathogenic yeasts and was the most active against *Cryptococcus neoformans*.

2. Golubev, W.I. and Churkina, L.G. 1997. Sensitivity to mycocins and morphological and physiological characteristics of authentic strains of *Rhodotorula mucilaginosa* synonyms. *Mikrobiologiya* **66**:254-261.

Rh. mucilaginosa strains differ in their sensitivity to mycocins produced by species of *Bensingtonia*, *Rhodotorula*, *Sporidiobolus* and *Sporobolomyces*. In some cases their sensitivity patterns correlate with morphological and physiological properties. Cluster

analysis using these characteristics revealed that authentic strains of species (the name of which were reduced to synonyms of *Rh. mucilaginosa*) form separate groups.

3. Golubev, W., Okunev, O., and Golubev, V. 1997. Biocontrol of postharvest rots of apple with *Cryptococcus humicola*. Abstr. 18th Int. spec. Symp. on Yeasts "Yeast Nutrition and Habitats" (August 24-29, 1997, Bled, Slovenia), p. 8-02.

Killer strains of *Cr. humicola* inhibited conidiospore germination in *Botrytis cinerea* and *Penicillium expansum*. The

treatment of fruits with cell suspensions of *Cr. humicola* resulted in two-fold reduction of rot lesion size.

4. Dmitriev, V.V., Gilichinskii, D.A., Faizutdinova, R.N., Shershunov, I.N., Golubev, W.I., and Duda, V.I. 1997. Occurrence of viable yeasts in 3-million-year-old permafrost in Siberia. *Mikrobiologiya* **66**:655-660.

Total yeast counts were about 9000 CFU/g in some layers of permafrost rocks. The yeasts isolated were identified as *Cryptococcus albidus*, *Sporobolomyces roseus* and *Rhodotorula*

spp. The strains of the latter genus are similar to *Rh. mucilaginosa* and *Rh. muscorum* but differ from them in some diagnostic characteristics.

5. Dmitriev, V.V., Gilichinsky, D.A., Faizutdinova, R.N., Ostroumova, N.V., Golubev, W.I., Duda, V.I. 1997. Yeasts in late Pliocene-early Pleistocene Siberian permafrost. *Cryosphere of the Earth* **1**:67-70.

Yeast isolates from permafrost were identified as *Cryptococcus albidus*, *Sporobolomyces roseus* and *Rhodotorula* spp. most similar

phenotypically to *Rh. mucilaginosa* and *Rh. muscorum*.

6. Golubev, W.I. 1998. Mycocins (Killer toxins). In: *The Yeasts. A Taxonomic Study* (Kurtzman, C.P., Fell, J.W., eds.). Elsevier Sci. B.V., Amsterdam, pp. 55-62.

Contents: 1. Introduction. 2. Characteristics of mycocins. 3. Taxonomic implications of sensitivity to mycocins.

4. Taxonomic implications among the ascomycetous yeasts. 5. Taxonomic implications among the basidiomycetous yeasts.

7. Golubev, W.I. 1998. *Xanthophyllomyces* Golubev. In: *The Yeasts. A Taxonomic Study*. (Kurtzman, C.P., Fell, J.W., eds.). Elsevier Sci. B.V., Amsterdam, pp. 718-719.

II. G. L. Hennebert, 32 Rue de l'Élevage, B-1340 Ottignies-LLN, Belgium <henneber@pops1.agro.ucl.ac.be>.

The following are summaries of two recently submitted papers.

1. Gouliamova, D.E. & G.L. Hennebert. 1998. Phylogenetic relationships in *Saccharomyces cerevisiae* complex species. *Mycotaxon* **66** (In press).

Phylogenetic relationships in *Saccharomyces cerevisiae sensu lato* between the neotype strain *S. cerevisiae sensu stricto* CBS 1171 and the type or neotype strains *S. pastorianus* CBS 1538, *S. bayanus* CBS 380, *S. paradoxus* CBS 432, *S. chevalieri* CBS 400, *S. uvarum* CBS 395, *S. carlsbergensis* CBS 1513 and *S. ellipsoideus* CBS 1395 were inferred from the nucleotide sequences of domain D2 of 25S rDNA (572 nucleotides) and of region ITS1-5.8SrDNA-ITS2 (510 nucleotides). Two methods were applied: the maximum parsimony (or minimum evolution) method and the Kimura's distance matrix (or number of nucleotide substitutions) method, before the construction of the phylogenetic trees with bootstrap using Felsenstein's Phylip 1993. Results show that D2 region of 25S rDNA with a maximum of 14 substitutions between type strains is far less discriminatory than

ITS1-5.8SrDNA-ITS2 with up to 60 substitutions, in order to segregate the components of the *S. cerevisiae* complex. The phylogenetic relationship inferred from analyses of ITS1-5.8S rDNA-ITS2 sequences also correlate better than analyses of D2 region with the results in DNA-DNA reassociation. Synonymy of *S. cerevisiae s.s.*, *S. ellipsoideus* and *S. chevalieri* in one cluster and of *S. pastorianus* and *S. carlsbergensis* in another cluster is confirmed. *S. paradoxus*, *S. bayanus* and *S. uvarum* segregate clearly as distinct species. Sequencing the internal transcribed spacers region appears to be a valid tool for a further study of the remaining 51 type strains available of the reputed synonyms of *Saccharomyces cerevisiae* in order to elucidate that complex taxonomy.

2. Gouliamova, D.E, G.L. Hennebert, M.T. Smith & J.P. van der Walt. Diversity and affinities among species and strains of *Lipomyces*. Antonie van Leeuwenhoek (submitted April 1998).

Phylogenetic relationships of the yeast genus *Lipomyces* were studied using sequences of fragments of 5.8S rDNA gene and of ITS2 region (total 300 nucleotides) of 13 lipomycetous strains including 7 type strains of described species (*Lipomyces starkeyi*, *L. kononenkoae*, *L. mesembrius*, *L. tetrasporus*, *L. spencer-martinsiae*, *Smithiozyma japonica* and *Waltomyces lipofer*) and undescribed taxa, and originating from distant geographical locations (Japan, Mauritius, Nigeria, North America, Russia, South Africa,

Trinidad, Western Europe). Parsimony and distance analyses were performed and the deducted tree topology confirmed the results of nDNA reassociation. The analyses segregate the 13 isolates into five major clades of which those of *Smithiozyma japonica* and *Waltomyces lipofer* are the most remote. Results confirm that analyses of ITS sequences corroborate better nDNA complementarity data and are superior to those of the 18S rDNA for estimating relationships between closely related species or species clusters.

III. Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604. Communicated by C.P. Kurtzman.

Recent publications.

1. Dien, B.S., C.P. Kurtzman, B.C. Saha, and R.J. Bothast. 1996. Screening for L-arabinose fermenting yeasts. *Appl. Biochem. Biotechnol.* **57/58**:233-242.

Utilization of pentose sugars (D-xylose and L-arabinose) derived from hemicellulose is essential for the economic conversion of biomass to ethanol. Xylose fermenting yeasts were discovered in the 1980's, but to date, no yeasts have been found that ferment L-arabinose to ethanol in significant quantities. We have screened 116 different yeasts for the ability to ferment L-arabinose and have

found the following species able to ferment the sugar: *Candida aurangiensis*, *Candida succiphila*, *Ambrosiozyma monospora*, and *Candida* sp. (YB-2248). Though these yeasts produced ethanol concentrations of 4.1 g/L or less, they are potential candidates for mutational enhancement of L-arabinose fermentation. These yeasts were also found to ferment D-xylose.

2. Kurtzman, C.P. . 1996. Transfer of *Hansenula ofunaensis* to the genus *Pichia*. *Mycotaxon* **59**:85-88.

Hansenula represents a synonym of the genus *Pichia*, and because of this, *Hansenula ofunaensis* is transferred to *Pichia*. An

emended physiological characterization of this yeast species is provided.

3. Kurtzman, C.P. and C.J. Robnett. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26s) ribosomal DNA gene. *J. Clin. Microbiol.* **35**:1216-1223.

Clinically important species of *Candida* and related taxa were compared from extent of nucleotide divergence in the 5' end of the large subunit (26S) ribosomal DNA gene. This rDNA region is sufficiently variable to allow reliable separation of all known

clinically significant species. Of the 204 species examined, 22 were shown to be synonyms of earlier described taxa. Phylogenetic relationships among the species are presented.

IV. Department of Environmental Biology, University of Guelph, Room 3218, Bovey Building, Guelph, Ontario Canada N1G 2W1. Communicated by H. Lee <hlee@uoguelph.ca>.

The following is the abstract of a paper published recently.

1. Kostrzynska, M., C.R. Sopher, and H. Lee. 1998. Mutational analysis of the role of the conserved lysine-270 in the *Pichia stipitis* xylose reductase. FEMS Microbiol. Lett. **159**:107-112.

Xylose reductase catalyzes the NAD(P)H-dependent reduction of xylose to xylitol and is essential for growth on xylose by yeasts. To understand the nature of coenzyme binding to the *Pichia stipitis* xylose reductase, we investigated the role of the strictly conserved Lys270 in the putative IPKS coenzyme binding motif by site-directed mutagenesis. The Lys270Met variant exhibited lower enzyme activity than the wild-type enzyme. The apparent affinity of the variant for NADPH was decreased by 5- to 16-fold, depending on the substrate used, while the apparent affinity for NADH, measured using glyceraldehyde as the substrate, remained

unchanged. This resulted in 4.3-fold higher affinity for NADH over NADPH using glyceraldehyde as the substrate. The variant also showed 14-fold decrease in K_m for xylose, but only small changes were observed in K_m values for glyceraldehyde. The wild-type enzyme, but not the Lys270Met variant, was susceptible to modification by the Lys-specific pyridoxal 5'-phosphate (PLP). Results of our chemical modification and site-directed mutagenesis study indicated that Lys270 is involved in both NADPH and D-xylose binding in the *P. stipitis* xylose reductase.

V. Laboratorium voor Microbiologie, Wageningen Agricultural University, P.O. Box 8033, 6700EJ Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@algemeen.micr.wau.nl>.

The following paper appeared recently.

1. Middelhoven, W.J. 1998. The yeast flora of maize silage. Food Technol. Biotechnol. **36**:7-11.

A literature review of yeast species prevailing in various silages is given. The yeast flora of maize silage is dominated by *Candida lambica* (*Issatchenkia orientalis*), *C. milleri*, *Saccharomyces*

exiguus (*Candida holmii*) and *Sacch. dairensis*. Particular attention is paid to the role of these species in the aerobic deterioration of maize silage, and to ways of preventing aerobic spoilage of silage.

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

Recent publications or those which are due to be published.

1. E. Minárik. 1998. Should wines with low acidity undergo biological degradation? (in Slovak). Vinohrad **36**(3): in press.

Bacterial malic acid decomposition by spontaneous or controlled malolactic fermentation in wine of northern vine growing regions is an important procedure by which not only malic acid is eliminated but risks of possible unfavourable microbiological

changes may be decreased. It is underlined to leave the wine on yeast sediment in the course of malolactic fermentation to support malolactic bacteria. The wine should not contain more than 3-4 g/l reducing sugars to assure full biological stability.

2. Jungová, O. 1998. Autochthonous microflora of viticultural regions of Slovakia (in Slovak). Vinohrad **36**(1):15-16.

Positive influence of autochthonous yeast strains used for pure wine yeast preparations in grape must fermentations could be proved in vintages 1996 and 1997. Very good fermentation ability and lower volatile acid formation was registered in comparison

to yeast strains originating from other vine growing regions. Profound selection of autochthonous yeast strains may contribute to increased wine stability and quality.

3. E. Minárik. 1998. By-products of bacterial acid decomposition in wine (in Slovak). Vinohrad **36**(1):17-18.

Yeast and malolactic bacteria produce by-products during alcoholic and malolactic fermentations in wines. The pathways of acetoin and 2,3-butandiol are discussed. It is suggested that diacetyl, a volatile diketone, is possibly responsible for buttery off-flavour and taste in wine. Diacetyl formation in wine depends on

the yeast strain, on malolactic fermentation and citric acid concentration of the wine, and, last not least, on the starter strain of *Leuconastoc oenos*. Practical aspects of wine quality are discussed.

4. E. Minárik. 1998. Influence of bentonite preparations for coupage wines intended for sparkling wine production (in Slovak). *Vinohrad* **36**(2):34-35.

In primary grape must fermentations which will be used for coupage wine, the amounts of nutrients for wine yeasts, especially nitrogen compounds, are decreased. In order to provide protein stability of the wine only a reduced bentonite addition is proposed to avoid eliminating all thermolabile proteins. Bentonite will be

also used in the coupage wine to provide good settling and removal of the yeast deposit after finished secondary fermentation. Thiamine and diammonium phosphate addition prior to secondary fermentation may be useful for sensory quality of the sparkling wine.

5. Hronský, V. Z. Dömöény, & F. Malík. 1998. Volatile wine constituents formation depending in fermentation conditions (in Slovak). *Vinohrad* **36**(2):38-40.

Volatile profile formation in young wine samples after fermentation had finished, wines which fermented by spontaneous microflora and pure wine yeast starters, had been investigated. Results of experiments showed optimum temperature for higher alcohol formation by spontaneous yeast flora at 21 °C and at 24 °C by active dry wine yeast starters. The dominant ester portion is

represented by ethylacetate, 2-methylpropylacetate and ethylhexanoate. Higher 2-methylpropylacetate contents occur only in wines ferments by the spontaneous microflora. Valeric acid showed the highest concentration of organic and fatty acids. Isobutyric acid was found in wines fermented at 6 °C by active dry wine yeasts.

VII. National Institute of Bioscience and Human Technology AIST, Higashi 1-1 Tsukuba, Ibaraki 305 Japan. Communicated by S. Nwaka <nwaka@nibh.go.jp>.

The following are recent publications of our institute.

1. Fujita, K., Iwahashi, H., Kodama, O., and Komatsu, Y. 1995. Induction of heat-shock proteins and accumulation of trehalose by TPN in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Comm.* **216**:1041-1047.

TPN (Tetrachloroisophthalonitrile), a kind of disinfectant, affected growth in *Saccharomyces cerevisiae*. Exposed to TPN under sublethal conditions, 70-, 90-kDa protein and hsp104 were induced. Each of them was not uniformly induced; namely, the 70-kDa protein was more sensitive to TPN among other proteins. Trehalose was also accumulated depending on the concentration.

The degree of thermotolerance in yeast cells pretreated with 1.0 mg/l TPN was about 100-fold greater than that in the untreated control cells. Under this condition, TPN-inducible proteins were synthesized but trehalose was not accumulated. TPN-inducible proteins and trehalose were significantly synthesized with 10.0 or 100.0mg/l TPN treatment; however, thermotolerance was not acquired.

2. Fujita, K., Iwahashi, H., Kodama, O., and Komatsu, Y. 1996. Induction of heat-shock proteins in the presence of thiuram in *Saccharomyces cerevisiae* for evaluating the potential toxic risks in pollutants. *Water Res.* **30**:2102-2106.

Thiuram [Bis (dimethyldithiocarbamoyl) disulfide], a kind of disinfectant, affected growth and induced lethality as a chemical stressor in *Saccharomyces cerevisiae*. Examining the protein profiles by SDS-PAGE and Western-blot analysis, yeast cells which were incubated with thiuram for 90 min at 30 °C induced a number of proteins including hsp104, hsp90 and hsp70 according to the concentrations. Using the *hsp104* null mutant and its parent strain,

thermotolerance with the prior 10 mg/l thiuram treatment in the parent strain was greater than that in the untreated control at 20 min, but that in the *hsp104* strain was almost same as the untreated control over the long term. Trehalose was not accumulated by thiuram. These follow that hsp104, which is typically induced by moderate heat-shock treatment, is mostly induced in the presence of thiuram also.

3. Komatsu, Y., Kodama, O., and Fujita, K. 1996. Heat-shock treatment reduces in situ temperature in yeast at sub-lethal high temperature *Cell. Mol. Biol.* **42**: 839-845.

Cells from the yeast *Schizosaccharomyces pombe*, once heat-shocked and then treated with ultra-centrifugation showed a significant increase in tolerance compared to non heat-shocked control cells as estimated by colony forming unit (CFU). Fluorescence microscopical observation of these treated cells when stained with DAPI revealed that in non heat-shocked cells the

chromatin regions were dislocated to one end due to the acceleration force of gravity. However, prior heat-shocked cells or 2.0 M glycerol suspended cells showed normal localization. From these results, it has been postulated that the interior part of the heat-shocked cells becomes viscous, and that in situ the temperature of the cell interior part might be reduced under sublethal high temperature.

4. Fujita, K., Iwahashi, R., Kawai, and Komatsu, Y. Hsp104 expression and morphological changes associated with disinfectants in *Saccharomyces cerevisiae*: environmental bioassay using stress response. Water Sci. Tech. In press.

The present study is concerned examining with the induction of a lacZ gene under Hsp104 protein element (HSP104-lacZ) control in the presence of some disinfectants for proposing a novel method of evaluating the toxic assessment of pesticides. On exposure of *Saccharomyces cerevisiae* to Thiuram [Bis (dimethyl-dithio-carbamoyl) disulfide], TPN [Tetrachloroisophthalonitrile], Captan [N-(trichloromethyl-thio)cyclohex-4-ene-1,2-dicarboximide], and Oxine-copper [Bis(quinolin-8-olato-O, N,) copper] under sublethal

conditions; HSP104-lacZ was sensitively expressed by detecting the relative β -galactosidase activity in the same way of that in heat-shock treatment. It follows that not only measuring the growth phase or the induction of synthesized proteins, but also detecting the level of gene expression shows these chemical stress response in *Saccharomyces cerevisiae*. Thus, this method is useful for evaluating the toxicity assessment of pesticides rapidly and conveniently.

VIII. Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Avenida Belgrano y Pasaje Caseros, 4000 S.M. de Tucuman, Argentina. Communicated by J.F.T. Spencer.

We are just beginning a new project for continuous production of galactosyl lactose from lactose by the basidiomycetous yeast species, *Bullera singularis*. When the yeast is grown on lactose, the glucose moiety is metabolized and the galactose moiety is transferred to another molecule of lactose. Another galactose residue may be

added to the trisaccharide under some conditions. Galactosyl lactose may be added to commercial infant formulas to promote the growth of bifidobacteria in the intestinal tract, which changes the microflora in the direction of that of breast-fed infants. There may be similar uses in the treatment of intestinal upsets in adults as well.

IX. Department of Food Science and Technology, University of California, Davis, CA 95616, USA. Communicated by H.J. Phaff <hjphaff@ucdavis.edu>.

The following paper is in press.

1. Phaff, H.J., A Vaughan-Martini,¹ & W.T. Starmer.² 1998. *Debaryomyces prosopidis* sp. nov., a yeast occurring in exudates of Mesquite trees. Int. J. Syst. Bacteriol.

¹Dipartimento di Biologia Vegetale, Università degli Studi, Perugia, Italy 06121.

²Department of Biology, Syracuse University, Syracuse, New York 13244.

Nine strains were studied of a new haploid species of the genus *Debaryomyces* Lodder & Kreger-van Rii that had been isolated from exudates of mesquite (*Prosopis juliflora*) trees in southern Arizona and from *Drosophila carbonaria* that breeds in these exudates (Ganter et al., 1986). Their physiological characteristics, life cycle, and nuclear DNA base composition (approximately 37.5 mol% G+C) led to their original classification as *Debaryomyces hansenii* (Zopf) Kreger-van Rii (Ganter et al. 1986). However, the two taxa are at most distantly related based on DNA reassociation

values that indicated low base sequence complementarity. The two taxa also have distinctly different electrophoretic karyotypes. *Debaryomyces hansenii* has two varieties, *D. hansenii* var. *hansenii* and *D. hansenii* var. *fabryi*. *Debaryomyces prosopidis* can be differentiated phenotypically from both varieties by lack of growth on cellobiose after two weeks of incubation and from the variety *hansenii* by a higher maximum temperature for growth. The type strain of *Debaryomyces prosopidis* sp. nov. is strain UCD-FST 84-100 = D8VPG 7010 = CBS 8450 = ATCC 201611.

X. Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan. Communicated by Y. Yamada.

The following paper has appeared recently.

1. Yamada, Y. T. Higashi, S. Ando, and K. Mikata. 1997. The Phylogeny of strains of species of the genus *Pichia* Hansen (Saccharomycetaceae) based on the partial sequences of 18S ribosomal RNA: the proposals of *Phaffomyces* and *Starmera*, the new genera. Bull. Fac. Agric. Shizuoka Univ. 47:23-35.

Sixteen strains of *Pichia* and *Saturnispora* species were examined for their partial base sequences in positions 1451 through 1618, 168 bases, of 18S rRNA. Of the sixteen strains of *Pichia* *P. membranaefaciens*, the type species of the genus *Pichia*. The calculated number of base differences was 3-1. On the other hand, the strains of the six species, *P. bovis*, *P. euphorbiae*, *P. onychis*, *P. pijperii*, *P. quercuum*, and *P. triangularis* were closely related to the type strain of *P. anomala* (*H. anomala*, type species of genus

and *Saturnispora* species examined, the strains of the four species, *P. cactophila*, *P. fermentans*, *P. nakasei*, and *P. norvegensis* were closely related phylogenetically to the type strain of *Hansenula*). The calculated number of base differences was 4-2. The strains of the only two species, *P. angophorae* and *P. salictaria* represented 13-5 base differences with the type strains of *P. membranaefaciens* and *P. anomala*. The type strain of *Saturnispora saitoi* had eight base differences, when compared

with that of *P. membranaefaciens*. The type strains of *P. amethionina*, *P. opuntiae*, and *P. thermotolerans*, viz., the cactus yeast species, were phylogenetically quite distant: the base differences were 23-14 in *P. amethionina*, 31-28 in *P. opuntiae*, and 32-27 in *P. thermotolerans*. The type strain of *P. amethionina* had 40-39 base differences with those of *P. opuntiae* and *P.*

thermotolerans. Between the type strains of *P. opuntiae* and *P. thermotolerans*, there were six base differences. Based on the sequence data obtained, the new genera *Phaffomyces* and *Starmera* were proposed for *P. opuntiae* and *P. thermotolerans* as well as *P. amethionina*, respectively.

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

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

1. Santos, E. A., de Oliveira, R. B., L. C. Mendonça-Hagler, and A. N. Hagler. 1996. Yeasts associated with cashew, caju, umbu, and mango fruits typical of the semiarid region of northeastern Brazil. *Rev. Microbiol.* **27**: 33-40.
2. Valente, P., G. A. Lemos, F. C. Gouveia, D. Pimentel, D. van Elsas, L. C. Mendonça-Hagler, A. N. Hagler. 1996. PCR amplification of the ITS region for differentiation of *Saccharomyces* cultures. *FEMS Microbiology Letters* **137**:253-256.
3. Morais, P. B., C. A. Rosa, J. Abranches, L. C. Mendonça-Hagler & A. N. Hagler. 1996. Yeasts vectored by *Drosophila quadrum* (calloptera group) in tropical rain forests. *Rev. Microbiol.* **27**:87-91.
4. Abranches, J., P. B. Morais, C. A. Rosa, L. C. Mendonça-Hagler, and A. N. Hagler. 1997. The incidence of killer activity and extracellular proteases in tropical yeast communities. *Can. J. Microbiol.* **43**:328-336.
5. Valente, P., F. C. Gouveia, G. A. Lemos, D. Pimentel, D. van Elsas, L. C. Mendonça-Hagler, A. N. Hagler. 1997. ITS length as a molecular tool for identification of the genus *Metschnikowia*. *J. Gen. Appl. Microbiol.* **43**: 179-181.
6. Hagler, A. N., L. C. Mendonca-Hagler and J. B. Silva. 1997. Ascomycetous yeast communities in coastal forest ecosystems of southeast Brazil. *Progress in Microbial Ecology.* pp. 189-195.
7. Mendonça-Hagler, L. C., A. N. Hagler, C. A. G. Soares, F. V. de Araujo, & P. R. Peres Neto. 1997. Yeasts as a model of microbial diversity in coastal marine habitats in Rio de Janeiro, Brazil. *Progress in Microbial Ecology.* pp. 237-244.
8. Soares, C. A., M. Maury, F. Pagnocca, F. A. Araujo, A. N. Hagler, and L. C. Mendonça-Hagler. 1997. Yeast communities in dark muddy intertidal estuarine sediments of Rio de Janeiro, Brazil. *J. Gen. Appl. Microbiol.* **43**:265-272.
9. Araujo, F. V., R. J. Madeiros, L. C. Mendonça-Hagler, & A. N. Hagler. 1998. A preliminary note on yeast communities of bromeliad-tank waters of Rio de Janeiro, Brazil. *Rev. Microbiologia.* In Press.
10. Abranches, J., H. N. Nóbrega, P. Valente, L. C. Mendonça, & A. N. Hagler. 1998. A Preliminary note on yeasts associated with rodents and marsupials of atlantic forest fragments in Rio de Janeiro, Brazil. *Rev. Microbiologia.* In Press.
11. Abranches, J., P. Valente, H. N. Nóbrega, F. A. S. Fernandez, L. C. Mendonça-Hagler, and A. N. Hagler. 1998. Yeast diversity and killer activity dispersed by fecal pellets from marsupials and rodents in a southeast Brazilian tropical habitat mosaic. *FEMS Microbiol. Ecol.*: In Press.

The following graduate theses were recently defended.

12. Glauber Lemos - M.Sc. 1996. Hibridização nDNA/nDNA de Espécies de Leveduras do Gênero *Saccharomyces*. Biotecnologia Vegetal, Univ. fed. Rio de Janeiro.
13. Douglas de Souza Pimentel - M.Sc. 1996. Caracterização de Comunidades de Leveduras Isoladas em Habitats Tropicais Aplicando o Tamanho da Região ITS do rDNA. PPG Ecologia, Univ. fed. Rio de Janeiro.
14. Luciana A. I. de Azeredo. M. Sc. 1996. Comunidade de Leveduras Associadas á Cana-de-açúcar (*Saccharum officinarum* Linneau.) na Região de Campos RJ. Instituto de Microbiologia Prof. Paulo de Goes, Univ. fed. Rio de Janeiro.
15. Regina A. Menez M. Sc. 1997. Mapeamento físico do rDNA em leveduras do gênero *Metschnikowia*. PPG Genética, Inst. Biol. Univ. fed. Rio de Janeiro. (co-orientação com Dr. Orilio Leoneini, Dept. Genética, UFRJ).
16. Fabio Castro Gouveia M. Sc. 1998. Estudo sobre a região espaçadora interna transcrita de rDNA em leveduras dos gêneros *Saccharomyces* e *Metschnikowia*. Inst. Microbiol. Prof. Paulo de Goes, Univ. fed. Rio de Janeiro.

XII. Department of Microbiology, Attila József University, P.O. Box 533, H-6701 Szeged, Hungary. Communicated by J. Kucsera <kucsera@sol.cc.u-szeged.hu>.

List of recent publications:

1. Pfeiffer, I., Kucsera, J., Varga, J., Parducz A., and Ferenczy, L. 1996. Variability and inheritance of double-stranded RNA viruses in *Phaffia rhodozyma*. *Curr. Genet.* **30**: 294-297.
2. Kucsera, J., Gacser, A. and Pfeiffer, I. 1997. Comparison of killer pheno- and genotype in the genus *Saccharomyces*. 18th ISSY, Yeast Nutrition and Natural Habitats, Bled, Slovenia, Abstract Book, p. 8-19.
3. Pfeiffer, I., Kucsera, J. and Ferenczy, L. 1997. dsRNA viruses and their effect on the fitness of the host in *Phaffia rhodozyma* 18th ISSY, Yeast Nutrition and Natural Habitats, Bled, Slovenia, Abstract Book, p. 8-20.
4. Gyantar M., Pfeiffer, I. and Kucsera, J. 1997. Isolation of dsRNA associated virus-like particles from *Cryptococcus hungaricus* CBS 6569 strain. *Acta Microbiol. Hung.* **44**:427-428.
5. Kucsera, J., Gacser, A. and Pfeiffer, I. 1997. Comparative analysis of killer properties of *Saccharomyces dairensis* and *Saccharomyces cerevisiae*. *Acta Microbiol. Hung.* **44**:418-419.
6. Lukacsi, A., Pfeiffer, I. and Kucsera, J. 1997. Comparative study of *Saccharomyces dairensis* and *Saccharomyces castellii* strains. *Acta Microbiol. Hung.* **44**:427.
7. Kucsera, J., Pfeiffer, I. and Ferenczy, L. 1998. Homothallic life cycle in the diploid red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). *Antonie van Leeuwenhoek* **73**:163-168.

XIII. National Collection of Yeast Cultures, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK. Communicated by I.N. Roberts <ian.roberts@bbsrc.ac.uk>.

NCYC is now co-ordinated with other UK microbial resource collections through the UK National Culture Collection (UKNCC). A central web site with comprehensive strain data, quality assurance and research information will be available shortly. Distribution of cultures and associated collection services are handled by ReNo

Ltd, a wholly-owned subsidiary of the Institute of Food Research. It is very much hoped that these developments will enhance the services that NCYC has provided since its foundation in 1946. More information is available at <http://www.ifrn.bbsrc.ac.uk/ncyc/>.

Recent publications:

1. James, S.A., Cai, J., Roberts, I.N., & Collins, M.D. 1997. A Phylogenetic Analysis of the genus *Saccharomyces* based on 18S rRNA gene sequences: description of *Saccharomyces kunashirensis* sp. nov. and *Saccharomyces martiniae* sp. nov. *Int. J. Syst. Bacteriol.* **47**:453-460.

2. James, S.A., Roberts, I.N., & Collins, M.D. 1998. A Phylogenetic analysis of the Genus *Williopsis* based on 18S rRNA gene sequences. *Int. J. Syst. Bacteriol.* **48**:591-596.
3. Roberts, I.N. 1998. Yeasts in natural and artificial habitats. Spencer and Spencer(Eds). Book Review. *Trends in Microbiology* **6**(3).
4. James, S.A., Roberts, I.N., Collins, M.D. 1998. A phylogenetic analysis of the genus *Arxiozyma* based on 18S rRNA gene sequences. *Int. J. Syst. Bacteriol.* (In Press).

XIV. Division of Industrial Microbiology, Department of Food technology and Nutritional Sciences, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Communicated by J.C. Verdoes <Jan.Verdoes@algemeen .im.wau.nl>.

Successful defense of a doctoral dissertation.

1. J. Wery. 1997. Genetics of the yeast *Phaffia rhodozyma*. Ph.D. dissertation. Wageningen Agricultural University.

The aim of this thesis is to study the genetics of *Phaffia* and to develop a genetic transformation system for this yeast. The genetic properties of *Phaffia* were studied on the gene and genome level. As a first step the molecular structure of the *Phaffia* actin gene was analyzed. Actin genes are highly conserved throughout nature, and as such they have been used for the classification of significantly diverging eukaryotic groups, like (in-) vertebrates, plants and fungi. We anticipated that the analysis of the primary structure of *Phaffia* actin gene and comparison with the actin genes from fungi, including 2 ascomycetous filamentous fungi, 2 basidiomycetous yeasts and 5 ascomycetous yeasts would provide further phylogenetic information on this yeast. It was found that the *Phaffia* actin gene encoded a protein consisting of 375 amino acids. In addition 4 (non-coding) intervening sequences were present. Comparison of both the coding DNA sequence and its predicted protein product with their fungal counterparts, revealed that least homology was found with the ascomycetous yeasts, like *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. It was also shown, that based on these comparisons *Phaffia* is closer related to the filamentous ascomycetous fungi *Thermomyces lanuginosus* and *Aspergillus nidulans*, whereas most homology was found with the basidiomycetous yeast *Filobasidiella neoformans* (perfect stage of *Cryptococcus neoformans*).

In addition to the phylogenetic analysis of the actin exons, the architecture of the introns (splice site consensus sequences, size, position in the gene) was compared. It was shown that the *Phaffia* introns most resembled that of *Filobasidiella neoformans*, whereas least resemblance occurred with the ascomycetous yeasts. This result was in agreement with the actin exon homology studies. Furthermore, the presence of multiple introns in the *Phaffia* actin gene resembled the situation in the actin genes from *F. neoformans* and the filamentous fungi, whereas the ascomycetous yeasts only carry one intron in their actin genes. Similar results were obtained by (phylo-) genetic analysis of the five introns containing *Phaffia* glyceraldehyde-3-phosphate dehydrogenase encoding gene (*gpd*). The genomic organization of the multiple rDNA genes in *Phaffia*

was elucidated. It was found that *Phaffia* carries the ribosomal DNA (rDNA) genes in three clusters, of 12, 14 and 35 copies, on three different chromosomes. This supports the trend that in basidiomycetes the rDNA genes are distributed over different chromosomes, whereas in the ascomycetous yeasts and fungi the rDNA is mainly present on one chromosome. The significant differences on the gene and genome level with the ascomycetous yeasts affected the strategy for the development of a transformation system for *Phaffia*. Whereas several marker gene sequences or sequences for plasmid replication and maintenance can be readily interchanged between most ascomycetous species as a result of high homologies, it was shown that this was not the case for *Phaffia*. Therefore an almost entirely homologous transformation system was developed using plasmids carrying the dominant G418 resistance gene (Km^R), driven by either the *Phaffia* actin or the *gpd* promoter and a *Phaffia* rDNA fragment for homologous integration. It was found that the rDNA clusters could serve as a target for high copy number integration. This integrative transformation system was used to determine the ploidy of *Phaffia*, strain CBS 6938, by monitoring chromosomal shifts as a result of multiple integrations. It was found that this strain was haploid.

Plasmids carrying the *gpd* promoter driven Km^R gene transformed *Phaffia* with significant higher efficiencies than constructs with the actin promoter. Furthermore, the plasmid copy number and transformation efficiencies of the first were found to be influenced by the presence of the *gpd* terminator downstream the Km^R gene. It was shown that plasmid amplification occurred independent from selection pressure to an extent that appeared to be negatively related to the effectiveness of expression of the Km^R gene. This observation indicated that the rising metabolic burden, as a consequence of amplification, imposes limits to the number of plasmid copies. The effectiveness, stability, and plasmid amplifying properties of the *Phaffia* transformation system offer possibilities for the use of recombinant DNA technology in developing industrially attractive *Phaffia* strains.

XV. Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, and Fermentec S/C Ltda, Rua Treze de Maio, 768 - Sala 153, Edificio Sisal Center, 13400-900 Piracicaba, São Paulo. Communicated by H.V. Amorim.

The following summarizes some of our ongoing research.

1. Ferreira, L. V. & Amorim, H.V.

Two strains of *Saccharomyces cerevisiae* (PE-2 and VR-1) were submitted to fermentation of its reserve (trehalose and glycogen) at 40°C using yeast from the first and fourth fermentation cycle. It was observed the effects of different temperatures (35, 40 and 45°C), different initial pH (2.50, 3.50 and 4.50) at 40°C. During a 24 hours interval samples were collected for determination of ethanol, suspension of pH, yeast content and dry, yeast and wine nitrogen and yeast trehalose, glycogen and cell viability. The endogenous fermentation using yeast from one cycle result in higher ethanol content and higher level of yeast nitrogen. Trehalose was completely exhausted after 24 hours at 35 and 40°C, but at 45°C it was consumed after 2 hours for PE-2 and after 6 hours for VR-1 strain. Glycogen was not completely consumed, but probably

contributes for ethanol formation. As trehalose is consumed yeast cell viability decreases, while yeast nitrogen content increase, reaching a maximum between 2 and 8 hours, depending on the temperature, initial pH and yeast strain. If yeast is maintained under prolonged stressing conditions, cell autolysis occurs and nitrogen is lost to the medium, increasing from 200 to 1500 mg/l. Such endogenous fermentation allows a production of 40 to 67 L of ethanol per ton of dry yeast, with yeast nitrogen increasing of 25 and 27% for PE-2 and VR-1, respectively. Values of up to 20% of trehalose can be found in distillery yeast during the industrial process, and such high trehalose concentration may render yeast tolerant to the stressing factor imposed by the industrial.

XVI. Escola Superior de Agricultura Luiz de Queiroz - Departamento de Química, Universidade de São Paulo, Av. Pádua Dias n. 11, P.O. Box 09, CEP 13418-900 Piracicaba (SP), Brazil. Communicated by L.C. Basso.

The following is a summary of recently completed research.

1. Basso, L.C., Lopes, M.L., Basso, T.O., Fonseca, A.J. and Amorim, H.V. 1998. The relative importance of killer activity for the industrial fuel ethanol fermentation process.

The fuel-ethanol industry in Brazil is based on yeast reuse during a continuous season over 200 days with batch fermentation using 10-15% (v/v) of yeast resulting in fermentation time around 6-7 hours. Yeast is separated by centrifugation and reused in a subsequent fermentation, comprising nearly 3 fermentation cycles per day. We could demonstrate that yeast strains of *Saccharomyces cerevisiae* traditionally used in distilleries (baker's yeast and others selected strains – M-300A, IZ-1904 and NF, a non-flocculent strain), were not able to survive the intensive recycling of the industrial process, being totally replaced by wild strains of *S. cerevisiae* after a 20-40 days period. It has been suggested that the killer phenomenon could play an important role since by this type of antagonism killer strains could dominate at least in wine spontaneous fermentation. In the present work, by means of the karyotyping

technique, we follow up the yeast populations from 16 distilleries where the introduced starter strains (baker's yeasts Fleischmann and Itaiquara and selected strains PE-2, VR-1 and IZ-1904) were completely replaced by wild strains. These wild strains (139) were examined for their killer activities and only 4,3% showed to be killer upon a sensitive strain (*S. cerevisiae* AH-22) and none of them showed any killer activity on the replaced starter strains. Most of the wild strains and the introduced starters presented the neutral character in relation to each other. These results suggested that the killer activity would not be of significant importance in ensuring competitiveness of dominant strains in the industrial fermentation. Such competitiveness and dominance could be related to the capability of some wild strains to survive the stressing conditions of the industrial fermentation process.

XVII. Dipartimento di Protezione e Valorizzazione Agroalimentare sez. Microbiologia Università di Bologna. via F.lli Rosselli, 107 42100 Reggio Emilia, Italy. Communicated by C. Zambonelli <c.zambonelli@stpa.unibo.it>.

Recent publications.

1. Rainieri, S., C. Zambonelli, V. Tini, L. Castellari, and P. Giudici. The oenological traits of ~~the most~~ *Saccharomyces* strains. Am. J. Enol. Vitic. (In press).

Thermotolerant *Saccharomyces cerevisiae* strains possess several interesting fermentative properties compared to non-thermotolerant *Sacch. cerevisiae* strains: they give a higher glycerol yield and intense malo-alcoholic fermentation. They have the disadvantages, however, of producing high levels of acetic acid, lacking vigour, and not completing the fermentation process,

2. Giudici, P., C. Caggia, A. Pulvirenti, C. Zambonelli, and S. Rainieri. 1998. Electrophoretic profile of hybrids between cryotolerant and non-cryotolerant *Saccharomyces* strains. *Lett. Appl. Microbiol.* (In press)

The chromosomal DNAs of cryotolerant *Saccharomyces bayanus*, non-cryotolerant *Saccharomyces cerevisiae* strains and their intra and interspecific hybrids were separated by pulsed field electrophoresis (PFGE). The cryotolerant and non-cryotolerant strains gave distinctly different electrophoretic profiles. The hybrids cryotolerant x cryotolerant and non-cryotolerant x non-cryotolerant

therefore leaving high levels of residual sugar. Despite the many negative traits, which make them oenologically unsuitable, thermotolerant *Saccharomyces cerevisiae* strains demonstrate potential for oenological applications and provide a valuable genetic resource for yeast improvement programs.

were fertile and they gave the same electrophoretic karyotype as the respective parents. The cryotolerant x non-cryotolerant hybrids were sterile and gave electrophoretic karyotypes which showed both the bands the parents have in common and those they do not share.

3. Rainieri, S., C. Zambonelli, P. Giudici and L. Castellari. 1998. Characterisation of thermotolerant *Saccharomyces cerevisiae* hybrids. *Biotechnol. Lett.* (In press).

Thermotolerant *Saccharomyces* strains were crossed with mesophilic *Saccharomyces cerevisiae* and with cryotolerant *Saccharomyces bayanus*. The former hybrid is fertile confirming thermotolerant strains as *S. cerevisiae*. The spores from this hybrid,

though, possess a low germinability and give cultures that grow poorly. The hybrid cryotolerant x thermotolerant is sterile and show a new combination of the parental strains' traits improving their technological application.

Publication of a new edition.

4. Zambonelli, C. 1998. *Microbiologia e Biotecnologia dei Vini* (Microbiology and Biotechnology of Wines) EDAGRICOLE (300 pages, in italian).

PhD dissertations.

5. G. Montanari. 1996. *Microorganisms of traditional koumiss of central Asia.*

The first part of the theses introduces the origin of the koumiss: this milk beverage is made from the lactic and alcoholic fermentation of mare milk. It is linked to the history and to the habits of nomad people who breed mares in the Asiatic steppe. In the second part of the dissertation the characteristics of mare milk are considered as well as the chemical and microbiological composition of the koumiss in relation with the possibility of its industrial production and its diffusion in Western Europe, and in relation with its therapeutical properties. In the experimental part 94 samples of traditional koumiss from Kazakhstan are examined. The chemical

composition is in accordance with the data of other authors; the yeast flora composition shows the dominance of lactose non-fermenting yeasts and one of these, *Saccharomyces unisporus*, was found to be the principal responsible for the alcoholic fermentation of koumiss. *Sacch. unisporus*, tested in different natural and synthetic medium, causes slower alcoholic fermentations than *Sacch. cerevisiae*. It produces larger amounts of minor compounds of fermentation such as glycerol, succinic acid, acetic acid and of higher alcohols.

6. S. Rainieri. 1998. Interspecific and intraspecific *Saccharomyces* hybrids: oenological properties.

Saccharomyces sensu stricto strains were crossed inter and intraspecifically to ascertain their ability to hybridize and to improve their technological suitability. The parental strains were selected among cryotolerant *S. bayanus*, thermotolerant *S. cerevisiae* and mesophilic *S. cerevisiae*. Cryotolerant *S. bayanus* and thermotolerant *S. cerevisiae* strains possess interesting oenological properties, but some negative traits limit their applicability. Cryotolerant strains give highly acidic fermentations and produce wines with an excessively intense rosy bouquet due to the high production of β -phenylethanol. Thermotolerant strains have a low fermentative vigour and produce high amounts of acetic acid. Both types of strain have a high glycerol yield and interestingly display a different action on malate: cryotolerant strains can synthesise the compound whereas thermotolerant strains can degrade it up to 50% of the

starting concentration.

Three types of hybrids were obtained: interspecific hybrids *S. bayanus* x *S. cerevisiae* and *S. bayanus* x thermotolerant *S. cerevisiae* and intraspecific hybrid *S. cerevisiae* x thermotolerant *S. cerevisiae*. The hybrids were characterised determining their temperature profile (optimal temperature of growth and maximum temperature of growth) and their fermentation profile (production of glycerol, succinic acid, acetic acid, malate and higher alcohols in synthetic medium). Fermentations in must at different temperatures and vinification tests following both techniques with and without pomace were carried out to test their technological applicability. Interspecific hybrids resulted sterile; they showed the characteristics of the parents in new combinations which would have been hard to obtain otherwise: in particular the hybrid *S.*

bayanus x *S. cerevisiae* showed all the parental fermentation traits but at an intermediate level; the hybrid *S. bayanus* x thermotolerant *S. cerevisiae* showed all the positive traits coming from both parents but not the negative traits (high glycerol yield, intense degradation of malate, but low production of acetic acid

and β -phenylethanol). Both hybrids were found to be generally more vigorous or at least as vigorous as their parental strains. The intraspecific hybrid was fertile, but not fully and its fermentation characteristics were not so different from those of other *S. cerevisiae* strains.

XVIII. Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorobyovy Hills, Moscow 119899, Russia. Communicated by I.P. Babjeva.

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

1. Chernov I. Yu., Babjeva I.P., and Reshetova I.S. 1997. Synecology of yeasts in subtropical deserts. *Uspechi Sovremennoy Biologii (Advantages of the Modern Biology)* **117**(5):584-602 (in Russian).

The long-term investigations of yeasts in three subtropical deserts (Kyzylkum, Karakum, and Negev) are summarized. Total density of yeasts is shown to be depended mainly on the type of substratum (plants, litter, or soil) and secondly on biotope and geographical region. Almost absolute absence of ascomycetous species, sharp predominance of common *Cryptococcus albidus* and *Cr. laurentii*, high abundance of ballistosporogenous species, and the absence of some species typical for arctic and boreal habitats (*Trichosporon*, *Leucosporidium*) characterize the taxonomic

structure of yeast communities in Asian deserts. The main peculiarity of yeast communities in subtropical deserts is a significant reduction of its syntypological differentiation: one-two typically epiphytous yeast species are highly predominant in all substrata, while litter and soil life forms are very rare or absent. *Cr. albidus* is strictly predominant in Middle Asian deserts while *Cr. laurentii* dominate in the Negev desert. Morphological adaptations of these two species are shown to be corresponded with climatic characteristics of their habitats.

2. Babjeva I.P. and Reshetova I.S. 1998. Yeasts resources in natural habitats at polar circle latitude. *Food Technol. and Biotechnol.* **36**(1):1-6.

Yeast communities in 8 types of natural habitats in typical biotops of the White Sea coastal zone at the polar circle latitude are described. Approximately 60 species among 780 isolated yeast strains were identified. The greatest difference was revealed

between the groupings of yeasts inhabiting soils and various parts of living plants and those associated with insect habitats. Basidiomycetous species were prevalent in the former and ascomycetous species in the latter.

Several yeast species were isolated from new geographical points in our lab.

(1) Three samples of soil were collected in Dreznica, near Kobarid, Slovenian Alps, 1200 m, during excursion tour in 1997, August, 18th ISSY. The yeasts of the following species were isolated from these samples and deposited in the yeast collection of the Department of Soil Biology (DSB): *Debaryomyces hansenii* DSB Y-3654, *Debaryomyces vanriijiae* DSB Y-3656, *Cryptococcus aerius* DSB Y-3657, *Cryptococcus laurentii* DSB Y-3652, *Holtermannia*

corniformis DSB Y-3659, *Rhodotorula* sp. (nonpigmented) DSB Y-3658, *Williopsis saturnus* DSB Y-3655, *Lipomyces* sp. (ascospores not obtained), *Trichosporon cutaneum*.

(2) New strains of *Lipomyces tetrasporus* were isolated from soil samples collected in Northern Argentina and Burjatia (Asian part of Russia).

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

Recent publications.

1. Rösel, H. & G. Kunze. 1998. Integrative transformation of the dimorphic yeast *Arxula adenivorans* LS3 based on hygromycin B resistance. *Curr. Genet.* **33**: 157-163.

A transformation system has been developed for the dimorphic yeast *Arxula adenivorans* based on a stable integration of the donor DNA into the ribosomal DNA. For this purpose a cassette elongation factor EF-1 α , and the transcription termination region of the *Sacharomyces cerevisiae* *PHO5* gene. This cassette was fused into the 25S rDNA of *Arxula adenivorans*. Linearization

was constructed which contains the *E. coli hph* gene conferring hygromycin B resistance, fused to the 5' expression signals of the *Arxula adenivorans* *TEF1* gene, encoding the translation of this vector was required for high transformation frequencies. The vector was integrated in multiple copies into the 25S rDNA by homologous recombination. Copy number was not altered even

after the growth of transformants for 15 generations under non-selective growth conditions. Microscopical analyses revealed that integration of the transformed plasmid did not influence the

dimorphism, which is triggered at 42°C for both transformed and non-transformed cells.

2. Wartmann, T., H. Rösel, I. Kunze, R. Bode & G. Kunze. 1998. *AILV1* gene from the yeast *Arxula adeninivorans* LS3 - a new selective transformation marker. *Yeast* (in press).

The *ILV1* gene of the yeast *Arxula adeninivorans* LS3 (*AILV1*) has been cloned from a genomic library, characterized and used as an auxotrophic selection marker for transformation of plasmids into this yeast. One copy of the gene is present in the *Arxula* genome, comprising 1,653 bp and encoding 550 amino acids of the threonine deaminase. The protein sequence is similar (60.55%) to that of the threonine deaminase from *Saccharomyces cerevisiae* encoded by the gene *ILV1*. The protein is enzymatically active during the whole period of cultivation, up to 70 h. Maximal activities, as well as protein concentrations of this enzyme, were

achieved after cultivation times of 20-36 h. The *AILV1* gene is a suitable auxotrophic selection marker in transformation experiments using an *Arxula adeninivorans ilv1* mutant and a plasmid containing this gene, which is fused into the 25S rDNA of *Arxula adeninivorans*. One to three copies of the linearized plasmid were integrated into the 25S rDNA by homologous recombination. Transformants resulting from complementation of the *ilv1* mutation can be easily and reproducibly selected and in addition are mitotically stable. Therefore, the described system is to prefer to the conventional selection for hygromycin B resistance.

3. Sander, U., I. Kunze, M. Bröker & G. Kunze. 1998. Humoral immune response to a 200-kDa glycoprotein antigen of *Saccharomyces cerevisiae* is common in man. *Immunol. Lett.* (in press).

According to Heelan et al. (1991) patients suffering from Crohn's disease produce antibodies against a cell wall associated glycoprotein antigen gp200 of the yeast *Saccharomyces cerevisiae*, while healthy people do not. Here we show that antibodies against this glycoprotein gp200 can also be detected in the sera of healthy humans. The intensity of the antibody titer which is measured by immunoblot experiments is independent from the state of health. The *Saccharomyces cerevisiae* specific gp200 is a highly glycosylated protein localized not only in the cell wall but is additionally accumulated in the culture medium. Some of the tested

sera from Crohn's disease patients as well as from healthy adults also reacted with a 120 kDa glycoprotein which is to be found in preparations containing secreted proteins. Because the binding of antibodies is greatly reduced by periodate treatment of gp200 and by the 120 kDa polypeptide, it is very likely that their carbohydrate moieties are the antigenic determinants against which the specific human antibodies are directed. The human humoral immune response applies only to *Saccharomyces cerevisiae* antigens, because no analogous immune responses could be detected against antigens derived from the yeast *Arxula adeninivorans*.

XX. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England. Communicated by J.A. Barnett.

The following are recent publications.

1. Bitzilekis, S. & Barnett, J.A. 1997. Exponential growth rates of the yeast genus *Kluyveromyces*. *FEMS Lett.* **146**:189-190.
2. Barnett, J.A. 1997. A historical survey of the study of yeasts. **In** *Yeast Sugar Metabolism: Biochemistry, Genetics, Biotechnology and Applications*, pp. 1-33, edited by F.K. Zimmermann & K.-D. Entian. Lancaster, Pennsylvania: Technomic Publishing.
3. Barnett, J.A. 1997. Sugar utilization by *Saccharomyces cerevisiae*. **In** *Yeast Sugar Metabolism: Biochemistry, Genetics, Biotechnology and Applications*, pp. 35-43, edited by F.K. Zimmermann & K.-D. Entian. Lancaster, Pennsylvania: Technomic Publishing.
4. Barnett, J.A. 1997. The contribution of taxonomists to the understanding of yeast nutrition. *Food Technology and Biotechnology* **35**: 23 1-234.

The following is in the press.

5. Barnett, J.A. 1998. A history of research on yeasts. **i.** Work by chemists and biologists 1789-1850. *Yeast* (December).

Recent publications.

1. Caridi, A. and V. Corte. 1997. Inhibition of malolactic fermentation by cryotolerant yeasts. *Biotechnol. Lett.* **19(8)**:723-726.

White wines produced by some cryotolerant strains of *Saccharomyces cerevisiae* are more resistant to malolactic fermentation than those produced by normal strains: e.g. for two months of storage, the wines, inoculated with *Leuconostoc oenos*

or *Lactobacillus plantarum*, were fully stabilized with levels of 51-65 mg total SO₂/L and 5.70-5.75 g titratable acidity/L. The use of these yeasts in wine-making can decrease the quantities of sulfites added to stabilize wines.

2. Caridi, A., S. Rainieri, P. Passarelli, and C. Zambonelli. 1997. Effects of hybrids between cryo- and non-cryotolerant *Saccharomyces* strains on the composition of wines from southern Italy. (in Italian). *Vigne vini* **24(4)**:27-30.

Hybrids between cryo- and non-cryotolerant *Saccharomyces* may be used as an alternative to the cryotolerant strains especially in musts with a low acid content. They are at least as vigorous as their parents also in presence of SO₂. They increase the titratable

acidity and produce glycerol and other minor fermentation compounds at intermediate levels with respect to their parents. The presence of extraneous smell in wine, typical of the cryotolerant strains, is highly reduced and in some cases not even perceptible.

3. Caridi, A., C.M. Lanza, F. Tomaselli, A. Pulvirenti, and P. Giudici. 1998. Principal components analysis applied to chemical, physical and sensory evaluation of Greco and Mantonico wines. (in Italian). *Ind. Bevande* (in press).

The chemical, physical, and sensory data of 32 wines from dried grapes were elaborated by multivariate analysis to assess their global quality and to simplify the model. Four samples of Calabrian must from *white Greco* and *white Mantonico* cultivars were employed. Winemakings at 20-30 °C by eight strains of selected yeast for enological use, singularly or in cofermentation, were performed. The wines so obtained were added with SO₂, bottled, and analysed. Data were elaborated by principal components analysis. Color, sugars, and diethyl-malate were positively correlated

to the first principal component; decanoic acid and 2,3-butanediol were negatively correlated. The second principal component was, instead, positively correlated with many higher alcohols. For sensory data, to the first principal component was attributable the taste-olfactory sensation; to the second, the intensity and the duration of the sensation. A selection of the parameters to determine in wines can discriminate among those produced from the *white Greco* and the *white Mantonico* cultivars, independently on the employed yeast strain.

4. A. Caridi, V. Corte, and C. Zambonelli. 1998. Influence of the production yeast strain on the development of malolactic fermentation in white wine. *Food Technol. Biotechnol.* **36(1)**:63-68.

The aim of the research was to study the influence of the hybrid strain of *Saccharomyces* 12233 x 6167, its parents - *S. bayanus* 12233 and *S. cerevisiae* 6167 - and the control strain *S. cerevisiae* 220 on the growth of lactic bacteria in white wine. A number of winemaking cycles with three samples of must from white grape of typical Sicilian and Calabrian cultivars were carried out without the addition of SO₂. At the end of fermentation the wines were clarified and bottled, both with and without the addition of SO₂. The wines were stored at 15-20°C for 90 days. The wines showed different levels of malic acid degradation as influenced by their ethanol content, the yeast strain used as a starter, and the levels of residual SO₂. The results demonstrate that the wines

produced by the *S. cerevisiae* strains were essentially unable to inhibit the tart of malolactic fermentation, except when 80 mg/L of SO₂ were added to the wines. On the other hand, all the wines produced by the *S. bayanus* 12233 effectively prevented the growth of lactic bacteria with just 40 mg/L of SO₂ and, for one cultivar, also without the addition of SO₂. The wines produced by the hybrid strain of *Saccharomyces* had an intermediary behaviour; therefore, with a low addition of SO₂, this strain stabilises white wines and prevents an excessive production of acids. This system of white wine microbiological stabilisation reduces SO₂ and offers considerable advantages for the health of the consumer.

XXII. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@ulmus.riken.go.jp> and T. Nakase <nakase@ulmus.riken.go.jp>.

The seventh edition of the JCM strain catalogue will be published on December 1998. Our own catalogue is published once every 3 years. We can now accept your applications and inquiry by e-mail <curator@jcm.riken.go.jp> or Fax (+81 48 462 4617). You can

also get a wealth of information for microorganisms (yeasts, fungi, the yeast-like alga *Prototheca*, bacteria and archaea) from the JCM On-Line Catalogue. Search JCM Catalogue Database via INTERNET: <http://www.jcm.riken.go.jp/>

The following articles have been published recently.

1. Mikata, K. and Nakase, T. 1997. Surface structure of ascospores of genus *Nadsonia* Sydow. Microbiol. Cult. Coll. **13**:97-102.

The surface structure of ascospores of *Nadsonia commutata* IFO 10029T (JCM 10138) and IFO 10030 (JCM 10139), *N. fulvescens* var. *elongata* IFO 10894 (JCM 9991), and *N. fulvescens* var. *fulvescens* IFO 10895 (JCM 10023) was viewed under a

scanning electron microscope. Ascospores of *N. commutata* were covered with irregular protuberances, while the two varieties of *N. fulvescens* were ornamented with stellate spines.

2. Nakase, T. and Suzuki, M. 1997. *Candida gotoi*, new species of an anamorphic yeasts isolated from insect frass in bark of Japanese maple. Microbiol. Cult. Coll. **13**:109-112.

A strain of yeast isolated from insect frass of a Japanese maple (*Acer palmatum* Thunberg) in 1982 was found to represent a new species of anamorphic yeast and was described as *Candida gotoi* Nakase et Suzuki. This yeast was characterized by Q-8 as the major ubiquinone, glucan-mannan type cell wall, and mol% G+C of DNA of 41.6. Among species with Q-8, *C. gotoi* resembles

C. silvanorum in the mol% G+C and in taxonomic criteria commonly employed but is differentiated from it by a low DNA homology value of 19%. Practically, *C. gotoi* is distinguished from *C. silvanorum* by its ability to assimilate galactitol and 2-ketogluconic acid, and inability to assimilate melibiose.

3. Sugita, T. and Nakase, T. 1998. Molecular phylogenetic study of the basidiomycetous anamorphic yeast genus *Trichosporon* and related taxa based on small subunit ribosomal DNA sequences. Mycoscience. **39**:7-13.

Small subunit ribosomal DNA sequences of all species of the basidiomycetous anamorphic yeast genus *Trichosporon* were determined, and phylogenetic trees were constructed by the neighbor-joining and maximum likelihood methods. The sequence data showed that, with the exception of *T. pullulans*, the genus is monophyletic, although its members have two different major ubiquinones, Q9 and Q10. The genus can be divided phylogenetically into three major clusters. Species with Q10 as

the major ubiquinone constitute a single cluster, while those with Q9 form two clusters. *Trichosporon pullulans* was phylogenetically distinct from other taxa of the genus. It is located in a cluster containing *Cystofilo-basidium capitatum*, *Mrakia frigida*, *Xanthophyllomyces dendrorhous* and three species of Udeniomyces. This result suggests that *T. pullulans* does not belong to the genus *Trichosporon*.

4. Hamamoto, M., Kuroyanagi, T., & Nakase, T. 1998. *Fellomyces ogasawarensis* sp. nov. and *Fellomyces distylii* sp. nov., yeasts isolated from a plant in Japan. Int. J. Syst. Bacteriol. **48**:287-293.

We describe *Fellomyces ogasawarensis* and *Fellomyces distylii*, new yeast species isolated from a dead leaf of a plant (*Distylium lepidotum* Nakai, the family Hamamelidaceae), which was collected in the Ogasawara Islands that are isolated in the Pacific Ocean at about 1,000 km south of Japan. The phylogenetic

relationship of *F. ogasawarensis* and *F. distylii* with other members of the genus *Fellomyces* was estimated from 18S rRNA gene sequence analysis. The type strain of *F. ogasawarensis* is strain OK-81 (=JCM 9861) and that of *F. distylii* is strain OK-83 (=JCM 9862), respectively.

5. Haryono, B., Hamamoto, M., Kuswanto, K. R., and Nakase, T. Systematics of ballistoconidium-forming yeasts isolated from plants in Indonesia. Asian Network on Microbial Researches. 223-231. Gadjah Mada University, The Institute of Physical and Chemical Research (RIKEN), Science and Technology Agency Japan. 1998.

6. Takashima, M., Fungsin, B., Atthasampunna, P., Komagata, K., and Nakase, T. 1998. A taxonomic and phylogenetic study of ballistoconidium-forming yeasts isolated from leaves in Thailand. In Asian Network on Microbial Researches. 529-536. Gadjah Mada University, The Institute of Physical and Chemical Research (RIKEN), Science and Technology Agency Japan. 1998.

Fifteen ballistoconidium-forming yeasts were isolated from plants in Thailand. They were assumed to belong to the

genus *Sporobolomyces* and represented 5 new species.

XXIII. Collection of Pure Cultures of Industrial Microorganisms, Institute of Fermentation Technology & Microbiology 90-530 Lodz, Wolczanska 175, Poland. Communicated by H. Oberman and H. Stobinska.

The Culture Collection of the Institute of Fermentation Technology and Microbiology is registered in the Catalogue of the World Federation of Culture Collections (WFCC) as LOCK 105. The collection maintains 650 strains of yeasts, filamentous fungi and bacteria for traditional and modern biotechnological processes. It supplies production stains for fruit wine industry, alcohol industry

and breweries in Poland. The main research activities are as follows: - isolation, improvement and maintenance of industrially important yeasts, especially amyolytic and cellulolytic strains - killer toxin in certain strains of wine fermenting yeasts and attempt to transfer of this feature to other industrially important yeast strains.

This paper is soon to be recently published.

1. Kregiel, D. Amylases of yeast *Schwanniomyces* sp. Postepy Mikrobiologii (in Polish).

The review presents the results of recent studies on amyolytic complex of the yeast *Schwanniomyces occidentalis*. The genus *Schwanniomyces* was established by Klocker (1909) who isolated the yeast from soil in the Antilles. The strains belong to *Schwanniomyces* sp. secrete extracellular alpha-amylase, two forms of glucoamylase and probably a debranching enzyme. They

are the only yeasts known to be capable of saccharifying starch and fermenting it to ethanol. This fact lets include strains *Schwanniomyces* sp. as virtuous in contemporary biotechnology. The paper describes genes coding amylases and influence of environmental conditions on secretion of these enzymes.

The following abstract will be presented on the 19th ISSY "Yeast in the Production and Spoilage of Food and Beverages", 30 August - 3 September 1998, Braga, Portugal.

2. Drewicz, E., Kregiel, D., Oberman, and H., Kunicka, A. 1998. Growth, amyolytic and fermentative activities of yeasts in the presence of killer toxins.

The aim of the work was to investigate the effect of killer yeasts of *Saccharomyces cerevisiae* T158C and W11 on *Schwanniomyces occidentalis* Y671/6 strain with high amyolytic activity as well as on the wine yeast *Saccharomyces cerevisiae* W6 strain. The visible killer effect of *Saccharomyces cerevisiae* T158C and W11 strains in the mixed cultures with Y671/6 strain was pointed out. In the medium with glucose added amyolytic yeasts were characterized by higher susceptibility to killer toxin K1 expressed as rate of cells survival, but in the presence of starch the killer effect was considerably lower, 60% comparing to glucose-medium. The effect of killer yeasts in the mixed population

on amyolytic activity of Y671/6 strain was not observed. The wine yeasts W6 and killer wine yeasts W11 showed different survival rates and fermentative activity in the presence of the killer toxin K1 secreted by T158C strain. W6 strain which appeared sensitive in the preliminary examination, revealed higher resistance in the fermentation systems W6:T158C. In the W11:T158C system the killer effect of T158C yeast strain to another killer wine yeasts W11 was observed. In these fermentations the toxin of T158C strain was dominant. The previous experiments carried out by a plate test, showed mutual antagonistic effect of killer yeasts secreting type K1 and K2 toxins.

XXIV. Laboratoire de Biotechnologie, Faculté des Sciences et des Techniques, Université de Nantes, 2, rue de is Houssinière, F 44072 Nantes Cedex 3 France. Communicated by L. Simon.

The following paper is in press.

1. Simon, L., K. Bremond, B. Bouchet, D.J. Gallant, and M. Bouchonneau. 1998. Studies on the pullulan extracellular production and glycogen intracellular content in *Aureobasidium pullulans*. Can. J. Microbiol., in press.

Pullulan is a well known extracellular polysaccharidic production of the micromycete *Aureobasidium pullulans* coming into more and more frequent use in commercial and industrial applications. Nevertheless its cellular origin and biosynthesis pathway still remain uncertain. As pullulan synthesis is increasing during growth while glycogen production (a major reserve stored within the cytoplasm) is decreasing, it should be possible that the kinetics of production of these polysaccharides whose location differs might be correlated. In order to check this hypothesis we have performed biochemical analysis and microscopic studies of the biomass removed at regular intervals during the growth. The ultrastructural data have shown that the glycogen units were present

in all the different cellular types (conidia, swollen cells, chlamydozoospores) and at all the stages of the cellular development. Moreover analytical studies have shown that glycogen level was time dependant, decreasing in the early exponential stage whereas the extracellular pullulan content was increasing. The correlation coefficient (r) calculated between intracellular glycogen and extracellular pullulan levels by the square method is in good agreement to each other suggesting that these productions are inversely correlated. Ultrastructural and confocal fluorescence data allow to propose that glycolipids could be implicated in the pullulan biosynthetic pathway.

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

The following article, whose abstract appeared in the previous issue of the Yeast Newsletter, is now in print.

1. Lachance, M.A., Rosa, C.A., W.T. Starmer, B. Schlag-Edler, J.S.F. Barker, and J.M. Bowles. 1998. *Metschnikowia continentalis* var. *continentalis*, *Metschnikowia continentalis* var. *borealis*, and *Metschnikowia hibisci*, new heterothallic haploid yeasts from ephemeral flowers and associated insects. *Can. J. Microbiol.* **44**:279-288.

The following articles have been accepted for publication.

2. Rosa, C.A. and M.A. Lachance. 1998. The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. *Int. J. Syst. Bacteriol.* **48**: in press.

Seven strains of a heterothallic haploid yeast species were isolated from flowers of *Calystegia sepium* (hedge bindweed, Convolvulaceae) and associated sap beetles in the genus *Conotelus*. Conjugation was observed between some of the isolates and the type culture of *Candida bombicola*, resulting in evanescent asci with one ascospore with a convoluted surface. The sequences of

the D1/D2 variable domain of the large subunit of the ribosomal DNAs of three strains differed by only one or two bases from that of the type. We propose the new genus *Starmerella*, with the single species *Starmerella bombicola*, to accommodate the teleomorph of *C. bombicola*. The designated isotype is strain UWO(PS)97-118 (h⁻, CBS 8451).

3. Lachance, M.A., C.A. Rosa, W.T. Starmer, B. Schlag-Edler, J.S.F. Barker, and J.M. Bowles. 1998. *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae*, and *Candida lipophila*, five new related yeast species from flowers and associated insects. *Int. J. Syst. Bacteriol.* **48**: in press.

We describe the five new yeast species *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae*, and *Candida lipophila* to accommodate isolates recovered from flowers and floricolous insects of Australian *Hibiscus* trees, cosmopolitan morning glories (*Ipomoea* spp.), and Brazilian cereoid cacti. The new *Wickerhamiella* species are heterothallic, occur in the haploid condition, and are clearly separated reproductively from one another. Although they exhibit little physiological variation, they are easily delineated from *Wickerhamiella domercqiae*, the only species known previously by their resistance to cycloheximide and the production of strong extracellular lipases. *C. drosophilae* and *C. lipophila* share the latter property, but unlike the *Wickerhamiella* species, they fail to utilize nitrate as sole nitrogen source. Pulse-field electrophoresis

indicates that these yeasts have an unusually low number of chromosomes. The large subunit ribosomal DNA (D1/D2) sequences demonstrate a close relationship between the five species, and *Candida vanderwaltii* and *Candida azyma*. Their relationship with *W. domercqiae* is more distant, but all share, with some other *Candida* species, a single monophyletic clade. The type cultures are as follows: *W. australiensis* strains UWO(PS)95-604.3 (h⁺, CBS 8456) and UWO(PS)95-631.3 (h⁻, CBS 8457); *W. cacticola* strains UFMG96-267 (h⁺, CBS 8454) and UFMG96-381 (h⁻, CBS 8455); *W. occidentalis* strains UWO(PS)91-698.4 (h⁺, CBS 8452) and UFMG96-212 (h⁻, CBS 8453); *C. drosophilae* UWO(PS)91-716.3 (CBS 8459), and *C. lipophila* UWO(PS)91-681.3 (CBS 8458).

4. Rosa, C., M.A. Lachance, W.T. Starmer, J.F. Barker, J.M. Bowles, and B. Schlag-Edler. *Kodamaea nitidulidarum*, *Candida restingae*, and *Kodamaea anthophila*, three new related yeast species from ephemeral flowers. *Int. J. Syst. Bacteriol.* **48**: in press.

Three new yeast species were discovered during studies of yeasts associated with ephemeral flowers in Brazil, Australia and Hawaii. Their physiological and morphological similarity to *Kodamaea (Pichia) ohmeri* suggested a possible relationship to that species, which was confirmed by ribosomal DNA sequencing. *Kodamaea nitidulidarum* and *Candida restingae* were found in cactus flowers and associated nitidulid beetles in sand dune ecosystems (*restinga*) of South-eastern Brazil. Over 350 strains of *Kodamaea anthophila* were isolated from *Hibiscus* and morning glory flowers (*Ipomoea* spp.) in Australia, and from associated

nitidulid beetles and *Drosophila hibisci*. A single isolate came from a beach morning glory in Hawaii. Expansion of the genus *Kodamaea* to three species modified the existing definition of the genus only slightly. The type cultures are as follows: *K. nitidulidarum* strains UFMG-96-272 (holotype, h⁺, CBS 8491) and UFMG96-394 (isotype, h⁻, CBS 8492); *C. restingae* UFMG96-276 (CBS 8493); *K. anthophila* strains UWO(PS)95-602.1 (holotype, h⁺, CBS 8494), UWO(PS)91-893.2 (isotype, h⁻, CBS 8495), and UWO(PS) 95-725.1 (isotype, h⁻, CBS 8496).

5. Lachance, M.A., C.A. Rosa, W.T. Starmer, and J.M. Bowles. 1998. *Candida ipomoeae*, a new yeast species related to large-spored *Metschnikowia* species. *Can. J. Microbiol.* **44**: in press.

Numerous strains of an unusual asexual yeast species were isolated from flowers of morning glory (*Ipomoea* spp., Convolvulaceae) and associated drosophilids and sap beetles in the genus *Conotelus* sampled in Hawaii and in Brazil. The nutritional profile of this yeast is similar to those of *Metschnikowia hawaiiensis* and *Metschnikowia continentalis*, which share the same habitats. The cells are large, hydrophobic, and tend to

remained attached after budding, causing the colonies on agar media to have a convoluted appearance, reminiscent of popcorn. The sequences of the D1/D2 domain of large subunit ribosomal DNAs of strains from three different localities confirmed that a single species is involved, and that it is related to large-spored *Metschnikowia* species. The type strain is UWO(PS) 91-672.1 (CBS 8466).

The following are chapters in the recently published treatise, THE YEASTS, A TAXONOMIC STUDY.

6. Lachance, M.A. and H.J. Phaff. 1998. The genus *Clavispora*. In: C.P. Kurtzman and J.W. Fell, eds. *The Yeasts, a Taxonomy Study*, 4th Edition. Elsevier, Amsterdam, pp. 152-156.
7. Lachance, M.A. and H.J. Phaff. 1998. The genus *Sporopachydermia*. In: C.P. Kurtzman and J.W. Fell, eds. *The Yeasts, a Taxonomy Study*, 4th Edition. Elsevier, Amsterdam, pp. 398-402.
8. Lachance, M.A. 1998. The genus *Kluyveromyces*. In: C.P. Kurtzman and J.W. Fell, eds. *The Yeasts, a Taxonomy Study*, 4th Edition. Elsevier, Amsterdam, pp. 230-250.
9. Lachance, M.A. and W.T. Starmer. 1998. Ecology and Yeasts. In: C.P. Kurtzman and J.W. Fell, eds. *The Yeasts, a Taxonomy Study*, 4th Edition. Elsevier, Amsterdam, pp. 21-30.

The proceedings of the 7th International Microbial Ecology Congress are now in print.

10. Lachance, M.A. 1997. Ecology and evolution of yeasts in tropical cactus forests. In: *Progress in Microbial Ecology*. Martins, M.T. et al. (eds.), São Paulo, SBM/ICOME. pp. 181-187.

Forthcoming meetings

1998 Yeast Genetics and Molecular Biology Meeting, University of Maryland, July 28 - August 2, 1998

The 1998 "Yeast Meeting" will be held on the campus of the University of Maryland at College Park. The facilities provide excellent meeting space and superior air-conditioned housing with nearby dining. This East-coast venue is within driving distance of many major metropolitan areas, and is accessible from three international airports. In addition, the museums and monuments of Washington, D.C., are just a short Metro ride away. This meeting

provides an important forum for presenting and learning about recent research advances on *S. cerevisiae*, *S. pombe*, and related yeast species. As at past meetings, there will be platform and workshop sessions on a wide variety of timely subjects, including: Cell Growth and Development. Chromosome & Genome Structure & Function. Protein Localization & Degradation. Gene Expression. Cell Cycle & Mating. Contact:

Marsha Ryan
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<http://genome-www.stanford.edu/Saccharomyces/yeast98/>

Sixth International Mycological Congress - IMC 6, August 23-28, 1998, Jerusalem, Israel

For last minute information, contact:

IMC6 Congress secretariat
P.O. Box 50006
Tel Aviv 615002
Israel

Tel: 972 3 5140014
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Internet: Mycol@Kenes.ccmil.compuserve.com
WWW: <http://Isb380.plbio.lsu.edu/ima/index.html>

19th ISSY on Yeast in the Production and Spoilage of Foods and Beverages, University of Minho, Braga, Portugal, August 30 - September 3, 1998



We look forward to your contribution to make the 19th ISSY scientifically rich and memorable, and we would like to stimulate, in particular, the attendance of younger scientists. Join us in Braga next August to enjoy both the Symposium and the beauty and hospitality of the country.

Scientific programme. The Scientific Programme of the 19th ISSY will consist of five Plenary Lectures of wide interest, eight sessions with contributed papers in thematic workshops and poster sessions. Each session is planned to include three to four invited Lectures and two Short Oral Presentations selected among the poster abstracts.

Plenary Lectures. Isak Pretorius, South Africa. "From polysaccharide utilization to yeast cell differentiation and focculation". Paul Henscke, Australia. "New generation wine yeast: biotechnology in oenology". Manfred J. Schmitt, Germany. "Yeast killer viruses: tracers in eukaryotic cell biology and useful vehicles in gene technology". Claudina Rodrigues-Pousada, Portugal. "Responsiveness of *Saccharomyces cerevisiae* to environmental stress: role of the YAP gene family". **Van Uden Lecture.** Carlos Gancedo, Spain. "Reflections on yeast metabolism".

Sessions. Session 1. Food ecology and differential diagnosis of yeasts. Coordinators: Sally A. Meyer (USA) and Larry Beuchat (USA). Session 2. Food-borne yeasts: metabolism and regulation Coordinators: Hans van Dijken (The Netherlands) and Maria C. Loureiro-Dias (Portugal). Session 3. Alcoholic beverages and ethanol tolerance. Coordinators: Graham H. Fleet (Australia) and Graham G. Stewart (UK). Session 4. Yeast activity in fermented food. Coordinators: Alan Wheals (UK) and Jose Martinet Peinado (Spain). Session 5. Yeast in food spoilage: quality control in the production chain. Coordinators: Harmen Hofstra (The Netherlands) and Tibor Deak (Hungary). Session 6. Stress in food environments: yeast physiology and molecular biology. Coordinators: Bernard Prior (South Africa) and Johan Thevelein (Belgium). Session 7. Molecular typing and rapid identification methods. Coordinators: Cletus P. Kurtzman (USA) and Ann VaughanMartini (Italy).

Session 8. Genetic improvement of yeasts for food and beverage processing Coordinators: Morten Kiehlund-Brandt (Denmark) and Claude Gaillardin (France).

Registrations will be accepted right till the start of the meeting. For additional information, contact

19th ISSY Secretariat
Department of Biology
University of Minho, Campus de Gualtar
4709 Braga Codex, Portugal

Tel.: 351 (0)53 604317
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WWW: <http://www.bio.uminho.pt/congr/ISSY98>

**Symposium "Yeast as a cell factory", Vlaardingen, The Netherlands,
30 November - 2 December 1998**

For information on this symposium please consult our web site or write to:

Dr. Jack Pronk
Kluyver Laboratory of Biotechnology
Delft University of Technology
Julianalaan 67, 2628 BC Delft, The Netherlands.

Telephone; +31 215 783214
Fax: +31 215 782355
E-mail: <j.t.pronk@stm.tudelft.nl>
WWW: <http://www.ecyeastsymp.com>

**20th International Specialized Symposium on Yeasts (ISSY)
on Cell Surfaces and Membrane Phenomena, Smolenice, Slovak Republic, May 23-27, 1999**

Invitation. You are cordially invited to participate in the 20th ISSY on "Cell Surfaces and Membrane Phenomena" organized by Czech and Slovak Yeast Commission of the Czechoslovak Society for Microbiology, and Institute of Chemistry, Slovak Academy of Sciences, under the auspices of the Federation of European Microbiological Societies (FEMS). The symposium will take place in Smolenice near Bratislava, Slovakia. This is the fourth time the ISSY is organized in Smolenice castle. The 1st ISSY was held in Smolenice in 1971, the 9th ISSY took place here in 1983, while the 14th ISSY was hosted in 1990. Thus, the beautiful castle of Smolenice, located at the foothills of the Small Carpathian mountains, is well familiar with the yeast-related topics and the yeast scientists are familiar with the excellent conditions Smolenice castle provides for the meetings. The Small Carpathian region is well known for its viticulture which is also associated with yeasts. I believe the beauty of the Smolenice castle and its picturesque surroundings will provide an excellent opportunity for the scientific presentations and discussions as well as for the informal encounters.

Organizing Committee. Peter Biely (Chairman); Libor Ebringer, Mária Vršanská (Vice-Chairpersons); Grigorij Kogan (Secretary);

Mária Czigárová (Treasurer); Nade da Kolarova, Elena Sláviková.
Program Committee: Peter Biely, Vladimír Farkaš, Grigorij Kogan, Marie Kopecká, Karel Sigler, Július Šubík. Foreign members: Hans van Dijken, Graham H. Fleet, Bernard Prior and Peter Raspor.
Scientific Program: The program of the symposium will consist of invited plenary lectures, oral contributions and poster sessions on the following topics: *Membrane Transport, Biogenesis and Structure of Cell Wall and Yeast Cytoskeleton*.
Location and Accommodation: The 20th ISSY will take place in Smolenice castle that serves as the Congress House of the Slovak Academy of Sciences. Smolenice is situated ca. 60 km from Bratislava. All scientific events will take place in the castle facilities. All participants will be accommodated and catered in the castle as well. The transportation between Bratislava and Smolenice castle will be organized by the symposium service.

Further Information: The second circular will be distributed in September, 1998 and will include information on fees, scientific and social programs, as well as on transportation, registration and instructions for the abstract submission. Contact:

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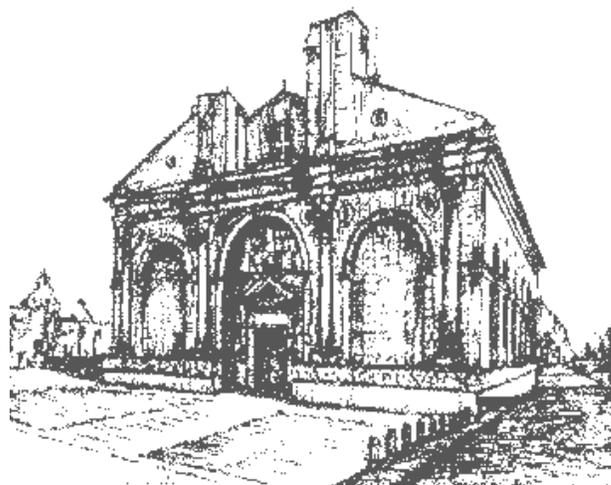
Nineteenth International Conference on Yeast Genetics and Molecular Biology, Rimini, Italy, 26-30 May, 1999

You are cordially invited to attend the Nineteenth International Conference on Yeast Genetics and Molecular Biology to be held in Rimini, Italy, on 26-30 May, 1999.

Scientific Committee: L. Frontali, L. Alberghina, C.V. Bruschi, L. Donini, I. Ferrero, G. Lucchini, A. Martini, M. Polsinelli, J. Pulitzer. **Organizing Committee:** L. Frontali, Chair, C.V. Bruschi, Secretary, N. Altamura, E. Berardi, I. Ferrero, C. Galeotti, G. Lucchini, E. Martegan, D. Porro.

Symposia. Cell cycle and checkpoints. DNA replication, recombination and repair. Signal transduction and growth control. Biotechnology and industrial applications. Cell architecture and morphogenesis. Nucleo-cytoplasmic interactions. Intracellular dynamics and transport. Telomeres, silencing and ageing. Genomics. Workshops and posters: Gene expression. Chromosome structure and function. Metabolism and metabolic regulation. Meiosis and sporulation. Organelles. New tools. Functional analysis of the yeast genome. Cell cycle and DNA replication. Recombination and repair. Biodiversity in yeasts. Signal transduction and stress response. Protein trafficking. Cell wall and morphogenesis. Others.

Location. Rimini is a historical city situated on the Adriatic coast. The symbol of its past is the Templo Malatestiano (see illustration) built by Leon Battista Alberti for Sigismondo Malatesta in the years from 1447 to 1470. Its unfinished facade is considered as a sort of "manifesto" of Italian Renaissance Architecture. In recent years Rimini has become a very popular sea-side resort. The hotels are known to offer reasonable rates and the quality of food is very good. The "Palacongressi", Italy's largest Conference Center, has a complete range of facilities including



several halls and wide spaces well-suited to our Conference. It is our intention to keep the cost of participation as low as possible, especially for students, as we are keen to attract young researchers to the conference. Rimini can be reached by train from Bologna (1 hr), Milan (3 hrs) and Rome (4 hrs), which have international airports. Excursions will be organized during and after the Conference to Ravenna (50 km) and to Urbino (70 km). A wide ranging programme of social events will be also offered to all the registered accompanying persons.

For further information, contact

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Yeasts 2000 - Tenth International Symposium on Yeasts Sunday, 27 August - Thursday 31 August 2000. Papendal, Arnhem, The Netherlands

The 10th International Symposium on Yeasts will bring together scientists from all disciplines involved in the study of yeasts and yeast-like organisms: physiologists, geneticists, taxonomists, molecular biologists, biotechnologists, food microbiologists and

medical mycologists. The Symposium will be structured for optimal interaction between scientists working in these fields, thus stimulating new developments in yeast research in the third millennium. Further information will follow in due course.

Brief News Item

The Hard Sciences and the Humanities, by W.N. Arnold

Readers with an interest in teaching, the humanities, or both might be interested in my recent article (1997). "The Hard Sciences and

the Humanities," published in *Biochemical Education* 25(4):211-214. For a reprint, contact

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The Yeasts, A Taxonomic Study, Fourth edition

Edited by C.P. Kurtzman, Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture Peoria, Illinois, USA and J.W. Fell, Rosenstiel School of Marine and Atmospheric Science University of Miami Key Biscayne, Florida, USA.

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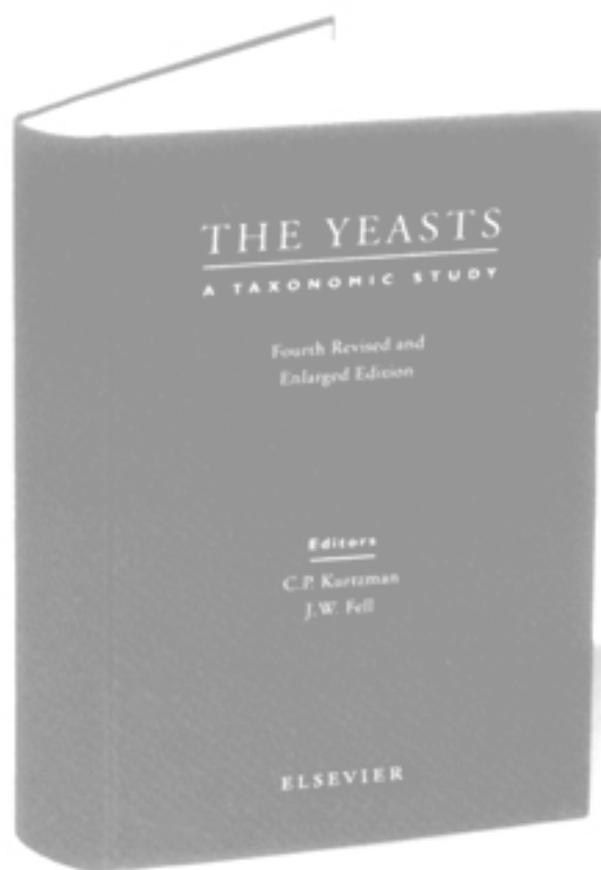
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