ISSN 0513-5222

Y E A S T

A Newsletter for Persons Interested in Yeast

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

JUNE 1997 **Volume XLVI, Number I**

Marc-André Lachance, Editor
University of Western Ontario, London, Ontario, Canada N6A 5B7

I. Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo and Fermentec s/c ltda., R. Treze de Maio, 768 s/43. CEP-13400-900, Piracicaba-SP, Brasil. Communicated by H.V. Amorim.

The following is completed research.

1. Alves, D. M. G.; Basso, L. C. & Amorim, **H.V.** 1997. The significance of temperature control in fuel alcohol fermentation.

Temperature is an important environmental aspect for all organisms and metabolic processes. Regarding the fuel alcohol production as performed in Brazil, the adequate temperature must provide high ethanol yield accompanied by the maintenance of yeast viability required for cell recycling. The temperature in tropical regions not seldom reaches 40ºC, also affecting the fermentation temperature. Data from distilleries point out a direct correlation between the increase of temperature and the raise of bacterial population, causing reduction in ethanol yield. In order to evaluate the effects of different temperatures on some biotechnological parameters of fuel alcohol fermentation, an experiment was carried out with three temperatures, 27, 33 and 38ºC. The fermentations were performed in 150 ml centrifuge tubes, using 10% (v/v) baker's yeast (*Saccharomyces cerevisiae*, Fleischmann) with semi-synthetic (15% total sugar). After fermentation (7-8 hours) yeast was separated from the fermented medium by centrifugation (800 x *g*, for 20 min.), weighed, analysed for cell viability and reused in subsequent fermentative cycles. Bacterial contamination was analysed in fermented medium prior centrifugation. The fermented medium was analysed for ethanol, glycerol and lactic acid. Trehalose was assessed in yeast at the beginning of the experiment and at the end of the last fermentative cycle. The results suggest basically the same tendencies of the distillery data, indicating that the decrease in ethanol yield is caused by an increase in glycerol production, despite of the lower cell growth and viability at 38 º C. In addition, the trehalose catabolism confirms the stressing condition that the yeast was submitted, not only by the high temperature *per se*, but also by the bacterial contamination and high concentrations of lactic acid produced by bacteria. The effective temperature control was showed to be necessary for the best performance in fuel alcohol fermentation.

II. Research Group on Black Yeasts. Centraalbureau voor Schimmelcultures, P.O. Box 273, NL-3740 AG Baarn, The Netherlands. Communicated by G.S. de Hoog.

The following manuscripts have been submitted since the appearance of the previous Yeast Newsletter:

1. Hoog G.S. de, Kuijpers, A.F.A. & Tweel, K. van den. 1997. Zeldzame schimmels: doodgewoon? Ned. Tijdschr. Med. Microbiol. (in press).
2. Feltkamp, M.C.W., Kersten, M.J., Lelie, J. van der, Hoog, G.S. de & Kuiper, E.J.. 1997. Fatal *Scedosporium prolificans* infection in a leukemic patient involving the brain, lung and skin. Eur. J. Microbiol. Infect. Dis. (in press).
3. Hoog, G.S. de: Significance of fungal evolution for the understanding of their pathogenicity, illustrated with agents of phaeohyphomycosis. 1997. Mycoses (in press).
4. Wedde, M., Müller, D., Tintelnot, K., Hoog, G.S. de & Stahl, U. 1997. PCR-based diagnostics of clinically relevant *Pseudallescheria/Scedosporium* strains. J. Med. Vet. Mycol. (submitted).
5. Verweij, P.E., Kasteren, M. van, Nes, J. van de, Hoog, G.S. de, Pauw, B.E. de & Meis, J.F.G.M. 1997. Fatal pulmonary infection caused by the Basidiomycete *Hormographiella aspergillata*. J. Clin. Microbiol. (in press).
6. Sterflinger, K., Baere, R. de, Hoog, G.S. de, Wachter, R. de, Krumbein, W.E. & Haase, G. 1997. *Coniosporium perforans* and *C. apollinis*, two rock-inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). Antonie van Leeuwenhoek (submitted).

The following papers have appeared since December:

7. Hoog, G.S. de. Risk assessment of fungi reported from humans and animals.1997 Mycoses 39: 107-117.
8. Wollenzien, U., Hoog, G.S. de, Krumbein, W. & Uijthof, J.M.J. 1997 . *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. Antonie van Leeuwenhoek 71: 281-288.

9. Hoog, G.S. de, Beguin, H. & Batenburg-van de Vegte, W.H. 1997. *Phaeotheca triangularis*, a new meristematic black yeast from a humidifier. Antonie van Leeuwenhoek 71: 289-295.

III. 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. Babjeva I.P. 1997. *Tausonia pamirica* gen.nov. sp.nov. - the psychrophilic yeast-like micromycete from Pamir. Mikrobiologia (Microbiology) (in Russian, in press).

Nine strains of unknown yeast-like fungi with maximum temperature of growth 18-19°C have been isolated from high-mountain soils of East and West Pamir. This organism is described as the species of the new genus of primitive obligate psychrophilic basidiomycetes with yeast-like growth.

2. Babjeva I.P. & Chernov I.Yu. 1995. Geographical aspects of yeast ecology. Physiol. Gen. Biol. Rev. **9**(3): Ecological Microbiology, 1-54

The original long-term investigations of geographic distribution of yeasts are reviewed in terms of typology of yeast adaptive complexes in natural substrata.

3. Babjeva I.P., Reshetova I.S. 1996. Taxonomic analysis of yeasts in the Far-Eastern regions of Russia. Mikologia i Phytipatologia (Mycology and Phytopathology) 30:10-18 (in Russian).

Taxonomic analysis of yeasts isolated from soils and other natural substrates in Far-Eastern regions of Russia was carried out and 45 species were found. Six species were found to be new for Russia. Geographical limits for some yeast species, especially for *Lipomyces* spp., were conformed.

4. Byzov B.A., Vu Nguen Thanh, Babjeva I.P., Tretyakova E.V., Dyvak I.A & Rabinovich Y.M. Killing and hydrolytic activities of the gut fluid of the soil millipede Pachyiulus flavipes C.L.Koch. Soil Biology and Biochem. (in press).

5. Byzov B.A., Kurakov A.V., Tretyakova E.B., Vu Nguyen Thanh, Nguyen Duc To Luu and Rabinovich Ya.M. Principles of the digestion of microorganisms in the gut of soil millipedes: specificity and possible mechanisms. Applied Soil Ecology (in press).

6. Chernov I.Yu. 1997. Microbial diversity: new possibilities of an old method. Mikrobiologia (Microbiology),, **66**:91-97 (in Russian).

The diluting plate method is known to be unreliable for detection of real ratio between microbial species in natural habitats. Nevertheless, several statistical conformities in yeast communities structure investigated by diluting plate method with differential counting of colonies are demonstrated, namely: i) the decreasing of alpha- and beta-diversity along a spatial-succession range; ii) the changing of distribution pattern from MacArthur model to geometric one along the same range; iii) the constancy of syntypological structure of yeast communities within a geographical zone in spite of taxonomic differences. Such conformities should be considered as indirect evidence of the fact that diluting plate method statistically reflects the real ratios between yeast species. Therefore the diluting plate method with differential counting of colonies is quite suitable for quantitative estimating of diversity of homogenous model microbial groups.

7. Zvyagintsev D.G., Babjeva I.P., Zenova G.M. & Polyanskaya L.M. 1996. Diversity of fungi and actinomycetes in soils and their ecological functions. Pochvovedenie (Soil Science) **6**:705-713 (in Russian).

For many years the authors investigated numerous microorganisms with the view of their geographical population in the main soil types. The long term data for mycelial and yeast-like fungi, and actinomycetes are summarized in the present report. A clearly expressed correlation is shown between taxonomic composition of microbial complexes and their ecological functions. Several environmental factors are shown to be responsible for structure of microbial complexes, which reflects adaptability of microorganisms to natural and climatic conditions of soil zones. Further discussed is the succession pattern, capable to make more complete accounting the microbial diversity of soils.

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

1. Golubev, W.I. & Nakase, T. 1996. Anti-*Cryptococcus* and anti-*Trichosporon* activity of *Bullera* species. Abstr. 3rd Int. Conf. "*Cryptococcus* & cryptococcosis" (Sept. 22-26, 1996, Paris), 173.

2. Golubev, W.I, Ikeda, R., Shinoda, T. & Nakase, T. 1996. Mycocinogeny in the genus *Bullera*: anti-tremellaceous yeast activity of killer toxin produced by *Bullera hannae*. J. Gen. Appl. Microbiol. **42**:471-479.

The type strain of *Bullera hannae* produces a thermolabile protease-sensitive fungicidal toxin that was specified as mycocin. This killer toxin did not act against ascomycetous, sporidiobolaceous and ustilaginaceous yeasts. Its killing pattern is restricted to tremellaceous yeasts and includes the species of *Fibulobasidium, Filobasidium, Holtermannia, Sirobasidium, Trimorphomyces,* and *Tsuchiyaea*. The members of the genera *Bulleromyces, Cystofilobasidium, Mrakia, Sterigmatosporidium,* and *Xanthophyllomyces* were insensitive to this mycocin. The anamorphic genera *Bullera, Cryptococcus, Fellomyces, Kockovaella, Trichosporon,* and *Udeniomyces* were heterogeneous in this respect. The taxonomic significance of the sensitivity to the mycocin of *B. hannae* is discussed.

3. Golubev, W. & Nakase, T. 1997. Mycocinogeny in the genus *Bullera*: taxonomic specificity of sensitivity to the mycocin produced by *Bullera* sinensis. FEMS Microbiol. Lett. **146**:59-64.

A strain of *Bullera* sinensis that secretes a fungicidal thermolabile and protease-sensitive toxin was found. Its killer phenotype was cureless. Ascomycetous, ustilaginaceous yeasts and the members of the Sporidiales are insensitive to the *B. sinensis* killer toxin. The mycocin acts against tremellaceous yeasts only, except for *Cystofilobasidium, Fellomyces* and *Trichosporon* spp. The genera *Mrakia, Bullera, Cryptococcus* and *Udeniomyces* are heterogeneous in sensitivity patterns. The different responses of some anamorphic species to this mycocin are often consistent with the origin of the strains and differences in sexual, chemotaxonomic and physiological characteristics between them.

V. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan. Communicated by M. Hamamoto <hamamoto@ulmus.riken.go.jp> and T. Nakase <nakase@ulmus.riken.go.jp>.

The Japan Collection of Microorganisms (JCM) has been operating as a culture collection of microorganisms since 1980, in the Institute of Physical and Chemical Research (RIKEN), a semi-governmental research institute supported by the Science and Technology Agency, Japan. JCM is directed by Dr. T. Nakase and has eleven researchers and seven supporting staff members. There is a wealth of information for microbiologists on the Internet provided by JCM. The URL for the JCM home page is *http://www.jcm.riken.go.jp/*.

Research of the yeast investigators in JCM is focused on the molecular systematics and the search of new taxa from various regions of the world. The philosophy of the research activities on yeast systematics in JCM is to combine physiological studies with biochemical and molecular techniques, and approaches through polyphasic analysis.

The research yeast group consists of four staff investigators and one postdoctoral fellow. Dr. T. Nakase covers general overview of the systematics of yeasts. Dr. M. Suzuki mainly carries out the taxonomic study of ascomycetous yeasts. Dr. M. Hamamoto focuses the systematics of basidiomycetous yeasts. Ms. Takashima focuses her research on the cell wall carbohydrate composition and DNA sequence analyses of ballistoconidium-forming yeasts. Dr. T. Sugita is now interested in the development of the molecular techniques for yeast taxonomy.

Besides the staff researchers of JCM, many visiting researchers from various countries have been carrying out their research in JCM recently. Dr. W. I. Golubev from All-Russian Collection of Microorganisms (VKM) in Russia who had stayed here for one year focused on the taxonomic evaluation of the mycocin produced by yeasts. Mr. B. Haryono from Gadjah Mada niversity in Indonesia for three and a half months studied the taxonomic study of yeasts isolated from plants collected in Indonesia. At present, Ms. Connie F. C. Gibas from University of the Philippines Los Baños in Philippines, Dr. V.N. Thanh from Vietnam, Dr. F.-Y. Bai from Institute of Microbiology, Academia Sinica in China, and Mr. B. Fungsin from Thailand Institute of Scientific and Technological Research in Thailand stay in JCM and collaborate with us on research of mutual interest in yeast systematics. Some of the above visiting researchers are supported by the Asian Network of Microbial Research (ANMR) of the Japanese Government.

The following articles have been published by our group from 1995 to the present.

1. Hamamoto M. & Nakase T. 1995. Ballistosporous yeasts found on the surface of plant materials collected in New Zealand. I. Six new species in the genus *Sporobolomyces*. Antonie van Leeuwenhoek **67**:151- 171.

2. Nakase T., Suh S.-O. & Hamamoto M. 1995. Molecular systematics of ballistoconidium-forming yeasts. Studies in Mycology **38.**

3. Suh S.-O. & Nakase T. 1995. Phylogenetic analysis of the ballistosporous anamorphic genera *Udeniomyces* and *Bullera*, and related basidiomycetous yeasts based on 18S rDNA sequences. Microbiology **141**:901-906.

4. Takashima M., Suh S.-O. & Nakase T. 1995. Phylogenetic relationships among species of the genus Bensingtonia and related taxa based on the small subunit ribosomal DNA sequences. J. Gen. Appl. Microbiol. **41**:131-141.

5. Takashima M., Suh S.-O. & Nakase T. 1995. *Bensingtonia musae* sp. nov. isolated from a dead leaf of *Musa paradisiaca* and its phylogenetic relationship among basidiomycetous yeasts. J. Gen. Appl. Microbiol. **41**:143-151.

6. Golubev W., Ikeda R., Shinoda T. & Nakase T. 1996. Mycocinogeny in the genus *Bullera*: Anti-tremellaceous yeast activity of killer toxin produced by *Bullera hannae*. J. Gen. Appl. Microbiol. **42**:471-479.

7. Hamamoto M. & Nakase T. 1996. Ballistosporous yeasts found on the surface of plant materials collected in New Zealand. The genera *Bensingtonia* and *Bullera* with descriptions of five new species. Antonie van Leeuwenhoek **69**:279-291.

8. Nakase T., Suzuki M., Hamamoto M., Takashima M., Hatano T. & Fukui S. 1996. A taxonomic study on cellulolytic yeasts and yeast-like microorganisms isolated from Japan. II. The genus *Cryptococcus*. J. Gen. Appl. Microbiol. **42**:7-15.

9. Prillinger H., Messner R., König H., Bauer R., Lopandic K., Molnar O., Dangel P., Weigang F., Kirisits T., Nakase T. & Sigler L. 1996. Yeasts associated with termites : A phenotypic and genotypic characterization and use of coevolution for dating evolutionary radiations in asco- and basidiomycetes. Syst. Appl. Microbiol. **19**:265-283.

10. Suh S.-O., Takashima M. & Nakase T. 1996. Phylogenetic study of the basidiomycetous anamorphic yeasts *Rhodotorula lactosa* and *R. minuta*, and related taxa based on 18S rDNA sequence. J. Gen. Appl. Microbiol. **42**:1-6.

11. Suh S.-O., Takashima M., Hamamoto M. & Nakase T. 1996. Molecular phylogeny of the ballistoconidium-forming anamorphic east genus *Bullera* and related taxa based on small subunit ribosomal DNA sequences. J. Gen. Appl. Microbiol. 42: 501-509.

12. Suh S.-O., Takematsu A., Takashima M. & Nakase T. 1996. Molecular phylogenetic study on stalked conidium-forming yeasts and related basidiomycetous yeast taxa based on 18S rDNA sequences. Microbiol. Cult. Coll. **12**:79-86.

13. Takashima M., Suh S.-O. & Nakase T. 1996. Group I introns found in nuclear small subunit ribosomal RNA genes of the ballistoconidiogenous anamorphic yeast *Bensingtonia ciliata* and *Bensingtonia yamatoana*. J. Gen. Appl. Microbiol. **42**:189-200.

14. Takashima M. & Nakase, T. 1996. A phylogenetic study of the genus *Tilletiopsis, Tilletiaria anomala* and related taxa based on the small subunit ribosomal DNA sequences. J. Gen. Appl. Microbiol. **42**:421-429.

15. Golubev W. & Nakase T. 1997. Mycocinogeny in the genus *Bullera*: taxonomic specificity of sensitivity to the mycocin produced by *Bullera sinensis*. FEMS Microbiol. Lett. **146**:59-64.

VI. Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Communicated by H. Lee.

The following is the abstract of a paper which was published recently.

1. Zhang, Y. & H. Lee. 1997. Site-directed mutagenesis of the cysteine residues in the *Pichia stipitis* xylose reductase. FEMS Microbiology Letters **147:**227-232.

Xylose reductase catalyzes the reduction of xylose to xylitol and is known to play a pivotal role in pentose metabolism in yeasts. We previously showed that a cysteine residue may be involved in binding of the coenzyme NADPH to the *Pichia stipitis* xylose reductase through chemical modification studies. The question arose as to which of the 3 cysteine residues in this enzyme may be involved in coenzyme binding. We cloned the *XYL1* gene encoding xylose reductase from P. stipitis into the phagemid pEMBL18(+) suitable for site-directed mutagenesis. Each of the three cysteine residues (Cys19, Cys27 and Cys130) was individually mutated to serine. All three Cys to Ser variants remained functional, but with reduced catalytic activity. Sensitivity of the *P. stipitis* xylose reductase to thiol-specific reagents was attributed to both Cys27 and Cys130 residues as substitution of either residue with Ser resulted in a significant but incomplete loss of sensitivity to PCMBS. The apparent K_m values of the Cys variants for NADPH, NADH and xylose did not differ from those of the wild type enzyme isolated from yeast by more than 4-fold. Our results suggest that none of the Cys residues are directly involved in NADPH binding, although Cys130 may reside in or near the coenzyme binding region and might play a role in coenzyme specificity.

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

Recent publication.

1. K. Riedel & G. Kunze. 1997. Rapid physiological characterization of microorganisms by biosensor technique. Microbiol. Res. (in press).

Eleven microorganisms, *Arxula adeninivorans* LS3, *Candida boidinii* DSM 70034, *Candida lactis-condensi* DSM 70635, *Pichia jadinii* DSM 2361, *Pichia minuta* DSM 7018, *Kluyveromyces lactis* DSM 4394, *Pseudomonas putida* DSM 50026, *Alcaligenes spec.* DSM 30002, *Arthrobacter nicotianae* DSM 20123 as well as *Issatchenkia orientalis* DSM 70077 and *Rhodococcus erythropolis* DSM 311 were characterized by the sensor technique by injection of 30 different substrates and substrate mixtures. The obtained data which are based on the determination of respiratory rate of microorganisms are similar to physiological characteristics obtained with conventional methods. In comparison to these conventional methods the sensor technique works much more rapid and permits quantification of the data. Therefore, the described technique provides an alternative method for the characterization of microorganisms.

VIII. Department of Food Microbiology and Toxicology and Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706. Communicated by E. A. Johnson.

1. W.A. Schroeder, P. Calo, M.L. DeClercq & E.A. Johnson. 1996. Selection for carotenogenesis in the yeast *Phaffia rhodozyma* by dark-generated singlet oxygen. Microbiology **142**:2923-2929.

Selection for carotenogenesis in *Phaffia rhodozyma* was achieved by exposure of yeast strains to dark chemical reactions that generate singlet oxygen. Incubation of a mixture of *P. rhodozyma* strains containing varying levels of carotenoids in hypochlorous acid or hydrogen peroxide resulted in weak selection for pigmented strains. However, the combination of hydrogen peroxide and hypochlorous acid was strongly selective for carotenogenesis and gave a monoculture of a carotenoid hyperproducer. Exposure of the yeast to ozone for 10 to 20 minutes also selected for a hyperproducing strain. These selections were relieved by 1,4- diazabicyclo [2.2.2]- octane, a specific quencher of singlet oxygen or by L-ascorbic acid. Continuous growth of *P. rhodozyma* on agar plates in an ozone/air atmosphere for 5 days decreased astaxanthin and total carotenoid levels and increased the levels of carotenoid biosynthetic intermediates. Repeated rounds of random mutagenesis followed by ozone exposure yielded mutant strains with higher pigmentation than control cultures. Our results support the hypothesis that a primary function of carotenoids in *P. rhodozyma* is to protect against singlet oxygen generated in the yeast's natural environment, and that a practical method for preventing strain degeneration during industrial fermentations may be achieved by generation of singlet oxygen using simple chemical supplements or by bubbling ozone through *P. rhodozyma* cultures during fermentation.

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

1. Jungová, O., Minárik, E. 1997. *Zygosaccharomyces bailii* - an osmotolerant spoiling yeast. Vinarsky Obzor **90**:1-2:l6-17 (in Czech).

The occurrence of haploid *Zygosaccharomyces bailii* in bottled wine is possible only by contamination during bottling. Total decontamination by final membrane filtration as well as decontamination of all contamination sources in the course of bottling may prevent contamination. It is underlined that contaminations by *Z. bailii* is often caused by concentrated grape must. Biotechnological aspects of this phenomenon are discussed.

2. Malík, F., Vollek, I,, Valkovičová, B., Vojteková, G. 1996. Contribution to the knowledge of the cellar microflora (in Slovak). Vinohrad **34**:l38-140.

118 representatives of the accompanying microflora of the wine cellar (22 yeast, 44 bacteria and 52 mould strains) were isolated from barrels, cellar walls and floors in different wineries in Slovakia and Moravia. A detailed survey on the occurrence of microorganisms and a brief description is presented.

3. Malík, F., Satko, J., Vollek, V. 1997. Some biochemical properties of wine yeasts *Saccharomyces cerevisiae* of the FV SAT Series. (in Slovak) Vinohrad **35**:14-16.

Acidification properties and respiration activity of new *Saccharomyces cerevisiae* isolates had been examined. The most vigorous acidification strength was observed with *S. cerevisiae* strain FV SAT 3 (pH_{20} = 1.83) and FV-SAT 5 (pH_{20}; = 1.74). The highest oxygen consumption of the endogenous respiration was registered with *S. cerevisiae* strains 13 RVV (22.15), FV SAT 3 (13.62) and 6 C(12,10) (calculated in $nmol.min^{-1}.mg^{-1}$).

4. Malík, F., Satko, J., Vollek, V. 1997. *Zygosaccharomyces rouxii* - contaminants of the concentrated grape must (in Slovak. Kvasný prumysl). **43**:39-41.

From concentrated grape must a contaminating yeast strain had been isolated and identified as *Zygosaccharomyces rouxii*. Basic morphological and some biochemical properties (acidifiction power, respiratory activity) were determined. This strain is not suitable for primary or secondary fermentations of must or wine.

X. State Institute for Genetics and Selection of Industrial Microorganisms, I Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov.

1. G. I. Naumov,1 E. S. Naumova,' & P. D. Sniegowski[1] Int. J. Syst Bacteriol, 1997. Differentiation of European and Far East Asian populations of *Saccharomyces paradoxus* by allozyme analysis. **47**:341-344.

[1]Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824-1325.

Allozyme electrophoresis was used to characterize 39 isolates belonging to the wild yeast species *Saccharomyces paradoxus* for variation at nine enzyme loci. The data revealed significant genetic differentiation between isolates from two geographically distinct regions, one including continental Europe and the other including the Russian Far East and Japan. The results are consistent with previous observations indicating that there is partial reproductive isolation between isolates collected from these regions, and they suggest the possibility that these two populations represent an early stage in speciation.

2. G.I. Naumov, E.S. Naumova, V.I. Kondratieva, S.A. Bula 1, N.V. Mironenko[2], L.C. Mendonca-Hagler[3] and A.N. Hagler[3]. 1997. Genetic and molecular delineation of three sibling species in the *Hansenula polymorpha* complex. System. Appl. Microbiol. **20**:50-56.

[1]Petersburg Nuclear Physics Institute, Russia. [2]All-Russian Plant Protection Institute, St-Petersburg-Pushkin, Russia.
[3]Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Genetic hybridization, molecular karyotyping and UP-PCR analysis showed that the taxonomic complex *Hansenula polymorpha* De Morais et Maia consists of three biological sibling species. *H. angusta* Teunisson *et al*. (= *Pichia angusta* Teunisson *et al*.) Kurtzman) is not synonymous with *H. polymorpha* and must be reinstated as a separate species. The third sibling species is apparently a new taxon associated with *Opuntia* cacti. The sibling species are able to cross with each other but their interspecific hybrids are sterile.

XI. Departamento de Bioquimica, Instituto de Química, Universidade Federal do Rio de Janeiro. CT Bloco A, Lab 547, 21949-900 Rio de Janeiro, RJ, Brasil. Communicated by A.D. Panek <anita@vms1.nce.ufrj.br>.

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

1. P.M.B. Fernandes, A.D. Panek & E. Kurtenbach[1]. 1997. Effect of hydrostatic pressure on a mutant of *Saccharomyces cerevisiae* deleted in the trehalose-6-phosphate synthase gene. FEMS. Microbiol. Lett. (accepted).

[1]Departamento de Bioquímica Médica, ICB, UFRJ, 21941-590 Rio de Janeiro, RJ, Brazil.

Mutants of *Saccharomyces cerevisiae* deleted in the trehalose-6-phosphate synthase gene (*tps1*) and their parental wild type cells were submitted to hydrostatic pressure in the range of 0 to 200 Mpa. Experimental evidence showed that viability for both strains decreased with increasing pressure and that *tps1* mutants, unable to accumulate trehalose, were more sensitive to hydrostatic pressure than the wild type cells. Additionally, both *tps1* and wild type cells in the stationary phase, when there is an accumulation of endogenous trehalose, were more resistant to pressure than proliferating cells. Under these conditions, mutant cells were also more sensitive to pressure treatment than the wild type. The present work also showed that low pressure pretreatment did not induce hydrostatic pressure resistance (barotolerance) in yeast cells.

2 P.M.B.Fernandes & A.D.Panek .1996. Mating pheromone response in *Saccharomyces cerevisiae* deleted in the trehalose synthase complex. Microbiology **143**:689-690.

Saccharomyces cerevisiae a and α haploid cells secrete a peptide mating factor that signals cells of opposite mating type to undergo many physiological changes. When haploid yeast cells are exposed to the mating factor for a prolonged period, cells become adapted to the presence of the pheromone and resume proliferation. Trehalose accumulates in cells exposed to non optimal physiological conditions and it enhances cell resistance to external stress conditions. The *TPS1* gene of *S. cerevisiae* codes for the small subunit of trehalose-6-phosphate synthase and mutants in this gene are unable to accumulate the disaccharide trehalose. Nevertheless, differences in *TPS1* and *tps1* isogenic yeast strains are broader than a mere proficiency or deficiency in trehalose accumulation. In order to study further the pleiotropic effects of the *tps1* mutation we have analyzed the effects of pheromone treatment on a *tps1* prototrophic mutant compared to the isogenic wild-type *TPS1* strain. Wild-type cells treated with a factor arrest in G1 with a typical cell topology known as shmoo cells. This phenotype is observed 1 hour after pheromone addition and after 8 h of treatment approximately 80% of the population is still arrested. Analyzing the *tps1* mutant treated with a factor we noticed that these cells were equally sensitive to the mating pheromone. However, 8 h after a factor had been added the number of shmoo cells had dropped to 40%. These results indicate that the gene mutation or the lack of trehalose-6-phosphate lead to a faster desensitization of the cell to the mating pheromone a factor. We investigated whether trehalose-6-phosphate itself would lead to the behavior of the wild-type strain in the adaptation to pheromone. We assayed a *tps2* mutant strain, deleted in the trehalose-6-phosphate phosphatase, which accumulates trehalose-6-phosphate in response to stress. When treated with a factor those cells showed the same profile as the wild-type, indicating a possible role of trehalose-6-phosphate in the recovery pathway.

XII. Toulouse Levure Club-Yeast Toulouse Club, Centre de Bioingenierie Gilbert Durand, UMR-CNRS 5504 Lab. Ass INRA, 31007 Toulouse Cedex 04. Communicated by J.M. François <fran_jm@insa-tlse.fr>.

The **Toulouse Levure Club** is an association of yeast researchers of Midi-Pyrenées, who meet one a year to exchange ideas and materials. The interest ranges from cell cycle to fermentation aspects including metabolism and recombinant technologies. Industrial groups Are also invited. The group is coordinated by Prof. Jean M. François.

The '**Cell cycle**' Group' is coordinated by Prof. Bernard Ducommun, Université Paul Sabatier, Institut de Pharmacologie et de Biologie Structurale, IPBS- CNRS UPR9062, 205, route de Narbonne, 31077 Toulouse Cedex <ducommun@ipbs.fr>. Theme: Cell cycle regulation in Schizosaccharomyces cerevisiae. Role of cdks and cdc25 inhibitors.

1. Leroy, D., Birck, C., Brambilla, P., Samama, J.P., and Ducommun, B. 1996. Characterization of human cdc2 lysine 33 mutations expressed in the fission yeast *Schizosaccharomyces pombe*. FEBS Letters **379**:217-221.
2. Tournier, S., Leroy, D., Goubin, F., Ducommun, B. and Hyams, J.S. 1996. Heterologous expression of the human cyclin-dependent kinase inhibitor p21Cip1 in fission yeast *Schizosaccharomyces pombe* reveals a role for PCNAin the chk1 cell cycle checkpoint pathway. Mol. Biol. Cell. **7**:651-662.

3. Belenguer, P., Pelloquin, L., Baldin, V., Oustrin, M-L. and Ducommun, B. 1995. The fission yeast nim1 kinase : A link between nutritional state and cell cycle control. Progress in Cell Cycle Research **1**:207-214 (Meijer, L., Guidet, S. and Tung, H.Y.L., eds) Plenum Press, New York, USA.

4. Belenguer, P., Pelloquin, L., Oustrin, ML. and Ducommun, B. 1997. Role of the fission yeast nim1 kinase in the cell cycle response tonutritional signals. B.B.R.C **232**:204-208.

Thesis

5. Leroy, D. 1996. Contribution à l'étude des relations entre la structure et les fonctions de la protéine kinase Cdc2. Thèse Université Paul Sabatier, Juin 1996.

The '**Biogenesis of RNA**' Group is coordinated by Michele Caizergues-Ferrer, Yeast Ribosomes Biogenesis Laboratory, LBME du CNRS, 118 route de Narbonne, 31062 Toulouse Cedex. Theme: Factors implicated in trans in ribosome biogenesis; implications of snoRNAs (small nucleolar RNAs) as guides of ribosomal RNA modifications, ribose methylation and pseudouridylation (cf 1,2 3,7); characterization of the components of these snpRNPs by genetic and biochemical approaches (cf 6); characterization of factors implicated in nucleolus structure (cf 4,5) by genetic and electron microscopy approaches.

6. Qu, L.H., Henry, Y., Nicoloso, M., Michot, B., Azum, M.C., Renalier, M.H., Caizergues-Ferrer, M. and Bachellerie, J.P. 1995. U24, a novel intron-encoded small nucleolar RNA with two 12 nt. long, phylogenetically conserved complementarities to 28S rRNA. Nucleic Acids res. 23:2669-2676.

7. Kiss-Làszlo, S., Henry, Y., Bachellerie, J.P., Caizergues-Ferrer, M., Kiss, T. (1996) Site-specific ribose methylation of preribosomalRNA: a novel function for small nucleolar RNAs. Cell **85**:1077-1088.

8. Ganot, P., Caizergues-Ferrer, M., Kiss T. 1997. The family of box ACA small nucleolar RNAs is defined by an evolutionarily conserved secondary structure and ubiquitous sequence elements essential for RNA accumulation. Genes & Development **11**:946-972.

In press:

9. Gulli, M.P., Faubladier, M., Sicard, H., Caizergues-Ferrer, M. 1996. Mitosis-specific phosphorylation of gar2, a fission yeast nucleolar protein structurally related to nucleolin. Chromosoma.

10. Léger-Silsertre, I., Gulli, M.P., Noillac-Depeyre, J., Faubladier M., Sicard, H., Caizergues-Ferrer, M., Gas, N. (1996) Ultrastructural changes in the *S.pombe* nucleolus following the disruption of the gar2+ gene which encodes a nucleolar protein structurally related to nucleolin. Chromosoma.

11. Venema, Y., Bousquet-Antonelli, C., Gélugne, J.P., Caizergues-Ferrer, M.,Tollervey, D. 1996. Rok1 is a potential RNA helicase required for pre-rRNAprocessing. Mol. Cell. Biol.

12. Bousquet-Antonelli, C., Henry, Y., Gélugne, J.P., Caizergues-Ferrer, M., Kiss, T. 1997. A small nucleolar RNP protein is required for pseudouridylation ofeukaryotic RNAs. EMBO J.

The '**Yeast metabolism and fermentation Group**' is coordinated by Prof. Jean M. François. Molecular Microbial Physiology group, Centre de Bioingenierie Gilbert Durand, Departement de Génie Biochimique et Alimentaire, INSA Toulouse. Theme and key words: regulation of carbon metabolism and of cell wall assembly in response to nutrient and to stress in *Saccharomyces cerevisiae*; metabolic regulation of reserve carbohydrates; molecular and biochemical studies on the yeast cell wall; metabolic engineering and strategy of fermentation.

13. Daran, J.M. & François, J. 1996. Récentes avancées dans les connaissances biochimiques et génétiques de la paroi des levures: Applications biotechnologiques et pharmacologiques. Regard sur la Biochimie **2**:20-31.

14. Estrada, E., Agostinis, P., Vandenheede, J.R., Goris, J., Merlevede, W., François,J., Goffeau, A. & Ghislain, M. 1996. Phosphorylation of yeast plasma membrane H+-ATPase by casein kinase 1. J. Biol. Chem. **271**:32064-32072.

15. Guillou, V., Queinnec, I., Uribelarrea, J.L & Pareilleux, A. 1996. On-line sensitive lightness measurement of cell mass in *Saccharomyces cerevisiae* culture. J. Biotechnol. **10**:19-24.

16. François, J., Blazquez, M.A., Rino, J. & Gancedo, C. 1997. Storage carbohydrates in the yeast *Saccharomyces cerevisiae*. Yeast sugar metabolism (F.K. Zimmermann & K.D Entian, eds.) pp. 285-311, Technomics Publishing Co, PA.

17. Parrou, J.L., Teste, M.A. & François, J. 1997. Effects of various types of stresses on reserve carbohydrates metabolism in *Saccharomyces cerevisiae*: Genetic evidences for a stress-induced recycling of glycogen and trehalose. Microbiology (in press).

18. Daran; J.M., Bell, W. & François, J. 1997. Physiological effects of genetic alterations leading to a reduced synthesis of UDP-glucose in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. (in press).

19. Gonzalez, B., François, J. & Renaud, M. 1997. A rapid and reliable method for metabolite extraction in yeast using boiled buffered ethanol. Yeast, (in press).

20. Parrou, J.L. & François, J. 1997. A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. Anal. Biochem. (in press).

Theses:

21. Guillou, V. 1996. Étude du comportement dynamique de *Saccharomyces cerevisiae* en culture continue dans la région oxydative des taux de croissance. These n°412, décembre 1996, INSA Toulouse.

22. Parrou, J.L. 1997. Phénomènes de stress chez *Saccharomyces cerevisiae*. Réponse transcriptionnelle des gènes impliqués dans le métabolisme des sucres de réserves. Thèse, mars 1997, INSA Toulouse.

The '**Yeast as a tool for heterologous production of molecules Group**' is coordinated by Dr. G. Loison, Sanofi Recherche, Genetic and Cell Biology Laboratory, Microbiology Department, Labege Innopole Voie N°1, BP137, 31376 Labege <gerard.loison@tls1.elfsanofi.fr>.

Theme: The Sanofi research laboratory is involved in genetic studies using recombinant microoganisms as tools to produce molecules of interest or as models to study the effects of molecules on cell biology. Recently published works include the following papers:

23. Joseph-Liauzun E., Farges, R., Le Fur G. Ferrara, P., & Loison, G. 1995. High level production of a human membrane protein in yeast: the peripheral type benzodiazepine receptor. Gene **155**:195-199.

23. Jara, P. , Delmas, P., Razanamparany, V., Olsen, L., Dupin, P., Bayol, A., Begueret, J. Loison G. 1995. Self-cloning in filamentous fungi. Application to the construction of endothiapepsin overproducers in *Cryphonectria parasitica*. J. Biotechnol **40**:111-120.

24. Valverde, V., Delmas, P., Kaghad, M., Loison, G., Jara, P. 1995. Secretion and maturation study of endothiapepsin in *Saccharomyces cerevisiae*. A first step toward improving its substrate specificity. J. Biol. Chem. **270**:15821-15826.

25. Silve, S., Leplatois, P., Josse, A., Dupuy, P. H., Lanau, C., Kaghad, M., Dhers, C., Picard, C., Rahier, A., Taton, M., Le Fur, G., Ferrara, P., Loison, G. 1996. The immunosuppressant SR 31747 blocks cell proliferation by inhibiting a steroid isomerase in *Saccharomyces cerevisiae*. Mol. Cel. Biol. **16**:2719-2727.

26. Silve, S., Dupuy, P.-H., Labit-Le Bouteiller,C., Kaghad, M., Chalon, P., Rahier, A., Taton, M., Lupker, J., Shire, D., Loison, G. 1996. Emopamil-binding protein, a mammalian protein that binds a series of structurally-diverse neuroprotective agents exhibits Δ8-Δ7 sterol isomerase activity in yeast. J. Biol. Chem **271**:2234-22440.

27. Legoux, R., Lelong,; P., Jourde, C.,Feuillerat, C., Capdevielle J., Sure, V., Ferran, E., Kaghad, M.,Delpech, B., Shire, D., Ferrara, P., Loison, G., Salome, M. 1996. N-acetyl heparosan lyase of *Escherichia coli* K5: gene cloning and expression. J. Bacteriol. **178**:7260-7264.

XIII. National Collection of Yeast Cultures, AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom. Communicated by I.N. Roberts.

Following a long-running government review the National Collection of Yeast Cultures (NCYC) is to remain at the Institute of Food Research, Norwich. A new catalogue will be published mid-late 1997. Details of the collection and collection services are also available at: *http://www.ifrn.bbsrc.ac.uk/ncyc/*. Recent publications include:

1. James, S A, Collins, M D, Roberts, I N. 1996. Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera *Zygosaccharomyces* and *Torulaspora*. Int. J. Syst. Bacteriol. **46**:189-194.
2. Cai, J, Roberts, I N and Collins, M D. 1996. Phylogenetic relationships among members of the ascomycetous yeast genera *Brettanomyces, Debaryomyces, Dekkera* and *Kluyveromyces* as deduced by small sub-unit ribosomal RNA gene sequences. Int. J. Syst. Bacteriol. **46**:542-549.
3. Roberts, I N, Weldon, L M, Bond, C J, Furze, J M, James, S A, Cai, J, Collins, M D. 1996. Genetic interrelationship amongst yeasts. Proceedings of the Eighth International Congress for Culture Collections, Veldhoven, The Netherlands.
4. Roberts, I N, Weldon, L M, Bond, C J, Furze, J M, James, S A, Cai, J, Collins, M D. 1996. The National Collection of Yeast Cultures: a diversity of databases. Systematics Association Meeting, University of Kent, UK.
5. James, S A, Cai, J, Collins, M D, Roberts, I N. 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.
6. James, S A, Collins, M D, Roberts, I N. 1997. A phylogenetic analysis of the genus *Williopsis* based on 18S rRNA gene sequences. Int. J. Syst. Bacteriol. (submitted).

XIV. Collection of Yeasts of Biotechnological Interest, Laboratoire de génétique Moléculaire et Cellulaire, INRA-INAPG- 78850 Thiverval-Grignon, France. Communicated by Nguyen Huu-Vang <clib@cardere.grignon.fr>.

1. S. Piredda & C. Gaillardin. 1994. Development of a transformation system for the yeast *Yamadazyma* (*Pichia) ohmeri*. Yeast **10**:1601-1612.

This communication describes the development of genetic tools for the yeast *Yamadazyma (Pichia) ohmeri*. Auxotrophic mutants was isolated after nystatin enrichment. LEU2 and URA3 were isolated by PCR and sequenced. DNA transformation was accomplished by electroporation mediated by plasmids p.LEU02 and p.URA03. The sequences are available for consultation under EMBL accession number Z35101 for Y.LEU2 and Z35100 for Y.URA3.

2. A. Romano, S. Casaregola, P. Torre, and C. Gaillardin. 1996. Use of RAPD and mitochondrial DNA RFLP for typing of *Candida zeylanoides* and *Debaryomyces hansenii* yeast strains isolated from cheese. System. Appl. Microbiol. **19**:255-264.

In order to evaluate the genetic diversity of the yeast flora in Northern Spain cheese, we adapted two molecular techniques devised for Saccharomyces, RAPD and RFLP of mitochondrial DNA to type 27 *Candida zeylanoides* strains and 28 *Debaryomyces hansenii* strains isolated from Roncal and Idiazabal cheese at different stages of manufacture in different dairies. RAPD with (GTG)5 primer although very reproducible, only defined 2 groups of strains for both species. On the other hand, mtDNA RFLP proved to be more discriminating and defined 5 groups of strains for both species. In addition, the strains from the minor groups in both species corresponded to specific groups defined by mtDNA RFLP. Overall, this analysis revealed an important genetic diversity in the biotopes tested.

3. Nguyen Huu-Vang & C. Gaillardin. 1997. Two subgroups within the *Saccharomyces bayanus* species evidenced by PCR amplificatiopn and restriction polymorphism of the non-transcribed spacer 2 in the ribosomal DNA unit. System. Appl. Microbiol. **20**, in press.

A PCR-RFLP method has been improved for the rapid identification of the four species of *Saccharomyces sensu stricto*. We used the NTS2-ETS sequence of the rDNA as target and the amplification reaction was realized by choosing a pair of primers that could anneal to the end and the beginning of the 5S and 18S conserved sequences, respectively. PCR products obtained from *Saccharomyces cerevisiae, S. bayanus, S. paradoxus* and *S. pastorianus* (synonym *S. carlsbergensis*) type strains displayed a clear polymorphism when digested with several restriction enzymes. Using only three enzymes: *Ban* I*, Alu* I and *Taq* I we were able to differentiate *Saccharomyces cerevisiae*, *S. paradoxus* and the *S. bayanus/S. pastorianus* complex which shared the same patterns. The *S. bayanus* taxon now regroups the former species *S. abuliensis, S. globosus, S. heterogenicus, S. intermedius, S. inusitatus, S. uvarum*. PCR-RFLP of these species showed that, except for the *S. abuliensis* and *S. uvarum*, they shared the same pattern as *S. bayanus* type strain. That led us to conclude that there are two subgroups in *S. bayanus* and that *S. abuliensis* and *S. uvarum* belong to a distinct subgroup. This is also true for the particular karyotypes found with strains from this group. Remarkably, wine strains that were previously identified as *S. bayanus* by genetic hybridization technique, as well as strains isolated from cider fermentation, belong to the *S. uvarum* subgroup.

4. Catalog of the Collection of Yeasts of Biotechnological Interest.

The first catalog of the Collection de Levures d'Intérêt Biotechnologique (CLIB) will be available shortly this year. This catalog is presented 6 years after the creation of the collection in 1991. More than 350 strains are now available. Strains held in the collection are type strains of some 75 different species and others are from particular biotopes as cheese, wine, cider or from particular habitats: many strains were isolated in Kamchatka. Strains are preserved in deep freeze and lyophilisation as recommended by the Microbial Information Network for Europe (MINE).

XV. Department of Microbiology, Attila József University, P.O. Box 428, H-6701, Szeged, Hungary. Communicated by I. Pfeiffer.

Extrachromosomal genetic elements of *Phaffia rhodozyma*.

Our experiments demonstrated that the basidiomycetous yeast species *P. rhodozyma* is petite positive. Petite mutants can be isolated only from strain CBS 5905. The O_2 consumption of the mitochondria and the cytochrome c oxidase test verified that mitochondria do not function in the petite mutants therefore they can be considered as "true petites". Petite mutants occur spontaneously (1%) but their frequency can be elevated to 74% by mutagenic treatment.

The buoyant density of the mitochondrial and nuclear DNA is the same so they can not be separated on CsCl isopycnic gradient. *Bal*31 treatment proved that the mtDNA is linear molecule, its molecular weight is determined by restriction enzyme digestion approximately 13 kb what is the smallest known fungal mtDNA. Amino acid sequences deduced from the sequence of the cloned *Xba*I fragment were compared with the available data in SwissProt data base. It showed sequence homology to the cytochrome oxidase of other organisms. We have found 100% homology at the Cu-binding motif of the proteins. Our study revealed the existence of dsRNA molecules of different sizes in four of the six examined *P. rhodozyma* strains. VLPs, 34x26 nm in size were isolated only from strains carrying four or three types of dsRNA, while no VLPs were detected in the strain containing only one type of dsRNA. Further study demonstrated that the 3.7 kb dsRNA molecule does not copurify with the VLP fraction, even in VLP-containing strains. These results suggest that this type of dsRNA does not form part of the VLP genome. Following the inheritance of the dsRNAs (VLPs) proved that their transmission through the basidiospores is very efficient, which is in agreement with the results obtained on other fungal species. Thus the mating process can also be effective in spreading yeast viruses. No phenotypic effect due to the presence of the VLPs could be detected in the descendants of the basidiospores. Furthermore, despite the results published earlier, we could not demonstrate any killer activity of the parental strains during extensive testing. The vegetative reproduction, the frequency of conjugation and sporulation of a virus-free strain and its virus-containing derivative were compared. The statistical analysis did not reveal significant difference between the vegetative reproduction of the two strains, however, the frequency of the conjugation and the sporulation differed significantly.

Five of the six examined strains contained several mitochondrially located DNA plasmids with the sizes 2.0-6.5 kb. They could be eliminated very efficiently from the cells by ethidium bromide treatment. The presence of the DNA plasmids is connected somehow with the cell division. The genetic background of this connection is still not known. The plasmids of the different strains showed strong homology to each other but no homology could be detected to plasmids of other fungal species.

Summarising, *P. rhodozyma* has a very complex extrachromosomal genetic system what can give many interesting new results in the near future.

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

The following is the abstract of a publication which will appear in Yeast in 1997.

Phylogenetic relationships between species from the genera *Kluyveromyces* and *Saccharomyces* and representatives of the Metschnikowiaceae (*Holleya, Metschnikowia, Nematospora*) including the two filamentous phytopathogenic fungi *Ashbya gossypii* and *Eremothecium ashbyii* were studied by comparing the monosaccharide pattern of purified cell walls, the ubiquinone system, the presence of dityrosine in ascospore walls, and nucleotide sequences of ribosomal DNA (complete 18S rDNA, ITS1 and ITS2 region). Based on sequence information from both ITS regions, the genera *Ashbya, Eremothecium, Holleya, and Nematospora* are closely related and may be placed in a single genus as suggested by Kurtzman (1995) J. Industr. Microbiol. 14, 523-530. In a phylogenetic tree derived from the ITS1 and ITS2 region as well as in a tree derived from the complete 18S rDNA gene the genus Metschnikowia remains distinct. The molecular evidence from ribosomal sequences suggests that morphology and ornamentation of ascospores as well as mycelium formation and fermentation should not be used as differentiating characters in family delimitation of the Saccharomycetales. Our data on cell wall sugars, ubiquinone side chains, dityrosine, and ribosomal DNA sequences support the inclusion of plant pathogenic, predominantly filamentous genera like *Ashbya* and *Eremothecium* or dimorphic genera like *Holleya* and *Nematospora* with needle-shaped ascospores within the family Saccharomycetaceae. After comparison of sequences from the complete genes of the 18S rDNA the genus *Kluyveromyces* appears heterogenous. The type species of the genus, *K. polysporus* is congeneric with the genus *Saccharomyces*. The data of Cai et al. (1996) Int. J. Syst. Bacteriol. 46, 542-549 and our own data suggest to conserve the genus *Kluyveromyces* for a clade containing *K. marxianus, K. dobzhanskii, K. wickerhamii, and K. aestuarii* which again can be included in the family Saccharomycetaceae. The phylogenetic age of the Metschnikowiaceae and Saccharomycetaceae will be discussed in the light of coevolution.

XVII. Kluyver Laboratory of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands. Communicated by J. Pronk <j.t.pronk.stm.tudelft.nl>.

Research in our group is currently focused on regulation of metabolic fluxes in *S. cerevisiae* and other industrial yeasts, with emphasis on pyruvate metabolism, metabolic compartmentation and redox metabolism. Within the BSDL (Biotechnological Sciences Delft-Leiden) research school, intensive collaboration exists with the Yeast Genetics group at Leiden University and the Bioprocess Engineering Group of Prof. Dr. Sef Heijnen in Delft. In the Netherlands, we participate in a large programme focusing on the regulation of glycolytic flux in yeasts and filamentous fungi. In the the EC Framework IV programme, the group coordinates the project "From gene to product in yeast - a quantitative approach", which involves engineers and biologists from 10 research groups (8 university groups and 2 major biotechnological industries from 8 European countries). Furthermore, the group participates in an EC Framework IV program on the industrial yeast *Kluyveromyces lactis*.

Recent publications:

1. van der Aart Q.J.M., Kleine K. and Steensma H.Y. 1996. Sequence analysis of the 43 kb CRM1-YLM9-PET54-DIE2-SMI1-PHO81-YHB4-PFK1 region from the right arm of *Saccharomyces cerevisiae* chromosome VII. Yeast **12**:385-390.
2. van den Berg M.A., de Jong-Gubbels P., Kortland C.J., van Dijken J.P., Pronk J.T. and Steensma H.Y. 1996. The two acetyl-CoA synthetases of *Saccharomyces cerevisiae* differ with respect to kinetic properties and transcriptional regulation. Journal of Biological Chemistry **271**:28953-28959.
3. Castrillo J.I., Kaliterna J., Weusthuis R.A., van Dijken J.P. & Pronk J.T. 1996. High-cell-density cultivation of yeasts in oxygen-limited batch cultures. Biotechnology and Bioengineering **49**:621-628.
4. Flikweert M.T., van der Zanden L., Janssen W.Th.M., Steensma H.Y., van Dijken J.P. & Pronk J.T. 1996. Pyruvate decarboxylase: an indispensable enzyme for growth *of Saccharomyces cerevisiae* on glucose. Yeast **12**: 247-257.
5. Gbelska Y., Horvathova K., van der Aart Q.J.M., Zonneveld B.J.M., Steensma H.Y. and Subik J. 1996. Isolation and molecular analysis of the gene for cytochrome c1 from *Kluyveromyces lactis*. Current Genetics **30**: 145-150.
6. van Heusden G.P.H., van der Zanden A.L., Ferl R.J. and Steensma H.Y. 1996. Four *Arabidopsis thaliana* 14-3-3 protein isoforms can complement the lethal *bmh1 bmh2* double disruption. FEBS Letters **391**: 252-256.

7. de Jong-Gubbels P., van Dijken J.P. & Pronk J.T. 1996. Metabolic fluxes in chemostat cultures of *Schizosaccharomyces pombe* grown on mixtures of glucose and ethanol. Microbiology **142**: 1399-1407.

8. Pronk J.T., Steensma H.Y., van Dijken J.P. 1996. Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast **12**: 1607-1633.

9. Vanrolleghem P., de Jong-Gubbels P., van Gulik W.M., Pronk J.T, van Dijken J.P. & Heijnen J.J. Validation of a metabolic network for *Saccharomyces cerevisiae* using mixed substrate studies. Biotechnology Progress **12**: 434-448.

XXVIII. Institute of Fermentation Technology and Microbiology, Centre of Industrial Microorganisms, Technical University of Lodz, Wolczanska 175, 90-530 Lodz, Poland. Communicated by H. Stobinska <ewa@mikrob.p.lodz.pl>.

The Culture Collection of the Institute of Fermentation Technology and Microbiology is registered in the Catalogue of World Federation of Culture Collection WFCC as LOCK 105. The following abstracts are our publications for 1997.

1. Stobinska H., Drewicz E., Kregiel D., Oberman H. 1997. Attempts to transform the killer factor of yeasts *Saccharomyces cerevisiae* to amylolytic yeasts *Schwanniomyces occidentalis*. Biotechnologia **1**(36):158-166 (in Polish).

The fermentative yeasts *Saccharomyces cerevisiae* capable of producing killer factor type K2 and amylolytic yeast *Schwanniomyces occidentalis* were used to obtain the somatic hybrids by means of fusion protoplasts according to Fournier. The resulting hybrids showed amylolytic and fermentation activity. They also were characterized by the ability to form spores and to biosynthesize the killer factor. Their stability was depended on the composition of the cultivation medium.

2. Stobinska H., Kregiel D., Oberman H. Fermentation and amylolityc activity of hybrids of *Schwanniomyces occidentalis* and *Saccharomyces cerevisiae* preserved by freezing. Polish J. Food and Nutrition Sci. (in English, in press).

The aim of the study was to determine applicability of yeast freezing at temperature -40'C in the mixture of milk and DMSO to storage of amylolytic-fermenting hybrids of *Schwanniomyces occidentalis* and *Saccharomyces cerevisiae*, and preservation of their enzymatic properties. Results obtained proved that the hybrids preserved by freezing were characterized by stability of fermentation characteristics only, while their amylolytic abilities decreased significantly by 47%.

The following will be presented as a poster at XXVIII Meeting KTCHZ PAN "Progress in Chemistry and Food Technology" (9-11 September 1997, Gdansk, Poland).

3. Kregiel D., Drewicz E., Stobinska H., Oberman H. 1997. The influence of killer yeasts on growth and amylolytic activity of *Schwanniomyces occidentalis*.

The influence of yeasts killer toxins on the amylolytic strain *Schwanniomyces occidentalis* Y671/6 was studied. This research work showed the killer effect of *Saccharomyces cerevisiae* and *Rhodotorula* strains on the sensitive strain Y671/6 in mix cultures. The sensitivity of the amylolytic strain depended on the kind of carbon source in the culture medium. In the complete medium with glucose the amylolytic strain was more sensitive to killer toxin, while in the same medium with soluble starch the killer effect was decreased by 60%. Killer strains didn't exert an influence on the amylolytic activity of *Schwanniomyces occidentalis*. The study on properties and activities of killer proteins will be continued.

XIX. Department of Microbiology and Enzymology, Kluyver Laboratory of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, The Netherlands. Communicated by W.A. Scheffers.

1. T. Boekhout,[1] A. van Belkum,[2] A.C.A.P. Leenders,[2] H.A. Verbrugh,[2] P.Mukamurangwa,[3] D. Swinne,[3] and W.A. Scheffers.[4] 1997. Molecular typing of *Cryptococcus neoformans*: Taxonomic and epidemiological aspects. Int. J. Syst. Bacteriol. **47**:432-442.

[1]Yeast Division, Centraalbureau voor Schimmelcultures, 2628 BC Delft.
[2]Department of Bacteriology, University Hospital Rotterdam, Rotterdam.
[3]Laboratory of Mycology, Institute of Tropical Medicine, Antwerp, Belgium.

Pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) analysis, serotype, and killer toxin sensitivity patterns of a wide range of saprobic, clinical, and veterinary isolates of both varieties of *Cryptococcus neoformans* were examined. *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* differed in chromosomal makeup, RAPD patterns, and killer sensitivity patterns. These results suggest that there are two separate species rather than two varieties. No clear genetic or phenotypic differences were observed among the clinical, saprobic, and veterinary isolates within each taxon. The serotypes differed substantially in their RAPD characteristics. Geographical clustering was observed among the isolates of *C. neoformans* var. *gattii*, but not among the isolates of *C. neoformans* var. *neoformans*. The isolates of each taxon that originated from restricted geographical areas often had identical or similar karyotypes and RAPD patterns, suggesting that clonal reproduction had occurred. The combination of PFGE and RAPD analysis allowed us to distinguish almost all isolates. This combination of techniques is recommended for further research on epidemiological, ecological, and population issues.

XX. Instituto de Investigaciones Biomedicas, CSIC. Arturo Duperier, 4. 28029-Madrid. Spain. Communicated by J. M. Gancedo.

The following papers have been published recently.

2. P. & R. Lagunas. 1997. Catabolite inactivation of the yeast maltose transporter requires ubiquitin-ligase npil/rsp5 and ubiquitin-hydrolase npi2/doa4. FEMS Microbiol. Letters **147**:273-277.

The maltose transporter in *Saccharomyces cerevisiae* is degraded in the vacuole after internalization by endocytosis when protein synthesis is impaired and a fermentable substrate is present. The possible implication of the ubiquitin pathway in this inactivation, known as catabolite inactivation, has been investigated. Using mutants deficient in npil/rsp5 ubiquitin-protein ligase and npi2/doa4 ubiquitinprotein hydrolase, we have shown that these two enzymes are required for normal endocytosis and degradation of the transporter. These facts indicate that the ubiquitin pathway is involved in catabolite inactivation of the maltose transporter. The results also revealed that both enzymes act in the internalization step of endocytosis.

2. I. Romero, A.M. Maldonado, & P. Eraso. 1997. Glucose-independent inhibition of yeast plasma-membrane H^+-ATPase by calmodulin antagonists. Biochem. J. **322**:823-828.

Glucose metabolism causes activation of the yeast plasma membrane H^+-ATPase. The molecular mechanism of this regulation is not known, but it is probably mediated by phosphorylation of the enzyme. The involvement in this process of several kinases has been suggested but their actual role has not been proved. The physiological role of a calmodulin-dependent protein kinase on glucose-induced activation was investigated by studying the effect of specific calmodulin antagonists on the glucose-induced ATPase kinetic changes in wild-type and two mutant strains affected in glucose regulation of the enzyme. Preincubation of the cells with calmidazolium or compound 48/80 impeded ATPase activity increase by reducing the V_{max} of the enzyme without modifying the apparent affinity for ATP in the three strains. In one mutant, *pmalT912A*, the putative calmodulin-dependent protein kinase phosphorylatable Thr-912 has been eliminated and in the other, *pmaI-P536L*, the H^+-ATPase is constitutively activated, suggesting that the antagonistic effect was not mediated by a calmodulindependent protein kinase and not related to glucose regulation. This was corroborated when the in vitro effect of the calmodulin antagonists on H^+-ATPase activity was tested. Purified plasma membranes from glucose-starved or glucose-fermenting cells from both *pmal-P890X*, another constitutively activated ATPase mutant, and wild type strains were preincubated with calmidazolium or melittin. In all cases, ATP hydrolysis was inhibited with an IC_{50} of 1μM. This inhibition was reversed by calmodulin. Analysis of the calmodulin-binding protein pattern in the plasmamembrane fraction eliminates ATPase as the calmodulin target protein. We conclude that H^+-ATPase inhibition by calmodulin antagonists is mediated by an as yet unidentified calmodulin-dependent membrane protein.

XXI. Department of Chemical Engineering, University of Melbourne, Parkville, Victoria, 3052, Australia. Communicated by N.B. Pamment.

Recent publications.

1. Stanley, G.A., Hobley, T.J. & Pamment, N.B. 1997. Effect of acetaldehyde on *Saccharomyces cerevisiae* and *Zymomonas mobilis* subjected to environmental shocks, Biotechnol. Bioengin. **53**:71-78.

2. Hobley, T.J. & Pamment N.B. 1997. Liquid injection gas chromatography and the ALDH assay overestimate free acetaldehyde in complex fermentation media, Biotechnol. Techniques **11**:39-42.

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

I am pleased to announce that Prof. C.A. Rosa, of the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, has obtained a research fellowship from Brazil's CNPq to join our laboratory recently for his research leave. He is conducting studies on the ecology and taxonomy of yeasts associated with plants and insects.

The following paper, whose abstract was given in the previous issue of the YNL, has now appeared.

1. M.A. Lachance & W.M. Pang. 1997. Predacious Yeasts. Yeast **13**:225-232.

The following will be presented at the 18th ISSY, Bled, Slevenia, in August 1997.

2. M.A. Lachance, A. Pupovac-Velikonja, B. Schlag, W.M. Pang, and C. Guptill. 1997. Yeasts as a yeast habitat.

Since the discovery that a yeast isolate from Australian *Hibiscus* (UWO[PS]95-697.4) could penetrate, kill, and gain nutritional benefits from other yeasts, we have investigated some aspects of the taxonomy, physiology, and morphology of yeast predation. Predation is present in a majority of species in the genus *Saccharomycopsis* as recently redefined by Kurtzman & Robnett (1995) on the basis of rDNA sequencing. The phylogenetic position of the Australian isolate, which is almost exclusively unicellular, is currently under scrutiny. All predaceous yeasts uncovered so far are naturally auxotrophic for organic sulfur, and we are verifying the hypothesis that organic sulfur may, under different conditions, serve as stimulus, reward, and inhibitor of predation. The role of organic sulfur may vary from one species to another. When mixed with *Metschnikowia* sp. (95-747.4) or with other species, the *Hibiscus* strain (95-697.4) never completely obliterated its prey, but the predator/prey ratio at equilibrium was higher in the presence of a small amount of methionine and suppressed by higher amounts. This yeast generally had better predatory activity on smaller prey species. When *Saccharomycopsis (Arthroascus) javanensis* was grown with *Saccharomyces cerevisiae* on synthetic medium devoid of organic sulfur, the two species established a stable equilibrium over several generations. Addition of L-methionine at concentrations of 1 µM or more enhanced predation significantly, leading to the elimination of prey cells after two subculturing cycles. Increasing the methionine levels up to 1% (67 *mM*) had no inhibitory effects. *Saccharomycopsis fermentans and S. schoenii* (also former *Arthroascus* species) were generally similar to *S. javanensis* with respect to predation. Other species, namely *Saccharomycopsis (Botryoascus) synnaedendrus, S. (Guilliermondella) selenospora, S. crataegensis, S. malanga*, *Candida amapae,* and two unknown hyphal yeasts exhibited predatory dimorphism, where predaceous hyphae differed somewhat in morphology from the normal thallus in the same culture by being smaller and more contorted. The exceptions were *S. fibuligera* and closely related *S. (Botryoascus) cladosporoides*, whose infection pegs arose at low frequencies from normal hyphae. Predation was not observed in other yeasts that had the potential to be predaceous. These included sulfur auxotrophs *Pichia amethionina, Saccharomycopsis capsularis, S. vini,* and a few anomalous auxotrophs. Among yeasts not requiring organic sulfur, all members of the genus *Ambrosiozyma (*syn. *Hormoascus) sensu* Kurtzman & Robnett, *Schizoblastosporion starkeyi-henricii, Sympodiomyces parvus,* and *Yarrowia lipolytica* gave negative results. It is tentatively concluded that predation may have co-evolved with the loss of sulfate transport in ancestral yeasts that gave rise to the genus *Saccharomycopsis sensu* Kurtzman & Robnett (1995) and possibly to some of its close relatives.

Reference: Kurtzman & Robnett. 1995. Molecular relationships among hyphal ascomycetous yeasts and yeastlike taxa. Can. J. Bot. **73**:S824-S830.

Forthcoming meetings

18th International Specialised Symposium on Yeasts - ISSY 1997. Yeast Nutrition and Natural Habitats. 24th-29th August, 1997, Bled, Slovenia.

The Symposium will be held on Bled, in beautiful alpine surroundings of the northern part of Slovenia, one of the young countries in middle Europe. The date of the conference, 24-29 August 1997, was chosen ideally to combine the attendance on 8th European Congress on Biotechnology in Budapest, Hungary and the 18th ISSY on Bled, Slovenia. Revised list of topics: Yeasts in conventional, nonconventional and extreme habitats. Organic and inorganic nutrition in yeasts. Physiology and energy metabolism in yeasts. Ecophysiology of yeasts. Yeasts as human and animal pathogens. Yeasts in food production and spoilage. Yeast interactions in natural environments. Extracellular enzymes in yeast nutrition and ecology. The deadline for submission of early registration is June 30. The second circular is now available. **For further information please contact:**

ISSY 97 Secretariat
Prof. dr. Peter Raspor
University of Ljubljana, Biotechnical Faculty
Jamnikarjeva 101,
1000 Slovenia

Tel: ++ 386 61 123 11 61
Fax: ++ 386 61 274 092
E-mail: ISSY97@bf.uni-lj.si

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

I take pleasure in inviting you to attend the Sixth International Mycological Congress - IMC 6 scheduled to take place from August 23-28, 1998 in Jerusalem at the ICC Jerusalem international Convention Center. You can expect excellent science combined with an enjoyable holiday. The Congress Program encompasses a wide array of themes structured of symposia sessions and workshops, daily plenary lectures, social activities and a special program for accompanying persons. Israel has a long tradition of Mycological and Phytopathological research that goes back to the beginning of the century. We have presently extensive investigations in Mycology including Medical Mycology, Phytopathology, Biotechnology and Symbiotic Systems. Jerusalem is a center of biblical, ancient and modern history, and the birthplace of great religions. The city is rich in archaeology, culture and natural beauty. It enjoys an ideal Mediterranean climate and is a Perfect place to combine science with travel. This international Congress will offer an opportunity to visit Israel's institutions and Centers for mycological research and establish personal contact with Israel's mycologists. Looking forward to welcoming you in Jerusalem!

Yours sincerely, Margalith Galun.

Organizing committee: M. Galun, President; I. Barash, Vice-President; Z. Eyal, General Secretary; A. Sztejnberg, Treasurer. **Scientific committee**: Y. Koltin, Chair, Y. Elad, R. Fluhr, Y. Hadar, T. Katan, M. Kupiec, I. Polacheck, O. Yarden. **International Mycological Association**: F. Oberwinkler, President; M. Blackwell, Secretary-General; M.E. Noordeloos, Treasurer.

Tentative Program: Opening address: Genomics and Mycology - S. Oliver (UK). Plenary lectures: Bioprospecting - L. Nisbet (UK); Molecular systematics and evolution - J.W. Taylor (USA); Gene regulation and morphogenesis - W. Timberlake (USA); Fungal diversity - D.L. Hawksworth (UK); Medical Mycology - J. E. Edwards (USA); Symbiosis and Parasitism, synonymous or distinct? - D.H.S. Richardson (Canada). Symposia and workshops: A. Fungal diversity; B. Cell biology; C. Fungal Genetics; D. Fungal Development and morphogenesis; E. Fungal-host interactions; F. Medical mycology; G. Technology; H. Ecology and biosystematics; Teaching Mycology; Computer networks and information systems; Specific taxonomic groups (We invite suggestions for workshops on specific taxonomic groups).

Travel programs of great interest for participants and accompanying persons are also planned.

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

Tel: 972 3 5140014
FAX: 972 3 5175674/5140077
Compuserve: ccmail: MYCOL@Kenes
Internet: Mycol@Kenes.ccmail.compuserve.com
WWW: http://Isb380.plbio.1su.edu/ima/index.html

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

For information on this symposium please consult the web site: *http://www.ecyeastsymp.com*, 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

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 Items

Change of address: T. Torok

My new mailing address is as follows:

Tamas Torok
4669 Setting Sun Dr.
Richmond, CA 94803
U.S.A.

Change of address: M.L. Suihko

Please note that the name and address of VTT (= Technical Research Centre of Finland) have changed as follows:

Dr. Maija-Liisa Suihko
VTT Biotechnology and Food Research
P.O.Box 1501
FIN-02044 VTT
Finland

Tel +358-9-456 5133
Fax +358-9-455 2028
Email: maija-liisa.suihko@vtt.fi

Yeasts on the Internet

I maintain an e-mail directory of all biologists interested in yeast. . If you wish to be included in this directory, please send me a short electronic mail message with your coordinates, and I will be happy to add you in. The list is maintained on the Internet, and can be retrieved with your favorite WWW browser from: *ftp://ncbi.nlm.nih.gov/repository/yeast/yeaster.lst*

I also coordinate the bionet yeast molecular biology newsgroup, for which you can get more information from this URL: *http://www.bio.net:80/hypermail/YEAST/*

If you have any questions about these two services, please contact:

B.F. Francis Ouellette,
GenBank Coordinator
NCBI/NLM/NIH Building 38A
Bethesda, MD 20894, USA

Tel: (301) 496-2477 ext 247
Fax: (301) 435-2433
E-mail: francis@ncbi.nlm.nih.gov