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Y E A S T

A Newsletter for Persons Interested in Yeast

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

Editorials

Ninth International Symposium on Yeasts, Sydney, Australia, 25-30 August 1996

The Ninth International Symposium on Yeasts was an unqualified success. The picturesque venue of the Sydney Harbour, scientific presentations of the highest quality, and the magnificent socient events held in various landmark buildings of Sydney all contirbuted to making this ISY memorable. Congratulations to Graham Fleet and his Organizing Committee for a superb job of coordination.

Dedication of the Herman J. Phaff Culture Collection, Davis, California, U.S.A., October 4 1996.

The official dedication of the Herman J. Phaff culture collection took place, last October, at the University of California, Davis. The Department of Food Science and Technology, UC Davis, brought together various individuals who at various times collaborated with Prof. Phaff throughout his career as a yeast explorer, to present scientific and personal tributes to their mentor. A list of speakers appears in this issue of the Yeast Newsletter. I congratulate the organizers and sponsors for bringing to fruition this marvellous idea, and wish the Collection a long and prosperous future.

M. A. Lachance, Editor

Thirty Years of the International Commission on Yeasts

This year we commemorate the thirtieth anniversary of the existence of the International Commission on Yeasts (ICY), whose role is "to establish effective liaison between persons and organizations concerned in yeast investigations, and between them and the practical users of results of investigations, including yeast culture collections". When and how did the history of ICY start? The creation of this body is linked to the second International Symposium on Yeasts which was held in Bratislava, Czechoslovakia, now the capital of Slovakia, in July 1966. The meeting was attended by 145 participants from 21 countries. During the Symposium the Czechoslovak representatives initiated the creation of an international organization which would stimulate scientific collaboration of people working with yeasts all over the world. A Council for International Collaboration in Yeast Science was founded. Dr. A. Kocková-Kratochvilová was appointed Chair and Dr. Erich Minárik (both from Czechoslovakia), Secretary of the Council. The other endowing members of the Council were: K. Beran (Czechoslovakia), A. Eddy (UK), P. Elinov (USSR), H. Klaushoffer (Austria), N.I. Kudrjavcev (USSR), U. Leupold (Switzerland), R. Muller (GDR), S. Nagai (Japan), O. Nečas (Czechoslovakia), H.J. Phaff (USA), C.F. Robinow (Canada), H. Suomalainen (Finland), T. Tsuchiya (Japan), L. Wickerham (USA), T. Wiken (Holland) and S. Windisch (GFR), all well recognized yeast researchers. During the meeting it was also agreed that the existing Yeast Newsletter, edited by H. Phaff at the University of California, would serve as the official publication of the Council.

In the following years the Council underwent changes in names and affiliations. In 1970, under the new name, International Commission on Yeasts and Yeast-like Microorganisms (ICY), it became a part of the Microbiology Division of the International Union of Biological Sciences (IUBS). In 1981 ICY also joined the Mycology Division of the International Union of Microbiological Societies.

The main activity of ICY is the organization International Symposia on Yeasts (ISY) at 3-5 years intervals, and, more frequently, International Specialized Symposia on Yeasts (ISSY). The main organizer of the ISY becomes Chair of the ICY until the next ISY.

On behalf of the readers and the editorial board of the Newsletter let us wish to the International Commission on Yeasts many successful activities and great achievements in years to come.

Peter Biely, Associate Editor

I. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC Delft, The Netherlands. Communicated by M.Th. Smith.

1. CBS publication: LIST OF CULTURES - Fungi and Yeasts, 34th edition, 1996, 617 pp, Hfl.35, (+ Hfl. 20, bank-fee + Hfl. 12,50 postage charge). Orders should be sent to:

Centraalbureau voor Schimmelcultures
Oosterstraat 1
P.O.Box 273
3740 AG Baarn, The Netherlands

Telephone +31(0)35-5481211
Telefax +31(0)35-5416142
Electronic Mail in Internet for information: INFO@CBS.KNAW.NL
Electronic Mail in Internet for ordering: SALES@CBS.KNAW.NL
Homepage on Internet:
HTTP://WWW.CBS.KNAW.NL/WWW/CBSHOME.HTML

II. 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 are completed research projects.

1. Basso, L.C. & Amorim, H.V. 1996. Toxic action of aluminium toward yeast fermentation.

Aluminium toxicity is considered to be an important growth limiting factor for crops in general and, for economical reasons, a large body of information are available regarding the metal toxic effects on plants. Deleterious effects of aluminium has also been reported on others biological systems, but very few data can be found in relation to yeasts. The present work was carried out to search toxic effects of aluminium towards yeast, by evaluating technological and physiological parameters related to ethanol fermentation process as it is performed in Brazil with yeast cell reuse. The results demonstrated a stressing effect of Al on yeast by causing reduction on growth, glycerol formation, cell viability as well as on yeast trehalose and glycogen contents. Meanwhile, ethanol yield increased during the first cycles since less sugar was deviated for biomass, glycerol and reserve carbohydrates. Such increased ethanol yield had no practical advantage for industrial process, since reduction in trehalose content rendered yeast unable to survive recycling. Similar results were obtained with two others industrial strains (TA and IZ-1904). Toxic effects could be demonstrated at aluminium concentration as low as 0.5 mM in semi-synthetic or sugar cane juice substrates. On the other hand, the presence of molasses attenuated the toxic effects suggesting a possibility of aluminium complexation by components of the medium. The stressing action became more pronounced the more yeast was reused, suggesting a cumulative effect of aluminium, and allowing to conclude that smaller concentration of the metal could be harmful when yeast was under prolonged recycling, as it is conducted in the industrial process. Aluminium concentration in industrial substrates (from several distilleries during the last two years) showed to be in the range 0.3 - 1.5 mM but in some occasion exceeded 5mM, when fermentation rate was very low, decreasing ethanol yield (no study was done to identify aluminium as the responsible for such problems). From these observations we conclude that toxic levels of aluminium could be present in industrial substrates and that its stressing action toward yeast is increased by yeast recycling as it is performed in industrial ethanol fermentation.

2. Alves, D.M.G. & Basso, L.C. 1996. Potassium as a stressing factor during alcohol fermentation.

Potassium is necessary for fermentations with yeast cell recycle in the range 700-1,200 mg/l. However, potassium concentration in sugar cane molasses not seldom reaches 24,000 mg/l, resulting in fermentation media with up to 10,000 mg/l. High potassium concentrations is known to stimulate glycerol formation in response to osmotic stress and to reduce succinic acid formation. Potassium uptake contributes to the external acidification during fermentation, while it allows the intracellular alcalinization. These are important aspects to be regarded in fermentation for fuel alcohol production, where maximum ethanol yield is searched. In order to evaluate the effects of high potassium levels upon yeast performing alcohol fermentation as well as the influence of molasses medium on this effect, two different experiments were carried out, with molasses medium and with semy-synthetic medium. The results showed higher glycerol production and lower succinic acid formation with high potassium concentrations (8000 mg/l) in both experiments, as expected. The also expected stressing effect on yeast was only observed with molasses medium, evaluated by the reduction in trehalose content, cell viability and yeast growth. Opposite results were observed in the experiment with semi-synthetic medium, which had lesser buffering capacity, suggesting a protective effect of potassium against external acidification. As a practical application of these observations we have the management of the industrial process according to different substrates, molasses or sugar cane juice i.e. with high or low buffering capacities, respectively, in order to avoid stresses on yeast caused by chemical characteristics of the medium.

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

1. D. Avram & A.T. Bakalinsky. Multicopy *FZF1* (*SUL1*) suppresses the sulfite sensitivity but not the glucose derepression or aberrant cell morphology of a *grr1* mutant of *Saccharomyces* cerevisiae .

An *ssu2* mutation in *Sacccharomyces cerevisiae,* previously shown to cause sulfite sensitivity, was found to be allelic to *GRR1,* a gene previously implicated in glucose repression. The suppressor *rgt1,* which suppresses the growth defects of *grr1* strains on glucose did not fully suppress the sensitivity on glucose or non-glucose carbon sources, indicating that it is not strictly linked to a defect in glucose metabolism. Because the *Cln1* protein was previously shown to be elevated in grr1 mutants, the effect of *CLN1* overexpression on sulfite sensitivity was investigated. Overexpression in *GRR1* cells resulted in sulfite sensitivity, suggesting a connection between *CLN1* and sulfite metabolism. Multicopy *FZF1,* a putative transcription factor, was found to suppress the sulfite sensitive phenotype of *grr1* strains, but not the glucose derepression or aberrant cell morphology. Multicopy *FZF1* was also found to suppress the sensitivity of a number of other unrelated sulfite-sensitive mutants, but not that of *ssu1* or *met20,* implying that *FZF1* may act through Ssu1p and Met20p. Disruption of *FZF1* resulted in sulfite sensitivity when the construct was introduced in single copy at the *FZF1* locus in a GRR1 strain, providing evidence that *FZF1* is involved in sulfite metabolism.

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

1. Bakker, J., Bellworthy, S.J., Hogg, T.A., Kirby, R.M., Reader, H.P., Rogerson, F.S.S., Watkins, S.J. & Barnett, J.A. 1996. Two methods of port vinification: a comparison of changes during fermentation and of characteristics of the wines. Amer. J. Enol. Vit. **47**:37-41.
2. Bitzilekis, S. & Barnett, J.A. 1996. Exponential growth rates of the yeast genus *Kluyveromyces*. *FEMS Microbiol. Lett.*, in the press.
3. Barnett, J.A., Payne, R.W. & Yarrow, D. 1996. Yeast Identification PC Program. 4th version, Norwich: Barnett.

This program is supplied on 3.5 inch disk for IBM PC & compatibles with MS/PC-DOS & 512 KB of free RAM. It is for use in industry, medical mycology & research. The program simplifies identifying yeasts and reduces time-consuming searches through identification keys or descriptions of species. After entering the results of tests and observations into the computer, lists can be obtained of the following: (1) all species with a matching set of characteristics (with probabilities); (2) yeasts with characteristics most nearly matching the entered set, with probabilities and details of the characteristics that differ; (3) further tests necessary to complete the identification. The program can also (a) allow for mistakes in the test-results; (b) display and compare descriptions of species; (c) select yeasts with particular characteristics. Results can be either entered from the keyboard or read from a file.

Version 4 provides a further improved interface, still allowing the use of popup menus and the mouse. The data base has also been completely updated, to include 19 new taxa and to take account of all the new information available since version 3 was published. Results of 7 new tests are included and some less useful tests omitted. DOS commands can be operated from within the program. The Commands in the program are as follows: BREAK to use a DOS command; COMMENT to label input; DATA to enter test results in any order; FORM to take results from a data form already stored on the computer; HELP to give information about the program and commands; IDENTIFY to identify species that match the entered results; LIST to enter results (tests are listed in turn and a result can be entered, or deleted, or the test can be skipped; MISTAKE to allow for mistakes in the results; NEXT to clear previous entries before entering a new set of results; OPEN to open (i) a file of data about extra species, or (ii) a transcript file, or (iii) a form containing results to be read by subsequent FORM commands, or (iv) a report file; QUIT to exit from the program; REPORT to produce a report containing details of the test results that have been entered, followed by the list of species that can give them; SELECT to select further tests to complete the identification of all species able to give the results already entered, some choice of these tests being possible; TRANSCRIPT to record input and output; VIEW to display (i) the description of a named yeast species, or (ii) the test results you have entered, or (iii) the differences between the unknown yeast and a particular species, or (iv) the differences between 2 species; WINDOW to switch between the use of commands and pop-up menus.

Prices: (a) Single-machine licence £150 or US$230; (b) Licence for up to 8 machines (within the same institute) £300 or US$460. Discount prices, if payment is made with the order by cheque in pounds sterling and drawn on a UK bank, net of charges: (a) Single-machine licence £125; (b) Licence for up to 8 machines (within the same institute) £250.

All communications about this program should be made to Dr J.A. Barnett, 36 Le Strange Close, Norwich NR2 3PW, UK.

V. Central Food Research Institute, 1022 Budapest, Herman Ottó út 15, Hungary. Communicated by P.A. Biacs.

1. A. Halász & M. Szakács-Dobozi. Proteolytic enzyme activity of S.cerevisiae baker's yeast and S. carlsbergensis brewer's yeast. Acta Aliment. **22**:193-209.

Investigation of the soluble proteinases from *S.cerevisiae* and *S.carlsbergensis* showed that the pH-enzyme activity profiles are significantly different. In baker's yeast proteinase B activity, for brewer's yeast carboxypeptidase Y activity seemed to be characteristic. SDS-PAGE separation of IEF fractions resulted in different protein prints for the investigated yeasts. Despite the serological similarity of the protein fractions representing proteinase B activity their SDS-PAGE molecular weight spectra are different. Soluble proteinases could be activated by adding surface active agents to sonicated samples however not to the plasmolysed ones. The cell wall disintegration method had also influence on the pH optimum of the soluble proteinase sample. Temperature optimum depends both on pH and cell wall disintegration method for both yeasts.

Cell wall bound proteolytic enzyme activity represents a remarkable level for *S.cerevisiae* and was dominant in case of *S.carlsbergensis,* temperature optimum and heat resistance of cell wall bound proteinases were different from those of the soluble ones.

2. K. Búsová, I. Magyar & F. Janky. 1994. Effect of immobilized yeasts on the quality of bottle-fermented sparkling wine. Acta Aliment. **23**:9-23.

To eliminate the difficult clarification process in bottle fermented sparkling wine production, four variations of Ca-alginate entrapment technique were applied for immobilization of a *Saccharomyces bayanus* starter strain. Biocatalyst prepared on the usual way of Ca-alginate entrapment technique (A) was compared to that prepared by coating the alginate heads with a cell-free alginate layer (D). Both types of biocatalysts were investigated in two levels of cell loading (1, 2) and compared to the traditional free-cell fermentation (K) as a control. Chemical analysis of sparkling wines after three months of aging on yeast showed little differences only in few parameters. The organoleptic quality of sparkling wines was not adversely affected by either types of biocatalysts. The cell retention capacity of biocatalysts characterized by the limpidity of sparkling wine was not satisfactory using biocatalysts without coating. A significantly belief cell retention could be achieved by the use of cell-free alginate coating, but the adhesion of the coat to the surface of the beads needs further improvement.

3. Á. Hoschke, J. Rezessy-Szabó, G. Vereczkey & M. Nagy-Gasztonyi. 1993. Bioengineering problems in the fermentation and down stream process of the industrial enzymes. Acta aliment. **22**:245.

The elaboration of the technology for enzyme production requires a complex research programme which includes the strain improvement, the optimization and the scale up of the fermentation, the concentration and the recovery of the product, purification of the enzyme according to demands. The problems of the biotechnological research are introduced by various topics: - the strain breeding of *Bacillus licheniformis* producing α-amylase and *Aspergillus niger* producing glucoamylase - the mathematical design of experiments and the scale up for the glucoamylase fermentation - the down stream processes for both the production liquid glucoamylase enzyme and the highly purified glucose oxidase preparations. Beside the technological solutions the economic points were also taken into consideration.

4. G. Vereczkey, L. Lelik & M. Gasztonyi. 1993. Production of bioactive compound with cultivation shiitake Lentinus edodes. Acta aliment. **22**:245.

Medical effects of the Shiitake mushroom have been studied by many cholesterol and antiviral or researchers, and remarkable effects to remove serum antitumor activities are certified. At the beginning of our experiments we wanted to produce Shiitake in submerged cultures. In order to enhance the biomass the three main components glucose, yeast extract, $(NH_4)_2SO_4$ - were added in various concentrations, and after the preliminary measurements a "Total Factor Plan" was made. On the other hand we wanted to make certain the occurrence of erytadenine (its medical effect is remarkable) in mycelia. Lentinus edodes strains Le-4 and Le-3343 were received from Czechoslovakia and Germany. More than one gram dried Shiitake mycelia was obtained from 100 cm^3 medium consisting of 3% glucose, 0.2% $(NH_4)_2SO_4$ and 0.8% yeast extract in case of strain Le-4, while strain Le-3343 produced 0.2-0.4 g dried mycelia under similar conditions. To obtain fungal mycelia a BIOSTAT system (B. Braun) has been used. Lentysine was isolated from Lentinus edodes with column chromatography [Amberlite IR-120 (H+) and Amberlite IRA-4nO (OH-)], for the identification and measuring the GC-MS and LC-MS technics were applied.

5. A. Maráz. 1993. Karyotyping of yeasts by pulsed field gel electrophoresis. Acta Aliment. **22**:246.

Pulsed Field Gel Electrophoresis (PFGE) developed by Cantor and Schwartz (1984) has given the possibility for the determination of karyotypes of yeast and fungal strains. One of the recently developed version of this method: RFE (Rotating Field Electrophoresis) was used in our laboratory for the determination of chromosome patterns and CLPs (Chromosome Length Polymorphism) of yeasts. Comparison of karyograms of different strains belonging to *S.cerevisiae* according to the currently accepted classification of yeasts by Kreger-van Rij (1984) was carried out. Considering the number and size of chromosomes the order of CLP increase compared with *S.cerevisiae* was as follows: *S.pastorianus, S.bayanus* and *S.paradoxus*. Electrophoretic karyotyping of somatic hybrids proved to be an efficient method for the selection of improved yeast strains when brewer's yeast strains with glucoamylase activity were constructed and selected. The isolated primary hybrid clones were further screened according to their karyograms, and clones which proved to be the most similar in their chromosome patterns to the parental brewer's yeast strain were selected.

6. I. Balogh, C. Scerri & A.E. Felice. 1993. Amplifying of *DEX* gene by PCR. Acta Aliment. **22**:247.

The *DEX* or *STA* genes are responsible for production of glucoamylase enzymes. These genes can be found in some yeast strains called *Saccharomyces diastaticus.* A gene bank containing the 2.6-4.4 kb long fragments has been created in *E.coli* by means of shuttle plasmid vector. The recombinant plasmids have been transferred into laboratory yeast strain. The transformants have been screened for degrading starch but the enzyme activity has not been detected. In our opinion the cloned fragments might not have had the promoter sequence. The whole *DEX2* gene which is 2753 bp long have been tried to amplify by PCR. For this reason two primers have been designed called P1 and P2 flanking on the two 5' ends of desired gene. An approximately 0.7 kb long product has been detected although 2.7 kb Long was expected. It probably happened because P2 primer had homology sequence 0.7 kb far from one of the 5' end. The specificity of PCR reaction has been tried to enhance by glycerol and two-temperature PCR, respectively but without any success. Since there is a unique restriction site of *Eco*RI inside of the gene two further primers called P3 and P4 have been designed on two sides of the above-mentioned restriction site. The gene has amplified in two pieces. Because these two fragments have an about 200 bp long overlapping sequence the whole gene has been constructed by recombinant PCR.

7. I. Pais, B. Janzsó, and Á. Suhajda. 1993. Production of yeast enriched with titanium. Acta aliment. **22**:247.

Under given conditions yeasts are able to accumulate large amounts of microelements in their cells and incorporate them into organic compounds. In our investigation titanium has been introduced *Saccharomyces cerevisiae* cells. The nutrient media has been supplemented with titanium-ascorbate solution: at 20 - 50 μg cm^{-3} Ti-concentration the titanium uptake resulted in 1500-6000 μg Ti per g dried yeast. The addition of Ti-ascorbate is more suitable in the exponential phase of yeast growth: in this case titanium is bound to the cell components instead of adsorption on the surface.

8. M. Novák-Fodor, P. Fodor, I. Pais, B. Janzsó and Á. Suhajda. 1993. Production of Zr containing yeast. Acta aliment. **22**:248.

Accumulation of Zr by *Saccharomyces cerevisiae* and *Candida utilis* species were examined. The Zr was added to the media by several ways in form of different compounds. The Zr concentration of the samples was measured with spectrophotometry, the other elements were detected with ICP. *Saccharomyces cerevisiae* proved to be superior in accumulating Zr in the cell, the highest concentration were reached by a complex made of $ZrOCl_2$ and ascorbic acid.

9. B. Hegyes-Vecseri, Á. Hoschke and P. Fodor. 1993. The role of microelements in beer fermentation. Acta aliment. **22**:248.

In the beer fermentation some microelements play positive, others play negative roles. In the Hungarian beers shortage of zinc was detected using inductively coupled plasma emission technique. The brewer's yeast consumed almost the whole amount of inorganic zinc at the first day of fermentation. For the separation of inorganic zinc and that bound in complex an ion-exchange technique was worked out. The method was controlled with consecutive dilution and standard addition technique. Using the method, in some cases during beer fermentation shortage of inorganic zinc was determined.

VI. Indiana University, Department of Biology, Jordan Hall, Bloomington, Indiana, USA 47405. Communicated by M. Celerin <mcelerin@bio.indiana.edu>.

1. M. Celerin, A.W. Day,[1] R.J. Smith,[1] & D.E. Laudenbach. In press. Immunolocalization of fimbrial epitopes in thin sections of *Microbotryum violaceum*. Can. J. Microbiol. (February, 1997)

[1]Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7.

Fungal fimbriae are long (0.5 to 20 μm), narrow (7 nm) surface appendages that have been observed on most members of the Mycota. Biochemical analyses have determined that fimbriae from *Microbotryum violaceum* are composed of 74 kDa glycoproteinaceous the reserves within the peripheral cytoplasm. Also, we show that fimbriae may not traverse the cell wall as previously believed, but may instead originate from within the outer lamella of the cell wall, possibly being anchored to the cell wall via other molecules. This model is subunits in which the protein moiety is fungal collagen. We present evidence for the localization of fimbrial subunits prior to their exportation from the cell. We term these internal, likely non-polymerized fimbriae "pro-fimbriae" and demonstrate the location of analogous to the animal extracellular matrix arrangement in which collagens are anchored to plasma membranes via other proteins such as fibronectin.

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

1. On April 3, 1996 J.M.J. Uijthof defended his PhD thesis entitled "Taxonomy and Phylogeny of the Human Pathogenic Black Yeast Genus *Exophiala* Carmichael." Copies of this thesis (120 pp.) can be obtained from the above address.

Other recent publications:

2. Ginter, G., Hoog, G.S. de, Psaid, A., Fellinger, M., Bogiatzis, A., Berghold, C. & Reich, E.M. 1995. Arthritis without grains caused by *Pseudallescheria boydii*. Mycoses **33**:369-371.
3. Göttlich, E., Hoog, G.S. de, Yoshida, S., Takeo, K., Nishimura, K. & Miyaji, M. 1995. Cell surface hydrophobicity and lipolysis as essential factors in human tinea nigra. Mycoses **38**:489-494.
4. Haase, G., Sonntag, I., Peer, Y. van de, Uijthof, J.M.J., Podbielski, A. & Melzer-Krick, B. 1995. Phylogenetic analysis of ten black yeasts using nuclear small subunit rRNA gene sequences. Antonie van Leeuwenhoek **68**:19-33.
5. Hoog, G.S. de, Gerrits van den Ende, A.H.G., Uijthof, J.M.J. & Untereiner, W.A. 1995. Nutritional physiology of type isolates of currently accepted species of *Exophiala* and *Phaeococcomyces*. Antonie van Leeuwenhoek **68**:43-49.
6. Hoog, G.S. de & Guarro, J. (eds). 1995. Atlas of Clinical Fungi. Baarn and Reus, 720 pp.
7. Hoog, G.S. de, Guého, E., Masclaux, F., Gerrits van den Ende, A.H.G., Kwon-Chung, K.J. & McGinnis, M.R. 1995. Nutritional physiology and taxonomy of human-pathogenic *Cladosporium-Xylohypha* species. J. Med. Vet. Mycol. **33**:339-347.
8. Hoog, G.S. de, Sigler, L., Untereiner, W.A., Kwon-Chung, K.J., Guého, E. & Uijthof, J.M.J. 1995. Changing taxonomic concepts and their impact on nomenclatural stability. J. Med. Vet. Mycol. **32** (Suppl.1):113-122.
9. Hoog, G.S. de, Takeo, K., Göttlich, E., Nishimura, K. & Miyaji, M. 1995. A human isolate of *Exophiala (Wangiella) dermatitidis* forming a catenate synanamorph that links the genera *Exophiala* and *Cladophialophora*. J. Med. Vet. Mycol. **33**:355-358.
10. Masclaux, F., Guého, E., Hoog, G.S. de & Christen, R. 1995. Phylogenetic relationships of human-patogenic *Cladosporium (Xylohypha)* species inferred from partial LS rRNA sequences. J. Med. Vet. Mycol. **33**:327-338.
11. Takeo, K., Hoog, G.S. de, Miyaji, M. & Nishimura, K. 1995. Conidial surface ultrastructure of human-pathogenic and saprobic *Cladosporium* species. Antonie van Leeuwenhoek **68**:51-55.
12. Tintelnot, K., Hunnius, P. von, Hoog, G.S. de, Polak-Wyss, A., Guého, E. & Masclaux, F. 1995. Systemic mycosis caused by a new *Cladophialophora* species. J. Med. Vet. Mycol. **33**:349-354.
13. Uijthof, J.M.J. & Hoog, G.S. de. 1995. PCR-Ribotyping of type isolates of currently accepted *Exophiala* and *Phaeococcomyces* species. Antonie van Leeuwenhoek **68**:35-42.
14. Wollenzien, U., Hoog, G.S. de, Krumbein, W.E. & Urzi, C. 1995. On the isolation, cultivation and taxonomy of microcolonial fungi occurring on and in marble and other calcareous rocks. Sci. Total Environm. **167**:287-294.
15. Yurlova, N.A., Mokrousov, I.V. & Hoog, G.S. de. 1995. Intraspecific variability and exopolysaccharide production in *Aureobasidium pullulans*. Antonie van Leeuwenhoek **68**:57-63.
16. Butin, H., Pehl, L., Hoog, G.S. de & Wollenzien, U. 1996. *Trimmatostroma abietis* sp. nov. (Hyphomycetes) and related species. Antonie van Leeuwenhoek **69**:203-209.
17. Figueras, M.J., Hoog, G.S. de, Takeo, K. & Guarro, J. 1996. Stationary phase development of *Trimmatostroma abietis*. Antonie van Leeuwenhoek **69**:217-222.

18. Uijthof, J.M.J., Figge, M.J. & Hoog, G.S. de. Molecular and physiological investigations of *Exophiala* species described from fish. Antonie van Leeuwenhoek (submitted).
19. Uijthof, J.M.J., Belkum, A. van, Hoog, G.S. de & Haase, G. *Exophiala dermatitidis* and the related species *Sarcinomyces phaeomuriformis:* physiology, ITS1-sequencing and the development of a molecular probe. Syst. Appl. Microbiol. (submitted).
20. Masclaux, F., Guého, E., Hoog, G.S. de & Villard, J. 1996. Intraspecific variability within species of *Cladophialophora* and *Cladosporium* using arbitrary primed PCR. J. Mycol. Méd. **6**:15-18.
21. Yurlova, N.A., Uijthof, J.M.J. & Hoog, G.S. de. 1996. Distinction of species in Aureobasidium and related genera by PCR-ribotyping. Antonie van Leeuwenhoek **69**:323-329.
22. Uijthof, J.M.J., Figge, M.J. & Hoog, G.S. de. Molecular and physiological investigations of *Exophiala* species described from fish. Antonie van Leeuwenhoek (in press).
23. Uijthof, J.M.J., Belkum, A. van, Hoog, G.S. de & Haase, G. *Exophiala dermatitidis* and the related species *Sarcinomyces phaeomuriformis:* physiology, ITS1-sequencing and the development of a molecular probe. Syst. Appl. Microbiol. (in press).
24. Wollenzien, U., Hoog, G.S. de, Krumbein, W. & Uijthof, J.M.J. *Sarcinomyces petricola,* a new microcolonial fungus from marble in the Mediterranean basin. Antonie van Leeuwenhoek (in press).
25. Hoog, G.S. de, Beguin, H. & Batenburg-van de Vegte, W.H.: Phaeotheca triangularis, a new meristematic black yeast from a humidifier. Antonie van Leeuwenhoek (in press).
26. Middelhoven, W.J. & Hoog, G.S. de. *Hormonema schizolunatum,* a new species of dothideaceous black yeasts from phyllosphere. Antonie van Leeuwenhoek (in press).
27. Mayser, P., Gründer, K., Qadripur, S., Köhn, F.-M., Schill, W.-B. & Hoog, G.S. de. Diagnostik, Klinik und Therapie der frühen Chromoblastomykose an einem Fallbespiel. Der Hautarzt (in press).
28. Hoog, G.S. de & Guého, E.: Agents of white piedra, black piedra and tinea nigra. In Topley & Wilson's Microbiol. Microb. Infect., ed. 9, vol. 4, chapter 12 (in press).
29. Roeijmans, H.J., Hoog, G.S. de, Tan, C.S. & Figge, M.J. Molecular taxonomy and GC/MS of metabolites of *Scytalidium hyalinum* and *Nattrassia mangiferae* (*Hendersonula toruloidea*). J. Med. Vet. Mycol. (in press).
30. Yoshida, S., Takeo, K., Hoog, G.S. de, Nishimura, K. & Miyaji, M. 1996. A new type of growth exhibited by Trimmatostroma abietis. Antonie van Leeuwenhoek **69**:211-215.
31. Hoog, G.S. de, Guého, E. & Smith, M.Th. Nomenclatural notes on some arthroconidial yeasts. Mycotaxon (in press).
32. Yurlova, N.A. & Hoog, G.S. de. A new variety of *Aureobasidium pullulans* characterized by exopolysaccharide structure, nutritional physiology and molecular features. Antonie van Leeuwenhoek (in press).
33. Hoog, G.S. de, Uijthof, J.M.J., Gerrits van den Ende, A.H.G., Figge, M.J. & Weenink, X.O. Comparative rDNA diversity in medically significant fungi. Microbiol. Cult. Coll. (in press).
34. Montijn, R.C., Wolven, P. van, Hoog G.S. de & Klis, F.M. β-glucosylated proteins in the cell wall of *Exophiala* (*Wangiella*) *dermatitidis.* (in press).

VIII. École Nationale Supérieure Agronomique de Montpellier, Chaire de Microbiologie Industrielle et de Génétique des Microorganismes. Place Pierre-Viala, 34060 Montpellier Cedex, France. Communicated by P. Galzy.

1. Briand D., Dubreucq E. and Galzy P. 1995. Factors affecting the acyltransfer activity of the lipase from *Candida parapsilosis* in aqueous media. J. Am. Oil Chem. Sec. **72**:1367-1373.

2. Lomascolo A., Dubreucq E. and Galzy P. 1996. Study of the Δ12-desaturase system of *Lipomyces starkeyi.* Lipids **31**:253-259.

3. Lecointe C., Dubreucq E. and Galzy P. 1996. Esters synthesis in aqueous media in the presence of various lipases. Biotechnol. Lett. **18**:869-874.

4. Janbon G., Magnet R., Bigey F., Arnaud A. and Galzy P. 1995. Karyotype studies on different strains of *Candida molischiana* by pulsed-field gel electrophoresis. Curr. Genet. **28**:150-154.

5. Gueguen Y., Chemardin P., Janbon G., Arnaud A. and Galzy P. 1996. A very efficient β-glucosidase-catalyst for the hydrolysis of flavor precursors process of wines and fruit juices. Agric. Food Chem. **44**:2336-2340.

IX. Instituto de Investigaciones biomédicas del CSIC, Arturo Duperier 4, 28029 Madrid, Spain. Communicated by J.M. Gancedo <jmgancedo@biomed.iib.unam.es>.

1. Vincent O. & Gancedo J.M. 1995. Analysis of positive elements sensitive to glucose in the promoter of the *FBP1* gene from yeast. J. Biol. Chem. **270**:12832-12838.

We have identified in the promoter of the *FBP1* gene from *Saccharomyces cerevisiae,* which codes for fructose-1,6-bisphosphatase, two elements which can form specific DNA.protein complexes and which confer glucose-repressed expression to an heterologous reporter gene. Complex formation and activation of transcription by either element require a functional *CAT1* gene and are not blocked by a *hap2-1* mutation, although this mutation interferes with maximal expression of the *FBP1* gene. A sequence from one of the elements acts as a weak upstream activating sequence, but its activity can be stimulated up to 10-fold by neighboring sequences. A further element of the promoter has been characterized, which forms a specific DNA.protein complex only when a nuclear extract from derepressed cells is used. This element does not activate transcription in a heterologous promoter. The DNA sequences of the three elements involved in protein binding, defined by DNase I footprinting, have no homology with consensus sequences for known activating factors.

2. Navas M.A. & Gancedo J.M. 1996. The regulatory characteristics of yeast fructose-1,6-bisphosphatase confer only a small selective advantage. J. Bacteriol. **178**:1809-1812.

The question of how the loss of regulatory mechanisms for a metabolic enzyme would affect the fitness of the corresponding organism has been addressed. For this, the fructose-1,6- bisphosphatase (FbPase) from *Saccharomyces cerevisiae* has been taken as a model. Yeast strains in which different controls on FbPase (catabolite repression and inactivation; inhibition by fructose2,6-bisphosphate and AMP) have been removed have been constructed. These strains express during growth on glucose either the native yeast FbPase, the *Escherichia coli* FbPase which is insensitive to inhibition by fructose-2,6-bisphosphate, or a mutated *E.coli* FbPase with low sensitivity to AMP. Expression of the heterologous FbPases increases the fermentation rate of the yeast and its generation time, while it decreases its growth yield. In the strain containing high levels of an unregulated bacterial FbPase, cycling between fructose-6-phosphate and fructose-1,6-bisphosphate reaches 14%. It is shown that regulatory mechanisms of FbPase provide a slight but definite competitive advantage during growth in mixed cultures.

3. Casal M., Blázquez M.A., Gamo F.J., Gancedo C. & Leao C. 1995. Lack of lactate-proton symport activity in *pck1* mutants of *Saccharomyces cerevisiae.* FEMS Microbiol. Lett. **128**:279-282.

Mutants of *Saccharomyces cerevisiae* without phosphoenolpyruvate carboxykinase activity showed no measurable lactate proton symport, while mutants without fructose-1,6-bisphosphatase had normal transport activity. Incubation of a *pck1* mutant, under derepression conditions in the presence of glycerol, restored the activity of the lactate-proton symport, with identical kinetic characteristics to that in the wild-type. For efficient lactateproton symport activity, not only is an external inducer such as lactic acid needed, but also a molecule derived from the acid metabolism may be necessary.

4. Casas C., Aldea M., Casamayor A., Lafuente M.J., Gamo F.J., Gancedo C., Ariño J. & Herrero E. 1995. Sequence analysis of a 9873 bp fragment of the left arm of the yeast chromosome XV that contains the *ARG8* and *CDC33* genes, a putative riboflavin synthase beta chain gene and four new open reading frames. Yeast **11**:1061-1067.

The DNA sequence of a 9873 bp fragment located near the left telomere of chromosome XV has been determined. Sequence analysis reveals seven open reading frames. One is the *ARG8* gene coding for N-acetylornithine aminotransferase. Another corresponds to *CDC33,* which codes for the initiation factor 4E or cap binding protein. The open reading frame *AOE169* can be considered as the putative gene for the *Saccharomyces cerevisiae* riboflavin synthase beta chain, since its translation product shows strong homology with four prokaryotic riboflavin synthase beta chains. The nucleotide sequence reported here has been submitted to the EMBL data library under the Accession Number X84036.

5. Casamayor A., Aldea M., Casas C., Herrero E., Gamo F.J., Lafuente M.J., Gancedo C. & Ariño J. 1995. DNA sequence analysis of a 13 kbp fragment of the left arm of yeast chromosome XV containing seven new open reading frames. Yeast **11**:12181-1288.

The sequence of a 13 kbp fragment located in the vicinity of the left telomere of chromosome XV (cosmid pEOA179) has been determined. Seven new open reading frames (ORFs) encoding polypeptides longer than 100 residues have been found (AOB629, AOA342, AOC231, AOE555, AOE236, AOA236 and AOE1045). Three of them show no identity with proteins deposited in the data banks. ORF AOB629 (629 amino acids) has some similarity with previously described ferric reductases from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe.* ORF AOA342 encodes a polypeptide reminiscent of dihydroflavonol-4-reductases from a number of plant species. AOE236 displays a high level of identity when compared with peroxisomal membrane proteins previously cloned from the methylotrophic yeast *Candida boidinii.* Finally, AOE1045 encodes a large protein (1045 residues) with some identity with a hypothetical 147 kDa protein identified during the sequencing of *Caenorhabditis elegans* chromosome 3. The complete nucleotide sequence of the 13 kbp fragment has been deposited at the EMBL data base (Accession Number Z48239).

6. Blázquez M.A., Gamo F.J. & Gancedo C. 1995. A mutation affecting carbon catabolite repression suppresses growth defects in pyruvate carboxylase mutants from *Saccharomyces cerevisiae.* FEBS Letters **377**:197-200.

Yeasts with disruptions in the genes *PYC1* and *PYC2* encoding the isoenzymes of pyruvate carboxylase cannot grow in a glucose-ammonium medium (Stucka et al. (1991) Mol. Gen. Genet. 229, 307-315). We have isolated a dominant mutation, *BPC-1,* that allows growth in this medium of yeasts with interrupted *PYC1* and *PYC2* genes. The *BPC1-1* mutation abolishes catabolite repression of a series of genes and allows expression of the enzymes of the glyoxylate cycle during growth in glucose. A functional glyoxylate cyle is necessary for suppression as a disruption of gene *ICL1* encoding isocitrate lyase abolished the phenotypic effect of *BPC1-1* on growth in glucose-ammonium. Concurrent expression from constitutive promoters of genes *ICL1* and *MLS1* (encoding malate synthase) also suppressed the growth phenotype of *pyc1 pyc2* mutants. The mutation *BPC1-1* is either allelic or closely linked to the mutation *DGT1-1*.

7. Blázquez M.A. & Gancedo C. 1995. Mode of action of the *qcr9* and *cat3* mutations in restoring the ability of *Saccharomyces cerevisiae tps1* mutants to grow on glucose. Mol. Gen. Genet. **249**:655-664.

Mutations in the *TPS1* gene, which encodes trehalose-6-P synthase, cause a glucose-negative phenotype in *Saccharomyces cerevisiae.* Antimycin A or disruption of the *QCR9* gene, which encodes one subunit of the cytochrome *bc1* complex, restore the ability to grow in glucose-containing media. Under these conditions the cell excreted a large amount of glycerol, corresponding to about 20% of the glucose taken up. Suppression appears to be achieved by diversion of accumulated glycolytic intermediates to the production of glycerol, thereby providing NAD+ and phosphate for the glyceraldehyde-3-P dehydrogenase reaction. Analysis of the mutation *sci1-1,* which also suppresses the glucose-negative phenotype of *tps1* mutants, showed that glucose transport was decreased in *sci1-1* mutants. The gene *SCI1* was cloned and its nucleotide sequence revealed it to be identical to *CAT3/SNF4.* The suppression mediated by *sci1-1* is attributable to a decrease in glycolytic flux.

8. Gamo F.J., Lafuente M.J., Casamayor A., Ariño J., Aldea M., Casas C., Herrero E. & Gancedo C. 1996. Analysis of the DNA sequence of a 15,500 bp fragment near the left telomere of chromosome XV from *Saccharomyces cerevisiae* reveals a putative sugar transporter, a carboxypeptidase homologue and two new open reading frames. Yeast **12**:709-714.

We report the sequence of a 15.5 kb DNA segment located near the left telomere of chromosome XV of *Saccharomyces cerevisiae.* The sequence contains nine open reading frames (ORFs) longer than 300 bp. Three of them are internal to other ones. One corresponds to the gene *LGT3* that encodes a putative sugar transporter. Three adjacent ORFs were separated by two stop codons in frame. These ORFs presented homology with the gene *CPS1* that encodes carboxypeptidase S. The stop codons were not found in the same sequence derived from another yeast strain. Two other ORFs without significant homology in databases were also found. One of them, O0420, is very rich in serine and threonine and presents a series of repeated or similar amino acid stretches along the sequence. The nucleotide sequence has been deposited in the EMBL data library under Accession Number X89715.

9. Lafuente M.J., Gamo F.J. & Gancedo C. 1996. DNA sequence analysis of a 10624 bp fragment of the left arm of chromosome XV from *Saccharomyces cerevisiae* reveals a RNA binding protein, a mitochondrial protein, two ribosomal proteins and two new open reading frames. Yeast **12**:1041-1045.

We have determined the sequence of a 10624 bp DNA segment located in the left arm of chromosome XV of *Saccharomyces cerevisiae.* The sequence contains eight open reading frames (ORFs) longer than 100 amino acids. Two of them do not present significant homology with sequences found in the databases. The product of ORF o0553 is identical to the protein encoded by the gene SMF1. Internal to it there is another ORF, o0555 that is apparently expressed. The proteins, encoded by ORFs o0559 and o0565 are identical to ribosomal proteins S19.e and L18 respectively. ORF o0550 encodes a protein with an RNA binding signature including RNP motifs and stretches rich in asparagine, glutamine and arginine. The nucleotide sequence determined has been deposited in the EMBL data library under Accession Number X95258.

X. Biology Department, Tennessee State University, 3500 John Merritt Blvd., Nashville, Tennessee, USA 37209. Communicated by P.F. Ganter <ganter01@harpo.tnstate.edu>.

1. P.F. Ganter & B. Quarles. Analysis of population structure of cactophilic yeast from the genus *Pichia*: *P.cactophila* and *P.norvegensis*. Can. J. Microbiol. (in press).

DNAs from 40 strains of *Pichia cactophila* and *P.norvegensis,* yeasts characteristic of cactus necroses, were compared using RAPD banding patterns and killer/sensitive phenotypes. Both species belong to the same species complex within the genus. The levels of between-strain RAPD variation were high in both species (higher in the apomictic *P.cactophila* than in the heterothallic *P.norvegensis*), although there is little variation in physiological abilities within either species. Although each species was a separate lineage, RAPD analysis confirms that the species are related. Within each species, RAPD variation was related to the geographic origin of the strains. *P.cactophila* strains from southern Florida were more related to those from Antigua than to those from north-central Florida. These results correlated well with the differences among strains' killer/sensitive phenotypes. PCA analysis indicated that each species phenotypes differ. Here too, strains from southern Florida were more similar to those from Antigua than to those from north central Florida. Previous work had identified differences in the cactophilic yeast communities from southern and north-central Florida and these results indicate that the differences are detectable at the population level as well.

XI. Unité de Mycologie, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15, France. Communicated by É. Guého.

1. E. Guého, G. Midgley[1] & J. Guillot.[2] 1996. The genus *Malassezia* with description of four new species. 1996. Antonie van Leeuwenhoek **69**:337-355.

[1]Department of Medical Mycology, St. John's Institute of Dermatology, St.Thomas' Hospital, Lambeth Palace Rd, London SEI 7EH, UK

[2]Unité de Parasitologie-Mycologie, École Nationale Vétérinaire d'Alfort, 7, avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex, France

The genus *Malassezia* has been revised using morphology, ultrastructure, physiology and molecular biology. As a result the genus has been enlarged to include seven species comprising the three former taxa *M.furfur, M.pachydermatis* and *M.sympodialis,* and four new taxa *M.globosa, M.obtusa, M.restricta* and *M.slooffiae.* The descriptions of all the species include morphology of the colonies and of the cells, together with ultrastructural details. The physiological properties studied were the presence of catalase, the tolerance of 37°C and the ability to utilize certain concentrations of Tween 20, 40, 60 and 80 as a source of lipid in a simple medium. Information is given for each of the taxa on mol%GC and also the rRNA sequence from the comparison previously described for the genus.

2. J. Guillot, E. Guého, M. Lesourd,[1] G. Midgley G. Chevrier, B. Dupont. 1996. Identification of Malassezia species. A practical approach. J. Mycol. Méd. **6**:103-110.

[1]Laboratoire d'Histologie et d'Embryologie, Faculté de Medecine, 1, rue Haute Reculée, 49000 Angers, France.

Background and objective. The genus *Malassezia* has recently been enlarged to include seven distinct species. Our purpose was to present a practical approach to the identification of all *Malassezia* yeasts from clinical material. **Materials and methods.** Representative isolates corresponding to the seven *Malassezia* species were examined. Yeast cell morphology was described. Ability to utilize individual Tweens (20, 40, 60 and 80) was assessed by the technique of diffusion. Catalase reaction was also determined. **Results and discussion.** All Malassezia species, except *M.furfur,* were proved to have stable morphological characteristics. Bottle-shaped yeasts might be globose (*M.globosa,* sometimes *M.furfur*), ovoid (*M.pachydermatis, M.sympodialis, M.restricta, M.slooffiae, M. furfur*) or cylindrical (*M.obtusa, M.furfur*). *M.pachydermatis* was confirmed to be non-lipid-dependent and could therefore be grown on conventional media. *M.restricta* was the only lipid-dependent species to lack catalase. Species were further distinguished by the four Tween assimilation patterns. **Conclusion.** The methods described in this study do not require elaborate equipment and yet represent a satisfactory identification scheme for *Malassezia* species.

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

1. Golubev, W.I. & Boekhout, T. 1995. Sensitivity to killer toxins as a taxonomic tool among heterobasidiomycetous yeasts. Studies in Mycology **38**:47-58.

Killer sensitivity patterns differ among phylogenetically diverse basidiomycetous yeasts. Taxonomic and phylogenetic applications and limitations of this character are discussed. Yeast and hyphal phases of a single organism may differ in sensitivity to killer toxins. The two varieties of *Cryptococcus neoformans* can be differentiated using the mycocin of *Cryptococcus laurentii.*

2. Golubev, W.I. & Nakase, T. 1996. Taxonomic specificity of sensitivity to the mycocin produced by *Bullera sinensis*. Microbiology and Culture Collections **12**:45.

3. Golubev, W.I. 1996. Russian Collection of Microorganisms (VKM). Proc. Asian Int. Mycol. Congress'96 (Ed. by T. Nakase & K. Takeo). Chiba Univ., Chiba. p. 38.

4. Kulakivskaya, T.V., Karamysheva, Z.N., Andreeva, N.A. & Golubev, W.I. 1996. For characterization of *Cystofilobasidium bisporidii* mycocin. Mikrobiologiya **65**:788-792.

The mycocin produced by *Cystofilobasidium bisporidii* was adsorbed rapidly by cells of sensitive yeast and caused permeability changes of cytoplasmic membrane. The spheroplasts of some yeasts (the whole cells of which were resistant) were sensitive to this mycocin having a molecular mass of about 16 kDa. Partisal purification of it resulted in decreased stability.

5. Golubev, W.I. 1996. Killer yeasts. Priroda **11**:49-53.

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

1. B.F. Johnson, B.Y. Yoo[1] & G.B. Calleja. 1995. Smashed fission yeast walls structural discontinuities related to wall growth. Cell Biophysics **26**:57-75.

[1]Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada.
[2]Diliman Institute, UP Campus, Diliman, Lunsod Quezon, The Philippines.

Twenty-three samples of fission yeast cells (*Schizosaccharomyces pombe*) were smashed by shaking them with glass beads. The samples represented all phases of the culture cycle, with the lag and log phases emphasized. Ruptured walls of the smashed cells were observed by phase-contrast and electron microscopy. Ruptures were tabulated with respect to their magnitudes and locations. Ruptures occurred not at random, nor at sites directed by geometry, but predominated in certain definable wall regions. These discontinuities were correlated with morphogenetic activities of the cell. Thus, the extensile end was found to be most fragile through most of the culture cycle. Also fragile was the nonextensile end, its edge more than its middle. Further, the data were applied to the testing of predictions from extant models (Johnson endohydrolytic softening model and Wessels presoftened-posthardened and crosslinking model) for hyphal tip extension. The frequency of rupture at the extensile (old) end of the cell was qualitatively predicted by both models; the frequency at the nonextensile (new) end was not predictable by either. Rupture frequencies and characteristics at other regions conformed to predictions by one or the other model, but rarely by both.

2. M.M. Kekez, P. Savic & B.F. Johnson. 1996. Contribution to the biophysics of the lethal effects of electric field on microorganisms. Biochim. Biophys. Acta **1278**:79-88.

The proposed model assumes that the criteria leading to the lethal breakdown of microorganisms suspended in a continuous medium depend on two parameters: (a) the applied electric field must exceed the critical field of membrane to create holes and (b) the Joule energy (deposited in the membrane) must exceed the minimum value beyond which the cell can not recover. The first parameter initiates (reversible) breakdown and the second one, the completion of the (irreversible) electrical breakdown leading to death of the cell. The number of cells surviving the electric field treatment is related to statistical distribution of cell size. Comparison between theory and the experimental results of Kinosita and Tsong (1977); Hülsheger et al. (1980, 1981, 1983); Rosembera and Korenstein (1990) and others is given.

3. M. Miyata,[1] H. Doi,[1] H. Miyata,[2] & B.F. Johnson. In press. Sexual co-flocculation by heterothallic cells of the fission yeast *Schizosaccharomyces pombe* modulated by medium constituents. Antonie van Leeuwenhoek.

[1]Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502 Japan.
[2]Nagoya Economic University. 61-1, Aza-Uchikubo, Inuyama 484, Japan.

Novel simple synthetic media for inducing sexual co-flocculation in a short time after mixing heterothallic fission-yeast (*Schizosaccharomyces pombe*) cells of h^- and h^+ were devised. The most effective of these, mannose synthetic medium (MSM) contains 0.4% mannose as a carbon source in addition to galactose, KH_2PO_4, (pH 4.0) and 4 vitamins. The addition of galactose to this medium suppressed the asexual self-flocculation but rather promoted the sexual flocculation. By using MSM, these heterothallic strains were revealed to be sexually ready through a long period of the log to stationary phases. Furthermore, a variety of C-sources and NH_4Cl at various concentrations in various media were examined for their effects upon sexual co-flocculation, conjugation and sporulation: it was found that the sugar concentration strictly affected the progress of the sequence of sexual reproduction at 26°C but not at 30°C and that sexual co-flocculation of the heterothallic strains was induced only under lower concentrations of C- and N-sources than that for the homothallic one.

XIV. Alkomohr Biotech Ltd., and Department of Biosciences, University of Helsinki, Biocentre, Viikinkaari 9, FIN-00710 Helsinki, Finland. Communicated by M. Korhola <matti.korhola@yeast.pp.fi>.

1. Naumov, G.I., Naumova, E.S., Turakainen, H. & Korhola, M. 1996. Identification of the α-galactosidase *MEL* genes in some populations of *Saccharomyces cerevisiae*: a new gene *MEL11*. Genet. Res. Camb. **67**:101-108.

In this report we mapped a new *MEL11* gene and summarize our population studies of the α-galactosidase *MEL* genes of *S. cerevisiae.* The unique family of structural *MEL* genes has undergone rapid translocations to the telomeres of most chromosomes in some specific *Saccharomyces cerevisiae* populations inhabiting olive oil processing waste (alpechin) and animal intestines. A comparative study of *MEL* genes in wine, pathogenic and alpechin populations of *S. cerevisiae* has been conducted using genetic hybridization analysis, molecular karyotyping and Southern hybridization with the *MEL1* probe. Five polymeric genes for the fermentation of melibiose, *MEL3, MEL4, MEL6, MEL7, MEL11*, have been identified in an alpechin strain CBS 3081. The new *MEL11* gene was mapped by tetrad analysis to the left telomeric region of chromosome I. In contrast, in wine and pathogenic populations of *S. cerevisiae, MEL* genes have been apparently eliminated. Their rare Mel^+ strains carry only one of the *MEL1, MEL2,* or *MEL8* genes. One clinical strain YJM273 was heterozygotic on the *MEL1* gene; its $mel1^0$ allele did not have a sequence of the gene.

2. Naumov, G.I., Naumova, E.S., Sancho, E.D. & Korhola, M.P. 1996. Polymeric *SUC* genes in natural populations of *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. **135**:31-35.

The comparative chromosomal locations of polymeric β-fructosidase *SUC* genes have been determined by Southern blot hybridization with the *SUC2* probe in 91 different strains of *Saccharomyces cerevisiae*. Most of the strains exhibited a single *SUC2* gene, but in some strains two or three *SUC* genes were found. All Suc^- strains carried a silent $suc2^0$ sequence. The accumulation of *SUC* genes was observed in populations derived from sources containing sucrose and seems to be absent in strains from sources promoting the *MEL* gene.

3. Aho, S., Arffman, A. & Korhola, M. 1996. *Saccharomyces cerevisiae* mutants selected for increased production of *Trichoderma reesei* cellulases. Appl. Microbiol. Biotechnol. **46**:36-45.

Trichoderma reesei endoglucanase I (EGI) was used as a reporter enzyme for screening mutagenized yeast strains for increased ability to produce protein. Sixteen haploid *Saccharomyces cerevisiae* strains, transformed with a yeast multicopy vector pALK222, containing the EGI cDNA under the *ADH1* promoter, produced EGI activity of 10^{-5}-10^{-4} g/l. On the average 93% of the total activity was secreted into the culture medium. Two strains with opposite mating types were mutagenized, and several mutants were isolated possessing up to 45-fold higher EGI activity. The best mutants were remutagenized and a second generation mutant, strain 2804, with an additional twofold increase in EGI activity was selected. The mutant strain 2804 grew more slowly and reached a lower final cell density than the parental strain. In the selective minimal medium, the strain 2804 produced 40 mg/l immunoreactive EGI protein, but only 2% was active enzyme. In the rich medium the secreted EGI enzyme stayed active, but without selection pressure the EGI production ceased after 2 days of cultivation, when the strain 2804 had produced 10 mg/l of EGI. A sevenfold difference was found between the parental and the 2804 strain in their total EGI production relative to cell density. The difference in favour of the mutant strain was also detected on the mRNA level. The 2804 mutant was found to be more active than the parental strain also in the production of *T. reesei* cellulases, cellobiohydrolase I, and cellobiohydrolase II.

4. Naumova, E.S., Naumov, G.I. & Korhola, M. 1996. Use of molecular karyotyping for differentiation of species in the heterogeneous taxon *Saccharomyces exiguus*. J. Gen. Appl. Microbiol. **42**:307-314.

A molecular karyotyping technique consisting of pulsed-field gel electrophoresis of intact chromosomal DNAs and following Southern hybridization with total *Hin*dIII-digested DNAs was used as a tool for yeast classification and identification. Significant differences in chromosomal patterns were found among 16 *Saccharomyces exiguus* strains. The complex taxon, classified into several groups of different karyotypes, showed a low degree of cross-DNA homology.

The following papers are in press:

5. Paalme, T., Elken, R., Vilu, R. & Korhola, M. 1996. Growth efficiency of *Saccharomyces cerevisiae* on glucose/ethanol media with smooth change in the dilution rate (A-stat). Enzyme Microbial Technol. **19**:000-000.

The effect of ethanol/glucose ratio on the growth characteristics (μ_{max}, $Q_{O_2}max$, Y_{XS}, Y_{XO_2}, etc.) of *Saccharomyces cerevisiae* on mineral media was studied. The maximum specific growth rate of the strain ,μ_{max} obtained in a continuous culture with a smooth increase of dilution rate (A-stat), was equal to 0.44 h^{-1} on glucose and to 0.13 h^{-1} on ethanol mineral medium. The maximum value of the specific respiratory rate with six different glucose/ethanol ratios remained in the range of 6-9 mmol O_2 h^{-1} g

dwt^{-1}. The residual concentration of ethanol up to concentrations of 4 g l^{-1} did not reduce the respiration rate markedly. The critical specific growth rate, μ_{crit}, (I_{eth} = 0) was 0.25 h^{-1}, which was independent on the ethanol/glucose ratio in the feed medium. Higher growth rates than $\mu > 0.25$ $^{h-}$ were supported by the fermentative use of glucose. The maximum growth yield, Y_{XS}, increased from 0.57 on pure glucose to 0.68 on pure ethanol (C-mol C-mol^{-1}). A practical maximum ratio of ethanol to glucose in Baker's yeast propagation was suggested to be 40%-50% (C-mol C-mol^{-1}).

6. Naumova, E.S., Turakainen, H., Naumov, G.I. & Korhola, M. 1996. Superfamily of α-galactosidase genes: *MEL* genes of the *Saccharomyces sensu stricto* species complex. Mol. Gen. Genet. **252**:000-000.

In order to study the molecular evolution of the yeasts grouped in the *Saccharomyces sensu stricto* species complex by analysis of the *MEL* gene family, we have cloned and sequenced two new species-specific *MEL* genes from *Saccharomyces* yeasts: *S. paradoxus* (*MELp*) and a Japanese *Saccharomyces* sp. (*MELj*). The clones were identified by hybridization homology to the *S. cerevisiae MEL1* gene. Both clones revealed an ORF of 1413 bp coding for a protein of 471 amino acids. The deduced molecular weights of the α-galactosidase enzymes were 52 767 for *MELp* and 52 378 for *MELj*. The nucleotide sequences of the *MELp* (EMBL accession no. X95505) and the *MELj* (EMBL accession no. X95506) genes showed 74.7% identity. The degree of identity of *MELp* to the *MEL1* gene was 76.8% and to the *S. pastorianus MELx* gene 75.7%. The *MELj* coding sequence was 75.1% identical to the *MEL1* gene and 80.7% to the *MELx* gene. The data suggest that *MEL1, MELj, MELp* and *MELx* genes are species-specific *MEL* genes. The strains studied each have only one *MEL* locus. The *MELp* gene is located on the *S. paradoxus* equivalent of the *S. cerevisiae* chromosome X; the *MELj* gene was on the chromosome that comigrates with the *S. cerevisiae* chromosome VII/XV doublet and hybridizes to the *S. cerevisiae* chromosome XV marker *HIS3*.

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

1. K. Riedel, M. Lehmann, K. Adler & G. Kunze. 1996. Physiological characterization of a microbial sensor containing the yeast *Arxula adeninivorans*. Antonie van Leeuwenhoek (in press).

The yeast *Arxula adeninivorans* LS3 is a suitable organism for use as part of a microbial sensor. In combination with an amperometric oxygen electrode the sensor offered a possibility for the physiological characterization of this yeast. About 300-400 measurements could be carried out with a single *Arxula* sensor. The microbial sensor was remarkably stable for over 35 days, when kept at 37°C during the operation time and at room temperature overnight. The physiological characteristics of *Arxula adeninivorans* LS3 obtained with the sensor technique were identical to the data obtained with the conventional techniques. However, the sensor technique makes it additionally possible to quantify the physiological data. So the substrates ribose, citric acid, glycerol, oil and benzoate produced signals lower than 10% in comparison to the glucose signal. Fructose, xylose, sucrose, maltose, gentianose, glucosamine, glutamic acid, tryptophan, butyric acid, lauryl acid and propionic acid reached 10-70%, galactose, alanine, glycine, lysine and methionine signals were similar to the glucose signal whereas acetic acid, ethyl alcohol, capron acid, capryl acid and caproic acid reached the highest signals up to 434%.

XVI. Faculté d'Oenologie, Université de Bordeaux II, 351, cours de la Libération, 33405 Talence, France. Communicated by F. Larue.

1. Masneuf I, Aigle M. & Dubourdieu D. 1996. Development of a polymerase chain reaction/restriction fragment length polymorphism method for *Saccharomyces cerevisiae* and *Saccharomyces bayanus* identification in enology. F.E.M.S. Microbiol. Lett. **138**:239-244.

Several yeast strains of the species *Saccharomyces cerevisiae, S.bayanus* and *S.paradoxus,* first identified by hybridization experiments and measurements of DNA/DNA homology, were characterized using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis of the *MET2* gene. There was no exception to the agreement between this method and classical genetic analyses for any of the strains examined, so PCR/RFLP of the *MET2* gene is a reliable and fast technique for delimiting *S.cerevisiae* and *S.bayanus.* Enological strains classified as *S.bayanus, S.chevalieri,* and *S.capensis* gave *S.cerevisiae* restriction patterns, whereas most *S.uvarrum* strains belong to *S.bayanus.* Enologists should no longer use the name of *S.bayanus* for *S.cerevisiae* Gal strains, and should consider *S.bayanus* as a distinct species.

2. Ness F., Lavallée F., Dubourdieu D., Aigle M. & Dulau L. 1993. Identification of yeast strains using the polymerase chain reaction. J. Sc. Food Agri. **62:**89-94.

Commonly used techniques for the identification of industrial yeast strains are usually time-consuming and cumbersome. Moreover, some of these methods may give ambiguous results. A novel strategy has been developed for identifying yeast strain employing polymerase chain reaction technology. Using customised oligonucleotides some regions of the yeast genome between elements are amplified to give an 'amplified sequence polymorphism' (Skolnick and Wallace, 1988) characteristic of the strains. With this technique it is possible to identify individual strains of *Saccharomyces cerevisiae.*

3. Doignon F & Rozes N. 1992. Effect of triazole fungicides on lipid metabolism of *Saccharomyces cerevisiae.* Lett. appl. Microbiol., **15**:172-174

The triazole fungicide (Flusilazole) modified the sterol content of *Saccharomyces cerevisiae.* The plasma membrane the short free fatty acids (C6 to C14) and the unsaturated free fatty acids increased in the cells, while the short free fatty acids fluidity was altered by the presence of methyl sterol which increased with the flusilazole concentration. On the other hand, decreased in the medium.

4. Rozes N, Garcia-Jares C, Larue F. & Lonvaud A. 1992. Differentiation between fermenting and spoilage yeasts in wine by total free fatty acid analysis J. Sc. Food Agric., **59**:351-357

Differentiation between fermenting and spoilage yeasts in wine was estimated by cellular fatty acid profiles. Forty-two strains of yeasts representing 17 genera were grown on a defined liquid medium for 48 h at 25°C in a rotary shaker. After saponification of yeast cells, free fatty acid extracts were analysed by gas chromatography. Multivariate analysis was performed by Principal Components Analysis (PCA) to define clusters of fatty acids and yeasts. Strains were characterised especially by long-chain fatty acids, palmitic (C_{16}) to linolenic ($C_{18:3}$) acid under aerobic culture. Nevertheless, most of *Saccharomyces cerevisiae* and also *Dekkera bruxellensis* (former names *D.intermedia* and *Brettanomyces lambicus*) synthesized medium-chain fatty acids, octanoic (C_8) acid to dodecanoic (C_{12}) acid. With this method it was possible to differentiate fermenting grape yeasts such as *S.cerevisiae* from spoilage yeasts on the basis of the absence of linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. However, the method seemed unreliable for the identification of strains, more particularly those of species of *S.cerevisiae*.

Ph.D. Theses. Faculty of Oenology. University of Bordeaux II

5. Isabelle Masneuf. 1996. Research on genetic identification of wine yeasts. Oenological applications.

The polymerase chain reaction method is used directly on entire yeast cells for genetic differentiation of wine yeasts. In conjunction with Restriction Fragment Length Polymorphism analysis (RFLP), PCR is useful for fast delimitation of fermenting species like *S.cerevisiae* and *S.bayanus* and also *S.paradoxus* species. Oenological strains until now classified as *S.bayanus* (ex. *oviformis*) give *S.cerevisiae* restriction patterns, whereas most of mel (+) yeasts (ex. *uvarum*) belong to *S.bayanus* species. PCR analysis of *S.cerevisiae* strains associated with the delta sequence distinguish most of the industrial yeast strains but the discrimination power of this method is inferior to pulsed field electrophoresis for identification of indigenous yeast strains. The PCR constitutes a rapid and sensitive tool for the control of yeast implantation in vinification. This method, combined with karyotype analysis, is applied for characterization of wild strains and wild species isolated from the wine producing areas of Bordeaux and Val de Loire. Finally, genetic analysis in combination with chromatographic analysis of aromatic substances responsible for the varietal typicity of Sauvignon wines are used for the study of the role of *S.cerevisiae* and *S.bayanus* yeast strains on sulphur varietal aroma revelation from the S-cysteine conjugate precursor present in grapes.

6. Virginie Moine-Ledoux. 1996. Research on yeast's mannoproteins' role facing proteic and tartric stability of wines.

White wine conservation on lies throughout several months goes with a clear improvement of their proteic stability manifesting itself by a large decrease of the bentonite's quantity necessary to their stabilisation. This phenomenon is not due to a decrease of the amount of thermosensitive proteins by means of protein hydrolysis, but to the protective effect of macromolecules released from the yeast's cell wall. Such a role is essentially attributable to a mannoprotein of 32 KDa, N-glycosyl, thermostable which is not adsorbed by bentonite and which corresponds to a fragment of parietal invertase of *Sacch. cerevisiae* released into wines during lies autolysis through the linked action of beta-glucanase from the cell wall and of yeast's vacuolar protease A. This mannoprotein can be produced industrially, thanks to its presence in an extract of yeast's mannoproteins using enzymatic hydrolysis from *Sacch. cerevisiae* cell wall with the aid of commercial preparation composed with ß-glucanases (GLUCANEX). The supplementation to a wine of this mannoproteins extract allows to diminish its sensibility to the proteic precipitation considerably as well as the amount of bentonite necessary to stabilise the wine. Moreover, the same preparation of mannoproteins which are extracted by the means of enzymatic hydrolysis of yeast's cell wall, inhibits the tartric acid's salts crystallisation in white wines as well as in red wine and rose. The inhibitor activity of crystallisation is due to the presence of mannoproteins sharply glycosylated, of molecular weight situated between 30 and 40 KDa.

7. Valérie Lavigne-Cruege. 1996. Research on volatile sulphur compounds produced by yeast during vinification and aging of dry white wines.

Perfecting simple analytical methods to determine dosage and identification in wines of heavy and light volatile sulphur compounds involved in reductive defects has enable us to study different parameters in the vinification and aging of white wines influencing the formation by yeast of these off-odoring compounds. It shows that insufficient clarification of must in the presence of excessive amounts of S02 is the principal cause of off-odors in wines during alcoholic fermentation. Thus, formation of methionol, the main sulphur compounds playing a preponderant role in this type of odor problem, is greatly increased in the presense of deposit from must. The unsaturated long-chain fatty acids it contains increases the tendency of the yeast to utilize the methionine, precursor of methionol in the must. It also shows that sulfite addition of the wine following alcoholic fermentation must be delayed in order to avoid formation of hydrogen sulfide. If such precautions are observed, wines vinified in barrel may easily be left on the yeast biomass and thus benefit from the numerous advantages of aging on lees, without any risk of unpleasant

reductive odors developing. However, prevention of this type of problem, arising frequently when white wines are kept for a long period on total biomass in large vats, necessitates the use of a special procedure involving temporary separation of the wine from its lees after sulfite addition. Finally, among the numerous volatile sulphur compounds present in wines, we identified for the first time two mercaptoalcohol acetates responsible for some of the roast meat aromas characteristic of certain white wines made to age.

XVII. VTT Biotechnology and Food Research, P.O. Box 1501, FIN-02044 VTT, Finland. Communicated by J. Londesborough <john.londesborough@vtt.fi>.

Area codes in Finland changed this October and our numbers are now:
Tel: +358 9 456 5101 or +358 9 456 5161
Fax: +358 9 455 2028 or +358 9 455 2103

1. Meinander, N., Hallborn, J., Keränen, S., Ojamo, H., Penttilä, M., Walfridsson, M., & Hahn-Hägerdal, B. 1994. Utilization of xylose with recombinant *Saccharomyces cerevisiae* harboring genes for xylose metabolism from *Pichia stipitis*. *In* Progress in Biotechnology 9. (Eds Alberghina, L., Frontali, L., & Sensi, P.) Proc. 6th Eur. Congr. Biotechnol., Florence 1993. Amsterdam: Elsevier, Part II, 1143-1146.
2. Hallborn, J., Gorwa, M.-F., Meinander, N., Penttilä, M., Keränen, S. and Hahn-Hägerdal, B. 1994. The influence of cosubstrate and aeration on xylitol formation by recombinant *Saccharomyces cerevisiae* expressing the *XYL1* gene. Appl. Microbiol. Biotechnol. **42**:326-333.
3. Meinander, N., Hahn-Hägerdal, B., Linko, M., Linko. P. & Ojamo, H. 1994. Fed-batch xylitol production with recombinant XYL1-expressing *Saccharomyce cerevisiae* using ethanol as a co-substrate. Appl. Microbiol. Biotechnol. **42**:334-339
4. Brennwald, B., Kearns, B., Champion, K., Keränen, S., Bankitis, V.P. and Novick, P. 1994. Sec9 is a SNAP-25-like component of yeast SNARE complex that may be the effector of Sec4 function in exocytosis. Cell **79**:245-258.
5. Jäntti, J., Keränen, S., Toikkanen, S., Kuismanen, E., Ehnholm, C., Söderlund, H. and Olkkonen, V. 1994. Membrane association of tail-anchored proteins: Localization of yeast syntaxin Sso2p in mammalian cells. J. Cell Sci. **107**:3623-3633.
6. Enari, T.-M. 1995. One hundred years of brewing research. J. Inst. Brew. Centennial edition p.33.
7. Stålbrand, H., Saloheimo, A., Vehmaanperä, J., Henrissat, B. & Penttilä, M. 1995. Cloning and expression in *Saccharomyces cerevisiae* of a *Trichoderma reesei* β-mannanase gene containing a cellulose-binding domain. Appl. Environ. Microbiol. **61**:1090-1097.
8. Ruohonen, L., Aalto, M.K. and Keränen, S. 1995. Modifications to the *ADH1* promoter of *Saccharomyces cerevisiae* for efficient production of heterologous proteins. J. Biotechnol. **39**:193-203.
9. Hallborn, J., Walfridsson, M., Penttilä, M., Keränen, S. and Hahn-Hägerdal, B. 1995. A short chain dehydrogenase gene from *Pichia stipitis* having D-arabitol dehydrogenase activity. Yeast **11**:839-847.
10. Enari, T.-M. 1995. State of the art of brewing research. Proc. 25th Congr. Eur. Brew. Conv. Brussels 1-11.
11. Walfridsson, M., Hallborn, J., Penttilä, M., Keränen, S., Hahn-Hägerdal, B. 1995. Xylose metabolising *Saccharomyces cerevisiae* overexpressing the *TKL1* and *TAL1* genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase. Appl. Environ. Microbiol. **61**:4184-4190.
12. Enari, T.-M. 1996. From Pasteur to modern brewing technology. Kemia-Kemi 23, 106-109.
13. Toikkanen, J., Gatti, E., Takei, K., Saloheimo, M., Olkkonen, V., Söderlund, H., De Camilli, P. and Keränen, S. 1996. Yeast protein translocation complex: isolation of two genes *SEB1* and *SEB2* encoding proteins homologous to Sec61 β-subunit. Yeast **12**:425-438.
14. Onnela, M.-L., Suihko, M.-L., Penttilä, M. and Keränen, S. 1996. Use of modified alcohol dehydrogenase, *ADH1,* promoter in construction of diacetyl non-producing brewer's yeast. J. Biotehnol. (in press)

15. Sizmann, D., Kuusinen, H., Keränen, S., Lomasney, J., Caron, M.G., Lefkowitz, R.J. and Keinänen, K. 1996. Production of adrenergic receptors in yeast. Receptors and Channels (in press).

16. Ruohonen, L., Toikkanen, J., Tieaho, V., Outola, M., Söderlund, H. and Keränen, S. 1996. Enhancement of protein secretion in *Saccharomyces cerevisiae* by overproduction of Sso protein, a late-acting component of the secretory machinery. Yeast (in press).

Four doctoral dissertations have been successfully defended:

17. J. Kronlöf. Immobilized yeast in continuous fermentation of beer. Espoo 1994 Technical Research Centre of Finland, VTT Publications 167. 96 p. + app. 47 p.

Continuous primary and secondary fermentation of beer with immobilized yeast was examined in laboratory scale and pilot scale packed bed reactors of cylindrical or tapered geometry. The purpose was to determine the basic prerequisites for rapid production of high quality beer in a continuous process. Secondary fermentation was the first objective of this work. The pilot scale secondary fermentation process consisted of three steps: Firstly, removal of yeast from green beer by centrifugation, secondly, decarboxylation of α-acetolactate by heat treatment and finally reduction of diacetyl in immobilized yeast bioreactors (50 dm^3). DEAE-cellulose was used as the carrier material. The quality of the beer was as good as that of conventional products. The residence time was only 2.5 hours, compared with several weeks in conventional fermentation. The sensitivity of the secondary fermentation system to potential brewery contaminants was examined in laboratory scale (1.6 dm^3). *Lactobacillus brevis, Pediococcus damnosus* and *Enterobacterium agglomerans* were incapable of contaminating the bioreactors at inoculation levels of 10^4-10^6 cells cm^{-3}. Wild yeasts, for example *Saccharomyces cerevisiae* (ex. *diastaticus*) were, however, able to attach and grow in the reactors, but even they were incapable of producing significant amounts of off-flavours in beer.

A study of the main fermentation was the second objective of this work. It was established that flavour formation could be controlled by choice of a suitable carrier material, moderate aeration and a two-stage process set-up. Porous glass was chosen for the carrier material in pilot scale (25 dm^3), resulting in a stable fermentation and a well balanced formation of acetate esters. Furthermore, less clogging problems were encountered with porous glass than with DEAE-cellulose. The residence time in most experiments was 40 hours to reach a final (apparent) attenuation of 80 %, but even higher flow rates could be maintained.

A genetically modified brewer's yeast with an α-*ald* (*budA*) gene from *Klebsiella terrigena* was used in the immobilized yeast system. The integrant strain is a producer of the enzyme α-acetolactate decarboxylase. Using this strain the formation of diacetyl was significantly reduced and the secondary fermentation could be considerably shortened or even omitted. No alterations in yeast metabolism were observed, except for a very slight decrease in yeast viability.

Simultaneously with the fermentation experiments, electrochemical measurements of viable biomass were performed both for suspended and for immobilized yeast samples. A capacitance probe was used for this purpose. The probe was well suited for monitoring of both suspended and immobilized yeast. Conventional methods did not give satisfactory results for immobilized yeast, probably because of the presence of non-viable matter (in gravimetric methods) or because of incomplete desorption of yeast cells (in cell counting methods).

18. H. Ojamo. Yeast xylose metabolism and xylitol production. Espoo 1994 Technical Research Centre of Finland, VTT Publications 176. 91 p.

A screening method was used for testing yeast strains in shake flask cultivations for their ability to convert xylose to xylitol. Of the 37 different strains studied by far the best were *Candida guilliermondii* C-6, *C.tropicalis* C-86 and *C.tropicalis* C-87. Of these strains C-6 was superior in a technical sense, being able to convert xylose to xylitol with a yield of 0.5 g g^{-1} at xylose concentrations at least up to 300 g l^{-1}, whereas the other two strains did not tolerate xylose concentrations more than 120 g l^{-1}.

Fermentation kinetics in xylose conversion were studied more closely with the strain C-6 both in shake flasks and in a fermenter. Oxygen availability was the key process variable. In order to quantify its effect on yeast metabolism, oxygen transfer characteristics for both shake flasks and a fermenter were determined. The rate of specific xylose uptake by the yeast was independent of the oxygen transfer rate above a certain threshold value. The growth of the yeast could be limited by oxygen limitation, under which conditions a typical overflow metabolism resulted in very efficient xylitol production. Under optimum conditions for oxygen transfer the yield of xylitol from xylose was 0.74 g g^{-1} and the rate of specific xylitol production was about 0.22 g $g^{-1}h^{-1}$. An initial xylose concentration of 200 g l^{-1} slowed down the xylose conversion, but this effect could be avoided by a fed-batch fermentation, in which the xylose concentration was controlled to 40-50 g l^{-1}. By this method the process time was decreased by 40% and the yield of xylitol was increased from 0.6 to 0.78 g g^{-1} compared with a batch fermentation. The metabolism of xylitol could also be limited by addition of the glycolytic and TCA-cycle inhibitor furfuraldehyde at a concentration of 0.6 ml l^{-1} under which conditions the limitation by oxygen was less critical for xylitol production.

Xylose metabolism was studied both by cultivation experiments and by simulation of a structured mathematical model. The model was constructed on the basis of the assumption of pseudo-steady-state of intracellular NADH, NADPH and ATP concentrations. The basis for xylitol accumulation appeared to be the high efficiency of the oxidative pentose phosphate cycle. This was verified by fermentation results, according to which the value of the respiratory quotient rose up to 10. The values of the activities or the affinities of the first two enzymes in xylose metabolism, xylose reductase and xylitol dehydrogenase, could not explain xylitol accumulation. The activity of xylitol dehydrogenase was four to sixfold

compared with that of xylose reductase, and the K_m-value of xylitol dehydrogenase for xylitol was not higher than 60 mM. Xylose reductase was strictly specific for NADPH and xylitol dehydrogenase for NAD, which both favour xylitol accumulation under oxygen limitation.

The structured mathematical model of xylose metabolism in the strain C-6 was combined to a model describing the performance of the fermenter. On the basis of the simulation using this combined model the fermentation could be optimized in relation to e.g. oxygen transfer. Xylitol production was also studied with a genetically modified *Saccharomyces cerevisiae* strain carrying a gene coding for xylose reductase in a vector under the constitutive *S.cerevisiae* PGK-promoter. By feeding this strain with a cosubstrate and xylose under carefully controlled conditions of dissolved oxygen concentration, yields of xylitol from xylose of over 0.95 g g^{-1} were achieved. Ethanol was used as the cosubstrate to regenerate the cofactor and for cell maintenance. The molar yield of xylitol on ethanol at the optimum dissolved oxygen concentration was about 1 mol mol^{-1}. Thus about half of the reducing power produced from ethanol was used for the reduction of xylose. Glucose inhibited xylose uptake very efficiently and was therefore not a suitable co-substrate.

19. M. Aalto. Cloning and characterization of genes encoding proteins involved in the terminal stages of vesicular traffic in the yeast *Saccharomyces cerevisiae*. Espoo, 1995 Technical Research Center of Finland, VTT Publication 255.

In eukaryotic cells, the compartmentalization of biochemical reactions requires a system for proper intracellular sorting of proteins destined for the different organelles of the cell. The secretory pathway consists of membrane-enclosed compartments through which proteins are transported either to the plasma membrane or to a final destination inside the cell. In the baker's yeast, *Saccharomyces cerevisiae*, the secretory pathway was first characterized through the use of temperature-sensitive mutants which block secretion and cell surface growth at the restrictive temperature, thus causing an abnormally large intracellular pool of secretory enzymes inside the cell.

The wild-type copy of the yeast *S.cerevisiae SEC1* gene was cloned by using its ability to restore the growth of a *sec1-1* temperature-sensitive mutant strain at the restrictive temperature. At this temperature, growth and secretion cease in *sec1-1* cells. Inside the cells, secretory vesicles accumulate, which contain fully matured secretory proteins destined for the plasma membrane or the outside of the cell. In this work it was found that *SEC1* is a single copy essential gene which encodes a large protein of hydrophilic nature. Database searches revealed that two other *SEC1*-related genes exist in yeast: *SLY1*, which is needed in vesicular transport between endoplasmic reticulum and Golgi apparatus, and *VPS33/SLP1*, which functions in the Golgi-to-vacuole transport. That related proteins function in different parts of the secretory pathway suggests that biochemically similar mechanisms are used at different stages of vesicular transport.

The yeast genes *SSO1* and *SSO2* were cloned by using their ability, when highly over-expressed, to partially restore the growth of *sec1-1* mutant. They suppress temperature-sensitive *SECI* mutations, but not a disruption of *SEC1*. Together, *SSO1* and *SSO2* perform an essential function in vesicular transport. *SSO1* and *SSO2* were found to be related to two other genes in yeast. One is *SED5*, which is involved in the transport of secretory vesicles between the endoplasmic reticulum and Golgi apparatus. The other is *PEP12*, which functions in transport from the Golgi to the vacuole. These three proteins are related to mammalian syntaxin proteins, which functions at the terminal stage of the vesicular transport in neuronal cells. The finding of duplicated members of the syntaxin family within the secretory pathway in yeast reinforces the notion that components of the secretory machinery have been duplicated at least twice during evolution, when new organelles emerged.

The *MSO1* gene was cloned by using its ability, when overexpressed, to restore the growth of *sec1-1*. It encodes a small protein of 211 amino acids, which has a hydrophilic nature. The gene itself is not an essential one, but a disruption of *MSO1* is lethal together with the *sec1-1* mutation. Using a two-hybrid analysis, I found that Msol protein binds to Secl protein. The interacting domains of the two proteins were mapped.

20. L. Ruohonen. Modulation of promoter and secretion efficiency for improved heterologous gene expression in the yeast *Saccharomyces cerevisiae*. Espoo 1995 Technical Research Center of Finland, VTT Publication 256.

The yeast *Saccharomyces cerevisiae* has certain advantages as a host for the production of heterologous proteins. It is a eukaryote, in which the production of proteins of higher eukaryotes may be more successful than in bacterial cells. Specific posttranslational modifications of eukaryotic proteins are likely to take place in yeast. Yeast has the capacity to secrete proteins outside the cell, which offers the possibility for the production of secretory proteins. However, the rather modest intrinsic secretory capacity of *S.cerevisiae* is a drawback of the yeast expression system. The glycosylation pattern of yeast secretory proteins differs from that of higher eukaryotes. Thus it is of interest to study the effects of yeast specific glycans on heterologous proteins.

The promoter of the yeast alcohol dehydrogenase I gene (*ADH1*) was among the first regulatory regions used for heterologous gene expression. Characterisation of the original promoter fragment has revealed an additional upstream promoter element, which was suggested to be responsible for the down-regulation of the promoter activity during the later stage of the fermentative growth cycle or during growth on non-fermentable carbon sources.

In the present study, the original *ADH1* promoter was modified in order to increase the production level of *Bacillus amyloliquefaciens* α-amylase used as a model secretory protein. The promoter (long *ADH1* promoter) was active only during fermentative growth on glucose or prolonged cultivations on ethanol. Two altered promoter constructions were made. Deletion of 1100 bp upstream from the original promoter resulted in activation of the modified promoter (short *ADHI* promoter) only after fermentative growth, i.e. during the ethanol consumption phase, or during growth on ethanol. When 300 upstream bp, containing a UAS element, were restored to the deleted promoter construction, activity of the promoter (middle *ADHI* promoter) reappeared during fermentative growth and

continued until well into the growth cycle. The activation during growth on ethanol was less delayed than with the original promoter. The primary carbon source affected the promoter activity during the growth cycle. The short promoter was active during respiratory growth on ethanol, whereas the original and middle promoter were activated during early growth on glucose. The concomitant appearance of a nonfunctional messenger RNA and disappearance of α-amylase activity during late fermentative growth showed that the upstream promoter element is responsible for the down-regulation of the original promoter. Once this upstream region was deleted the promoter was constitutively expressed. Gluconeogenesis may activate the original and middle promoter during growth on ethanol.

The bacterial signal peptide of α-amylase was functional and correctly processed in yeast cells. α-Amylase was transported through the entire yeast secretory route and N-linked core-glycans were added to the α-amylase during export. Interestingly, the presence of glycans did not abolish the enzymatic activity of α-amylase.

A significant increase in α-amylase production was obtained by promoter optimisation. The secretion capacity of yeast was enhanced when a component ot the yeast secretory machinery, the Sso2 protein, was simultaneously expressed with α-amylase in yeast cells. Varying the Sso2p level during the growth cycle using the original and middle ADHI promoter revealed a correlation between the amount of Sso2p and the level of secretion. This offers a means to improve secretion efficiency in yeast.

The VTT culture collection now contains about 1400 strains, including 385 yeasts, most of them industrial brewing and wine yeasts. There are also over 400 filamentous fungi and over 500 bacteria. The most recent catalogue was published in 1994:
Suihko, M.-L. VTT Culture Collection, Catalogue of strains, 3rd edition. Espoo 1994. The Technical Research Center of Finland, VTT Research Notes 1571. 148 p.

Copies of this catalogue and further information can be obtained from

Dr. M.-L. Suihko
Tel: + 358 9 456 5133;
Fax: + 358 9 456 2028;
E-mail: Maija-Liisa.Suihko@VTT.fi

XVIII. Department of Microbiology and Biotechnolgy, University of Horticulture and Food Industry, Somlói út 14-16, H-1118 Budapest, Hungary. Communicated by A. Maráz <h11636mar@ella.hu>.

1. Balogh, I. & Maráz, A. 1996. Presence of *STA* gene sequences in brewer's yeast genome. Letters in Appl. Microbiol. **22**:400-404

Saccharomyces cerevisiae var. *diastaticus* and brewer's yeast strains were characterized by pulsed-field gel electrophoresis. In many cases chromosome length polymorphism (CLP) was found. The chromosomes were hybridized with a DNA probe which was homologous with *STA* genes and the *SGA1* gene. Presence of the *SGA1* gene was detected in each strain used. Four brewing yeast strains were found to have homologous sequences with the *STA3* gene on chromosome XIV, despite the fact that these strains were not able to produce extracellular glucoamylase enzyme.

2. Balogh, I. & Maráz, A. 1996. Segregation of yeast polymorphic *STA* genes in meiotic recombinants and analysis of glucoamylase production. Can. J. Microbiol. (in press)

Hybrid yeast strains were constructed using haploid *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* var. *diastaticus* strains in order to get haploid meiotic recombinants having more than one copy of *STA1, STA2* and *STA3* genes. *STA* genes were localized on the chromosomes by PFGE blotting. Working gene dosage effects were found among *STA* genes in liquid starch medium indicating low levels of glucose repression. Growth of strains on starch, however, was not influenced by their *STA* copy number.

3. A. Geleta & A. Maráz. 1996. Ethanol inducible flocculation and its physiological background in *Schizosaccharomyces pombe* RIVE 4-2-1. FEMS Microbiol. Letters (submitted)

Flocculation of *Schizosaccharomyces pombe* RIVE 4-2-1 strain developed in the presence of ethanol both under aerobic and anaerobic growth conditions. Cell surface proteins which participated in cell to cell interaction were characterized by susceptibility of flocculation to different proteolytic enzymes, heat treatment, denaturing and thiol compounds and by inhibition of flocculation by sugars and derivatives. It was shown that a galactose-specific lectin was involed in this new type of flocculation.

The following conference abstracts have been published recently.

4. Maráz, A., Balla, É. & Végh, Gy. 1996. Preparation and storage of electrocompetent yeast cells used for cosmid transformation. 8th Intern. Congress of Mycology Division, IUMS Congresses '96, Jerusalem, Israel. Abstracts p57
5. Balogh, I. & Maráz, A. 1996. Expression level of chromosomal *STA* genes in hybrid yeast strains. 8th Intern. Congress of Mycology Division, IUMS Congresses '96, Jerusalem, Israel. Abstracts p53
6. Geleta, A. & Maráz, A. 1996. Cell wall ultrastructure of a flocculent *Schizosaccharomyces pombe* strain. 8th Intern. Congress of Mycology Division, IUMS Congresses '96, Jerusalem, Israel. Abstracts p116

7. Maráz, A., Végh, Gy., Zagorc, T., Smole Možina, S., Raspor, P., & Magyar, I. 1996. Development of killer yeast population during Tokaj wine fermentation. Food Micro '96, 16th Intern. Symp. of IUMS-ICFMH, Budapest. Book of Abstracts p105
8. Geleta, A., Maráz, A., & Magyar, I. 1996. L-malic acid degradation in wine with a flocculent *Schizosaccharomyces pombe* strain. Food Micro '96, 16th Intern. Symp. of IUMS-ICFMH, Budapest. Book of Abstracts p106
9. Smole Možina, S., Maráz, A. & Raspor, P. 1996. Molecular monitoring of yeast population kinetics during wine fermentations in Costal and Carst regions of Slovenia. Food Micro '96, 16th Intern. Symp. of IUMS-ICFMH, Budapest Book of Abstracts p88

The following thesis has been completed.

10. A. Geleta. 1996. Physiology and genetics of malic acid degradation and flocculation in *Schizosaccharomyces pombe*. PhD thesis. Department of Microbiology and Biotechnolgy, University of Horticulture and Food Industry, Budapest

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

1. Jungová, O., Minárik, E. 1996. Problems with *Zygosaccharomyces bailii* still exist. Vinohrad **34**(5): in press. (in Slovak).

Zygosaccharomyces bailii, a haploid chemoresistant spoilage yeast occurring in wineries on different equipment, first of all on the bottling line, may cause serious spoilage by haze even in membrane filtered bottled wines with or without residual sugar, as result of recontamination. Investigations in a Moravian and Slovak winery confirmed that only thorough decontamination of the winery equipment by surface active agents may entirely eliminate contaminating yeasts.

2. Satko, J., Malík, F., Vollek, V. 1996. Technological properties of wine yeasts of the series FV SAT (in Slovak). Vinohrad **34**:61-63.

Wine yeast strains of the series FV SAT were tested in conditions of grape must or grape wine fermentations. In all tested strains the yield coefficient values $Y_{P/S}$ varied in the interval 0.45 to 0.49 in all tested strains in the process of primary grape must fermentation. Significant differences were found in the yield coefficients $Y_{X/S}$. In secondary fermentation conditions all tested strains produced "dry" sparkling wines. Their yield coefficients $Y_{P/S}$ were approximately equal (0.47 to 0.46). The yield coefficients $Y_{X/S}$ of the wine yeasts of the series FV SAT have changed from 2.72×10^{-3} (*S.cerevisiae* FV SAT 3) to $10.76\ 10^{-3}$ (*S.cerevisiae* FV SAT 4) in conditions of the secondary fermentation.

3. Šajbidor, J., Malík, F., Buchtová, V. 1996. Changes in amino acid content during sparkling wine production (in Slovak). Zahradnictvi (Horticultural Science Prague) **23**: Accepted for publication.

XX. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, 1 Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gennadi@vnigen.msk.su>.

We thank Paul Sniegowski, Richard Lenski, James Tiedje (Center for Microbial Ecology, MSU, E. Lansing), Francis Molina (ATCC, Rockville), Amparo Querol (Dept. de Biotechnologia, IATA, Valencia), Federico Uruburu (CECT, Valencia) and Matti Korhola (Dept. of General Microbiology, University of Helsinki) for the possibility to do yeast research in their labs in 1995-1996.

The following are our publications for 1995 and 1996.

1. G.I. Naumov. 1995. Possibilities of breeding of eukaryotic microorganisms. Russian Biotechnology **1**:1-4.
2. G.I.Naumov, E.S. Naumova, A.N. Hagler, L.C. Mendonça-Hagler & E.J. Louis. 1995. A new genetically isolated population of the *Saccharomyces sensu stricto* yeast from Brazil. Antonie van Leeuwenhoek **67**:351-155.
3. G.I. Naumov, E.S. Naumova & E.J. Louis. 1995. Two new genetically isolated populations of the *Saccharomyces sensu stricto* complex from Japan. J. Gen. Appl. Microbiol. **41**:499-505.
4. G.I. Naumov, E.S. Naumova & M. Korhola. 1995. Karyotypic relationships among species of *Saccharomyces sensu lato*: *S.castellii, S.dairensis, S.unisporus* and *S.servazzii.* System. Appl. Microbiol. **18**:103-108.

5. G.I. Naumov, E.S. Naumova & M.N. Shchurov. 1995. Chromosomal polymorphism in a type species, *Pichia membranaefaciens:* twin species. Doklady Biological Sciences **343**:381-383.

6. E.S. Naumova, G.I. Naumov, P. Fournier & C. Gaillardin. 1996. Comparative karyotyping of yeast strains of the genera *Saccharomycopsis, Endomycopsella, Endomyces,* and *Yarrowia.* Microbiology-Engl. Tr. **65**:192-194.

7. E.S. Naumova, G.I. Naumov, E.D. Sancho & M. Korhola. 1996. Karyotyping of the *Saccharomyces exiguus* complex. Doklady Biological Sciences. **347**:177-179.

8. G.I. Naumov, E.S. Naumova, E.D. Sancho & M. Korhola. 1996. Polymeric SUC genes in natural populations of *Saccharomyces cerevisiae.* FEMS Microbiology Letters **135**:31-35.

9. G.I. Naumov, E.S. Naumova, H. Turakainen & M. Korhola. 1996. Identification of the α-galactosidase *MEL* genes in some populations of *Saccharomyces cerevisiae:* a new gene *MEL11.* Genet. Res. Camb. **67**:101-108.

10. G.I. Naumov, E.S. Naumova & E.D. Sancho. 1996. Genetic reidentification of *Saccharomyces* strains associated with black knot disease of trees in Ontario and *Drosophila* species in California. Can. J. Microbiol. **42**:335-339.

11. E.S. Naumova, G.I. Naumov & M. Korhola. 1996. Use of molecular karyotyping for differentiation of species in the heterogeneous taxon *Saccharomyces exiguus.* J. Gen. Appl. Microbiol. **42**:307-314.

A molecular karyotyping technique consisting of pulsed-field gel electrophoresis of intact chromosomal DNAs and following Southern hybridization with total *Hin*dIII-digested DNAs was used as a tool for yeast classification and identification. Significant differences in chromosomal patterns were found among 16 *Saccharomyces exiguus* strains. The complex taxon, classified into several groups of different karyotypes, showed a low degree of cross-DNA homology.

12. E.S. Naumova, H. Turakainen, G.I. Naumov & M. Korhola M. 1996. Superfamily of α-galactosidase *MEL* genes of *Saccharomyces sensu stricto* complex. Mol. Gen. Genet. **252**: (in press)

In order to study the molecular evolution of the yeasts grouped in the *Saccharomyces sensu stricto* species complex by analysis of the *MEL* gene family, we have cloned and sequenced two new species specific *MEL* genes from *Saccharomyces* yeasts: *S.paradoxus* (*MELp*) and a Japanese *Saccharomyces* sp. (*MELj*). The clones were identified by sequence homology to the *S.cerevisiae MEL1* gene. Both clones revealed an ORF of 1413 bp coding for a protein of 471 amino acids. The deduced molecular weights of the α-galactosidase enzymes were 52767 for *MELp* and 52378 for *MELj.* The nucleotide sequences of the MELp (EMBL accession no. X9505) and the MELj (EMBL accession no. X95506) genes showed 74.7% identity. The degree of identity of *MELp* to the *MEL1* gene was 76.8% and to the *S.pastorianus MELx* gene, 75.7%. The *MELj* coding sequence was 75.1% identical to the *MEL1* gene and 80.7% to the *MELx* gene. The data suggest that *MEL1, MELj, MELp* and *MELx* genes are species-specific *MEL* genes. The *MELp* gene is located on the *S.paradoxus* equivalent of *S.cerevisiae* chromosome X; the *MELj* gene was on the chromosome VII/XV doublet and hybridizes to the *S.cerevisiae* chromosome XV marker *HIS3*.

13. G.I. Naumov. 1996. Genetic identification of biological species in the *Saccharomyces sensu stricto* complex. J. Ind. Microbiol. (in press)

Studies on taxonomic and evolutionary genetics of the *Saccharomyces sensu stricto* complex are considered in light of the biological species concept. Genetic variability of some physiological properties traditionally used in yeast taxonomy is discussed. Genetic hybridization analysis and molecular karyotyping revealed six biological species in the *Saccharomyces sensu stricto* complex. DNADNA reassociation data are concordant with the data obtained by genetic analysis. A new system for naming the cultivated *Saccharomyces* yeast (groups of cultivars) is proposed.

14. G.I. Naumov, E.S. Naumova, Kondratieva V.I., Bulat S.A., Mironenko N.V., Mendonça-Hagler L.C. & A.N. Hagler. 1996. Genetic and molecular delineation of three sibling species in the *Hansenula polymorpha* complex. Syst. Appl. Microbiol. (in press)

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 inter-specific hybrids are sterile.

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

1. H.J. Phaff, J. Blue, A.N. Hagler[1], & C.P. Kurtzman.[2] *Dipodascus starmeri sp. nov.,* a new species of yeast occurring in cactus necroses. Int. J. Syst. Bacteriol. (Accepted for April 1997 issue).

[1]Instituto de Microbiologia, Universidade Federal de Rio de Janeiro, Brasil
[2]Microbial Properties Research, National Center for Agricultural Utilization Research, ARS/USDA, Peoria, IL 61604

In a previous publication describing the geographic distribution of yeasts associated with cactus necroses, 127 isolates were identified as strains of *Candida ingens* van der Walt & van Kerken on the basis of morphology and certain phenotypic characteristics. Here we show by DNA hybridization and additional phenotypic properties that these strains were misidentified and that they represent a minimum of three separate species that can be differentiated from *C.ingens* and from each other by utilization of 2-propanol or acetone, sensitivity to digitonin, utilization of L-lysine as sole nitrogen source, vitamin dependency, NaCl tolerance, lipolytic activity, and habitat. One of the new species is haploid and heterothallic and its teleomorph represents the genus *Dipodascus.* We describe *Dipodascus starmeri* sp. nov. The phylogenetic relationship of *D.starmeri* with other members of *Dipodascus* and its anamorph *Geotrichum* is estimated from rDNA nucleotide sequence divergence. The type strain, a heterothallic haploid isolate, is UCD-FST 72-316 (= CBS 780.96 = ATCC 20056 = NRRL Y-17816). The complementary mating type is UCD-FST 81-513.3 (= CBS 781.96 = ATCC 200547= NRRL Y-17817).

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

1. R. Messner, W. Schweigkofler, M. Ibl, G. Berg[1] & H. Prillinger. 1996. Molecular characterization of the plant pathogen *Verticillium dahliae* Kleb. using RAPD-PCR and sequencing of the 18SrRNA gene. J. Phytopathology **144**: in press.

[1]Universität Rostock, Fachbereich Biologie, D-18051 Rostock, Germany.

Thirty-four isolates of *Verticillium dahliae* Kleb. from nine different genera of dicotyledonous host plants and a broad range of geographic regions were analyzed genotypically. Random amplified polymorphic DNA (RAPD) markers were used for the estimation of the genetic variability within the species. Using four primers for the analysis, 79 distinct fragments were obtained. The derived phenogram clustered the isolates in two main groups: one consisted almost entirely of *V.dahliae* isolates from oilseed-rape (*Brassica napus*), the other group comprises isolates from a wide range of host plants. No correlation between geographic location of the isolates and the RAPD-pattern was observed. Sequencing of the gene for the 18SrRNA and calculation of the phylogenetic tree integrated the deuteromycetous fungus *V.dahliae* into the sexual system of the filamentous ascomyetes. In *V.dahliae* from *Brassica* and *Capsicum* a unicellular yeast-like growth stage was isolated using a Czapek-Dox medium. Purified cell walls of this unicellular yeast stage showed the glucose, mannose, galactose pattern (Glc: 72%, Man: 10%, Gal: 18%) and a ubiquinone Q-10 H2.

2. K. Lopandic. Ph.D. thesis.

Carbohydrate composition of purified cell walls was investigated in numerous ascomycetous and basidiomycetous yeasts and yeast-stages. Three different carbohydrate patterns have been established within the Ascomycetes: glucose-mannose; glucose-mannose-galactose; and glucose-mannose-galactose-rhamnose. The group showing glucose-mannose pattern is comprised predominantly of Hemiascomycetes (*Arxula, Candida p. pte., Debaryomyces, Holleya, Kluyveromyces, Mastigomyces, Nematospora, Metschnikowia, Pachytichospora, Pichia, Saccharomyces, Saccharomycopsis, Stephanoascus, Ashbya,* and *Eremothecium*). The glucose-mannose-galactose pattern was established in the Hemiascomycetes (*Candida p. pte., Geotrichum, Nadsonia, Schizoblastosporion, Waltomyces,* and *Yarrowia*), Euascomycetes (*Aureobasidium, Blastomyces, Capronia, Dothiora, Exophiala, Pringsheimia, Sydowia,* and *Verticillium*), and Archiascomycetes (*Saitoella*). The glucose-mannose-galactose-rhamnose group is comprised of Euascomycetes (*Ophiostoma, Sporothrix*), Archiascomycetes (Taphrina), and genera of uncertain taxonomic position (*Hyphozyma, Lecytophora*). Three carbohydrate patterns were detected within basidiomycetous yeasts and yeast-stages: glucose-mannose-galactose, mannose-glucose-galactose-fucose, and glucose-mannose-xylose. The glucose-mannose-galactose pattern exhibit phragmobasidiomycetous smut fungi (*Nannfeldtiomyces, Ramphospora, Schizonella, Sporisorium,* and *Ustilago*), a holobasidiomycetous genus, *Kordyana,* and the anamorphic genus *Tilletiopsis.* The auricularioid fungi like *Chionosphaera, Colacogloea, Kriegeria,* and *Platygloea,* two genera of uncertain taxonomic position, *Erythrobasidium* and *Occultifur,* a number of anamorphic yeasts like *Ballisto-sporomyces, Bensingtonia, Kurtzmanomyces, Rhodotorula, Sporobolomyces,* and *Sterigmatomyces* have predominant amounts of mannose, glucose, galactose and often fucose. Sporadically rhamnose can be find too. The carbohydrate pattern glucose-mannose-xylose exhibit different heterobasidiomycetous (*Atractogloea, Captotrema, Christiansenia, Cystofilobasidium, Filobasidiella*) and various anamorphic genera (*Bullera, Cryptococcus, Fellomyces, Kockovaella, Trichosporon, Tsuchiyaea*). Comparisons of the cell wall carbohydrate composition study with other investigations currently used in yeast systematics (e.g. ubiquinone analysis, enzymes, sequencing studies of ribosomal RNA and DNA) indicate that this approach is very useful in characterization and identification of yeasts at higher taxonomic levels (genera, families), but in estimation of phylogenetic relationships it exhibits limited potential. Thus, the ascomycetous group displaying the glucose-mannose-galactose

pattern combines budding, fission yeasts, and different orders of filamentous Ascomycetes pointing out that the phylogenetic value of galactose is rather low. Cell wall carbohydrate composition was analyzed in new isolates from lower termites and lichens.

3. O. Molnar. Ph. D. thesis.

Within the genus *Saccharomyces* a species concept comprising 10 genotypically distinct species (*S.bayanus, S.castellii, S.cerevisiae, S.dairensis, S.exiguus, S.kluyveri, S.paradoxus, S.pastorianus, S.servazzii, S. unisporus*) was confirmed by Random Amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) analysis. The species are differentiated from each other to the maximal extent with exception of the relationship between *S.bayanus* and *S.pastorianus.* In this case, the similarity value of 45% is significantly higher than the background noise (0-20%), but less than the values within species (83 to 100%). 76 strains including the type strains of 20 *Saccharomyces* species defined by phenotypic characteristics (e.g. *S.chevalieri, S.diastaticus, S.ellipsoideus*) proved to belong to *S.cerevisiae.* 14 strains (*e.g.* type strains of *S.globosus, S.heterogenicus, S.inusitatus, S.uvarum*) appeared to be genetically indistinguishable from *S.bayanus.* 6 strains including the type strains of *S.carlsbergensis* and *S.monacensis* were assigned to *S.pastorianus.* Two strains isolated from a peanut oil leaf-extract of *Hypericum perforatum* and two further strains obtained from different culture collections were identified to belong to *S.paradoxus.* Our RAPD-PCR analysis showed that it is impossible to separate the beer yeast from yeast fermenting grape and apple juices. Similarly, it is impossible to assign top fermenting yeasts exclusively to *S.cerevisiae* and bottom fermenting yeasts to *S.pastorianus.* Analyses of the ubiquinone system reinforced the homogeneity within the genus *Saccharomyces* (Q-6). Analysis of the coenzyme Q system and the monosaccharide pattern of purified cell walls were used for species characterization in the genus *Kluyveromyces.* All the type strains of the genus possess coenzyme Q-6 and the mannose-glucose ("*Saccharomyces* type") cell wall sugar pattern. With the help of RAPD-PCR analysis 17 species were separated: *K.aestuarii, K.africanus, K.bacillisporus, K.blattae, K.delphensis, K.dobzhanski, K.lactis* (anamorph *Candida sphaerica*), *K.lodderae, K.marxianus* (syn. *K.fragilis, K.bulgaricus, K.cicerisporus,* anamorphs *Candida macedoniensis, C.pseudotropicalis, C.kefyr*), *K.phaffii, K.piceae, K.polysporus, K.sinensis, K.thermotolerans* (syn. *K.veronae,* anamorph *Candida dattila*), *K.waltii, K.wickerhamii, K.yarrowii* (anamorph *Candida tannotolerans*). A strain of *K.drosophilarum* showed with the type strain of *K.lactis* only 63% similarity. The strain originally described as the type strain of *K.cellobiovorus* nom. nud. was excluded from the genus (Q-9), and found to be conspecific with the type strain of *Candida intermedia.* 85 yeast strains isolated from cheeses identified phenotypically by traditional physiological methods were investigated genotypically. In 66 yeast strains the results obtained phenotypically agreed well with the genotypic investigation. Using the diazonium blue B test and a polyphasic molecular approach including ubiquinone analysis, yeast cell wall sugars, and RAPD-PCR the 66 yeast strains were assigned to 39 different species. In addition 10 further yeast isolates could be identified genotypically at the species level. *Debaryomyces hansenii, Galactomyces geotrichum* group A, *Issatchenkia orientalis, Kluyveromyces marxianus, Saccharomyces cerevisiae, Yarrowia lipolytica,* and *Candida catenulata* appeared to be the most common species. The species *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and *Debaryomyces fabryi* Ota were reinstated. Our RAPD-PCR data reinforced that the species *Galactomyces geotrichum* is heterogenous. Nine strains remained unidentified.

The RAPD-PCR technique was employed in the identification of different *Metschnikowia* and *Ustilago* species. Using a polyphasic approach, some new representatives were identified at the genus and species level.

XXIII. Departamento de Microbiologia, Instituto de Ciências Biologicas, Universidade Federal de Minas Gerais, C.P. 486, Belo Horizonte-MG, 31270-901, Brazil. Communicated by V.R. Linardi <linardiv@mono.icb.ufmg.br> & C.A. Rosa <carlrosa@oraculo.lcc.ufmg.br>.

1. Linardi, V.R., Dias, J.C.T. & Rosa, C. A. 1996. Utilization of acetonitrile and other aliphatic nitriles by a *Candida famata* strain. FEMS Microbiol. Letters, **144**:67-72.
2. Morais, P.B., Rosa, C.A., Abranches[1], J., Mendonça Hagler[1], L.C. & Hagler[1], A.N. 1996. Yeasts vectored by *Drosophila quadrum* (*Calloptera* group) in tropical rain forests. Rev. Microbiol. **27**:87-91.
[1]Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ.
3. Morais, P.B., Resende, M.A., Rosa, C.A. & Barbosa, F.A.R. 1996. Occurrence and diel distribution of yeasts in a paleo-karstic lake of the Southeastern Brazil. Rev. Microbiol. **27**:167-172.
4. Morais, P.B., Rosa, C.A., Linardi, V.R., Carraza, Nonato, E. 1996. Fuel alcohol production *Saccharomyces* strains isolated from tropical forests. Biotechnol. Lett. **18**: In press.
5. Morais, P.B., Rosa, C.A., Linardi, V.R., Pataro, C. & Maia, A.B.R.A. 1996. Characterization and succession of yeast populations associated with spontaneous fermentations during the production of Brazilian sugar-cane Aguardente. World J. Microbiol. Biotechnol., In press.
6. Franzot, S. & Hamdan, J.S. 1996. *In vitro* susceptibilities of clinical and environmental isolates of *Cryptococcus neoformans* to five antifungal drugs. Antimicrob. Agents Chemother. **40**:822-824.

7. Rodrigues, A.C.P., Nardi, R.M., Bambirra, E.A., Vieira, E.C. & Nicoli, J.R. 1996. Effect of *Saccharomyces boulardii* against experimental oral infection with *Salmonella typhimurium* and *Shigella flexneri* in conventional and gnotobiotic mice. J. Appl. Bacteriol. 81: 251-256.

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

Two projects currently under way.

1. A.L Ragout, H. Borsetti, A. Martini,[1] A.V. Martini,[1] and J.F.T. Spencer. Yeasts isolated from sour bread starters.

[1]Sezione Microbiologia, Università di Perugia, Italy.

Raising of some sour breads depends on the natural yeast and bacterial flora. We are investigating the yeast flora of starters ("sponges") used in making salt-rising bread, which is made without additon of commercial yeast. If the starter is incubated at 37°C, microbial action begins after 4-6 hr, and examination of the mix reveals heavy growths of bacteria and yeasts. We will determine the upper limits of temperature and the pH range for growth of the starter culture. Enough acid is formed to reduce the pH far enough to kill the yeasts if the starter is allowed to incubate longer. We intend to determine the range of species of Saccharomyces-like yeasts participating in the flora of the sponge, and possibly isolate desirable temperature- and acid-tolerant strains of industrially useful yeasts.

2. A.L. Ragout, H. Borsetti and J.F.T. Spencer. Continuous culture of mixtures of the flocculent yeast *Saccharomyces diastaticus* NCYC 625 and *Lactobacillus* species.

Mixed cultures of lactic acid bacteria and yeasts are commonly found as fermenting and raising agents in Indian and other Oriental fermented foods, as well as in speciality breads such as white sour dough breads, sour rye breads, and salt-rising breads. Studies of the nature of the interaction of the lactic acid bacteria and the yeasts in the foods are rare. We have investigated the interaction between the yeast *Saccharomyces diastaticus* (*Saccharomyces cerevisiae* NCYC 625) and species of *Lactobacillus* in continuous culture. We will determine the effect on total biomass yield, product (acid) formation and utilization, effects of pH, and of embedding of the bacteria in the yeast flocs. We will use a carbon source which was not utilized by the yeast (lactose) but which was utilized and converted to lactic acid by the bacteria. This by-product was utilized by the yeast for growth and biomass formation, so that the metabolism of the yeast was dependent on the activity of the other member of mixed culture, the bacteria. The effect of embedding of the bacteria in the yeast flocs was determined using a non-flocculent yeast strain for comparison. We will determine the effect on the bacteria in this simulated immobilizing system to investigate the effect on the performance of the bacteria in immobilized cultures.

XXV. Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Wolczanska 175, 90-530 Lodz, Poland. Communicated by H. Stobinska (Fax: 36 59 76).

The Centre of Industrial Microorganisms Collection LOCK is operating as part of the Institute of Fermentation Technology and Microbiology at the Technical University of Lodz in Poland. Its aims are: (1) to collect, preserve and distribute strains of yeasts used in traditional fermentations in Poland, (2) to study choice methods of storage for amylolytic, cellulolytic and killer yeasts. LOCK is using traditional and long-term methods of storage for maintenance of yeast cultures.

1. Stobinska H., Drewicz E., Kregiel D., Oberman H. In press. Trials of transformation of a killer factor from *Saccharomyces cerevisiae* to the amylolytic yeast *Schwanniomyces occidentalis,* Biotechnologia (in Polish).
2. Stobinska H., Kregiel D., Oberman H. In press. Enzymatical activity of *Schwanniomyces occidentalis* and *Saccharomyces cerevisiae* hybrids during storage in frozen state. Polish J. Food Nutr. Sci. (in English).

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

The content of the poster abstract given in the last issue has been amended considerably in the light of more recent results. The abstract of the following paper summarizes some of the most significant findings. In addition, we have now identified a total of 8 predacious species, and are studying the evolutionary, ecological, and physiological significance of yeast predation.

1. M.A. Lachance & W.M. Pang. Predacious Yeasts. Yeast (in press).

Haustorium-mediated predation was observed in seven yeast species. *Arthroascus javanensis*, *Botryoascus synnaedendrus*, *Guilliermondella selenospora*, *Saccharomycopsis fibuligera*, and three hitherto unknown species penetrate and kill other yeasts. These yeasts share an unusual requirement for organic sulphur. One isolate recovered from Australian *Hibiscus* was studied in detail and found to attack a broad range of prey species, including ascomycetous and basidiomycetous yeasts as well as moulds. Predation was most effective when growth was on a solid surface and the medium was poor in complex nutrients. Organic sulphur (exemplified by methionine) was identified as a key factor. It serves as a nutritional benefit to the predator and, depending on the concentration, acts as either an inhibitor of predation or possibly a signal for detection of prey. Sampling of a yeast habitat with a medium selective for selenium-resistant yeasts indicated that auxotrophic and predacious yeasts might be more widespread than anticipated.

International Commission on Yeasts

1. Minutes of the Business Meeting of the International Commission on Yeasts

The business meeting of the International Commission on Yeasts was convened on August 29, 1996. A lunch was sponsored by Graham Fleet's department (The Department of Food Science and Technology, University of New South Wales, Sydney, Australia).

Commissioners and delegates present: Lucia de Figueroa & Frank Spencer (Argentina); Graham Fleet (Australia), H-G. Prillinger (Austria), Leda Mendonça-Hagler (Brazil), Bryon Johnson & André Lachance (Canada), Tom Nielsen (for Karen Oxenbø ll, Denmark), Matti Korhola (Finland), Pierre Galzy (France), Alessandro Martini (Italy); Alexander Rapoport Latvia); Teun Boekhout & Hans Van Dijken (for W. A. Scheffers, Netherlands), Maria Loureiro-Dias & Isabel Spencer-Martins (Portugal), Peter Raspor (Slovenia), Peter Biely (Slovakia), James Du Preez & Bernard Prior (S. Africa), David Berry (for John Johnston) & Graham Stewart (UK), H. J. Phaff, C.P. Kurtzman and Sally Meyer (USA).

1. The Minutes of the previous ICY meeting (August 27, 1992) were approved as distributed and corrected in the Yeast Newsletter.

2. Previous ISSY and IUMS meetings:

Since the last general meeting of the ICY two International Specialized Symposia on Yeasts (ISSY) were held. ISSY-16, "Metabolic compartmentation in yeasts", was organized by W.A. Scheffers and held in Arnheim, The Netherlands, August 23-26, 1993. ISSY-17 - "Yeast Growth & Differentiation: Biotechnological, Biochemical & Genetic Aspects" - was organized by David Berry and held in Edinburgh, Scotland, August 27-September 1, 1995. Both of these symposia were outstanding scientifically and socially and I wish to thank the organizers for all their efforts to make these events so worthwhile. In 1994 the general meeting of the International Union of Microbiological Societies (IUMS) was held in Prague, Czech Republic. ICY was represented by a symposium organized by Alessandro Martini entitled, "New Approaches to Yeast Taxonomy."

3. Since the last business meeting of the ICY, the following individuals have resigned from the Commission: Arnost Kotyk (Czechoslovakia), Karen Oxenbøll and A. Stenderup (Denmark), Pierre Galzy, J. M. Bastide and H. Heslot (France), John Johnston (UK) and W.A. Scheffers (Netherlands). I wish to thank all these individuals for their time, effort and support throughout the years of serving on the ICY. The Chair recommended that Dr. Pierre Galzy be offered emeritus status on the ICY because of his many years of service. He accepted and the Commissioners agreed.

4. The following individuals were recommended and elected as members of the International Commissioners on Yeasts:

Neville Pamment, University of Melbourne, (Australia)
Ken Watson, University of New England, Armidale, (Australia)
Mike Ingledew University of Saskatchewan, Saskatoon (Canada)
Maria Kopecka, Masaryk University Faculty of Medicine, Brno (Czech Republic)
Tom Busk Nielsen Novo Nordisk Bioindustry Ltd., Chiba-shi, Japan (Denmark)
Jørgen Stenderup, Statens Seruminstitut, Copenhagen (Denmark)
Guy Moulin, École Nationale Superieure Agronomique de Montpellier (France)
Michelle Mallie, Faculte de Pharmacie de Montpellier (France)
Eveline Guého, Institut Pasteur (France)
Hans van Dijken, Technical University, Delft (The Netherlands)
Barbel Hahn-Hägerdal, Lund University (Sweden)
David Berry, University of Strathclyde, Glasgow, Scotland (UK)

5. Future symposia: The tenth International Symposium on Yeasts (ISY-10) will take place in the year 2000. Offers to host the next symposium came from commissioners from Brazil, The Netherlands and South Africa. Since time did not allow a discussion of all the commissioners in attendance, the Chair appointed an ad hoc committee to meet immediately after the business meeting to discuss the next meeting site and make the decision. I wish to thank the commissioners from Brazil, The Netherlands, and South Africa for their willingness to organize ISY-10.

6. Future ISSY:

1997 ISSY-18. Peter Raspor is organizing a symposium to be held in Slovenia the last week of August, 1997. The topic is "Yeasts Nutrition and Natural Habitats."

1998 ISSY-19. The 19th ISSY is being organized by Cecilia Leão, Universidade do Minho, Braga, Portugal. The topic of this symposium is "Yeasts in the production and spoilage of foods and beverages."

1999 ISSY-20. The Czech and Slovak Yeast Commission will organize ISSY-20. The topic is "Surface Structures and Membrane Phenomena". The meeting will be held either at Smolenice Castle or at the facility of the Slovak Academy of Sciences in High Tatras.

7. The next general meeting of the International Union of

Microbiological Societies will take place in Sydney, Australia in August 1999. The ICY is expected to take part and should organize a symposium.

8. A copy of the By-Laws of ICY was obtained from A. Stenderup (Denmark). In general we are abiding by these. However, it is apparent that some changes should be made. A copy of the By-Laws will be mailed to all Commissioners for their review. We will follow the By-Laws in order to make the necessary changes. Also, the list of Commissioners needs revisement. Some members have retired and new commissioners need to be elected. I will continue to work on this along with the next ICY Chair.

9. I think I can speak for all of us in expressing our thanks and appreciation to Graham Fleet and the members of the organizing committee for their efforts in arranging ISY-9. We appreciate the fine programming and excellent science you have provided. The outstanding facilities of the convention center and the beauty of the city of Sydney are genuinely acknowledged.

10. The commission unanimously elected Graham Fleet as Chair of the ICY for the next four years. We wish him well in this position and look forward to working with him.

2. Other ICY news

A letter from T.-M. Enari (Biotechnology and Food Research, Finland) dated September 26, 1996 informed me of his retirement from his position and consequent resignation from the ICY.

A summary of the business meeting was presented by the Chair of the Commission at the closing of the ISY-9.

Announcements were made of the new commissioners elected to the International Commission on Yeasts (see Minutes of the Business Meeting) and the location of ISY-10, as well as future ISSY.

The tenth ISY will be held in The Netherlands.

Proposals came from Commissioners from Brazil, The Netherlands and South Africa. Location played a key role in the selection of the site. It was agreed that the European site may be better for attendance. Hans van Dijken and his colleagues at the Technical University in Delft will oversee the organization the next ISY.

The winners of the Poster Contest were announced. There were two winners and the prize was $500.00 each. The winning posters were "Yeast cell killing by the extracellular addition of part of HIV-1 Vpr: a model for indirect cell killing in AIDS" by Ian G. Macreadie, Chinnah K. Arunagiri, Dean R. Hewish and A. A. Azad, Biomolecular Research Institute, and Commonwealth Scientific and Industrial Research Organization, Division of Biomolecular Engineering, Victoria Australia and "Non-specific, haustorium-mediated predation in yeasts" by M.A. Lachance, Department of Plant Sciences, University of Western Ontario. Congratulations to all who did the science that made these posters possible.

A special thanks to all participants who attended ISY9. This was an outstanding meeting and we applaud Graham Fleet and his organizing committee for making it all possible.

Comments from the ICY Chair, 1992-1996

I wish to express my thanks to all of you who have assisted me during my time as the Chair of ICY. I appreciate your cooperation and encouragement. I want to acknowledge a very special thanks to Sandro Martini, the former Chair, for his help and support during the transition time. I thank Lex Scheffers and Dave Berry who organized ISSY-16 and 17 and Graham Fleet for organizing ISY-9. I think the programming at these symposia was outstanding. Also, I wish to thank Peter Raspor and Cecilia Leão for agreeing to organize ISSY-18 and 19 and Lex Scheffers and Hans van Dijken for offering to organize ISY-10. It has been an exciting four years and I thank you all very much. It has been a pleasure to serve as the ICY Chair and I look forward to working with Graham Fleet, ICY Chair, 1996-2000.

Sally A. Meyer, Chair, ICY 1992-1996

Recent meetings

Ninth International Symposium on Yeasts (ISY9)

The Ninth International Symposium on Yeasts (ISY9) was held concurrently with the 10th International Biotechnology Symposium in Sydney, Australia, 25-30 August. The ISY is held every four years as an activity of the International Commission for Yeasts (ICY) to foster interest in the science and technology of Yeasts. As a symposium with a general mission, it aims to promote interaction between scientists working in the many specialised aspects of yeast biology and technology.

ISY9 attracted 251 full-time, 14 day and 20 accompanying delegates, from 35 different countries, making it one of the largest ISY held in recent years. The scientific program consisted of 7 plenary presentations, 55 symposium papers, and 180 posters. Twenty two of the posters were selected for proffered oral presentation, with a bias towards contributions from younger scientists. The topics for plenary presentations covered a broad range of general interest topics. John Pringle examined the budding process in yeasts, with emphasis on the molecular mechanisms that determine the sites for bud formation. Clete Kurtzman addressed yeast taxonomy and described how sequencing of ribosomal DNA was the way forward for determining the taxonomic and phylogenetic relationships of yeast genera and species. Stephen Oliver reported that the 6000 genes within *Saccharomyces cerevisiae* have now been sequenced but, while this achievement represents an historical milestone in biology, the challenge ahead is to devise an experimental strategy to relate gene sequence to cell function. Tania Sorrell gave an update on the human pathogenic yeast, *Cryptococcus neoformans,* and considered its ecology, significance in immunocompromised individuals, virulence factors, and molecular technologies for probing its epidemiological profile. Lyne McFarland provided evidence to show that some yeast species such as *Saccharomyces boulardii* have the potential to improve human health through their use as probiotic or biotherapeutic agents. New developments in the exploitation of yeasts in the food and beverage industries were presented by John Friend, and further emphasised by Paul Henschke who showed how advances in understanding the ecology, biochemistry and molecular biology of yeasts, including development of genetically engineered strains, could substantially

improve the process of winemaking.

Each symposium session consisted of 3/4 presentations from leaders in particular areas of research, and were structured to provide overviews and discussions of latest advances in knowledge. Sessions on yeast taxonomy and ecology focused on the concept of biodiversity of yeasts in many habitats and the importance of DNA-based methods in studying yeast systematics. Sessions on biochemistry and physiology covered the enzymology, molecular biology and regulation of carbohydrate metabolism, transport, cell walls and stress responses. Gene expression was considered in topics covering meiosis, mitochondrial proteins, cell wall polysaccharides, phospholipid biosynthesis, response to copper ions and the production of a wide range of heterologous proteins. The medical significance of yeasts was discussed in a range of presentations covering *Cryptococcus* neoformans and *Candida albicans,* yeast infections in AIDS patients, antifungal therapies, molecular markers, and the role of biofilms in pathogenesis. The industrial applications of yeasts (three sessions) covered their roles in food spoilage, fermentations of wine, beer, bread and cocoa beans, production of flavour extracts and pigments, resistance to preservatives, production of ethanol from starch and lignocellulosic substrates and very high gravity fermentations. The proffered oral presentations extended the themes of the symposia.

Many favourable comments were received about the high quality of these presentations, as well as for the posters.

Congratulations to André Lachance (U. of Western Ontario) for his poster "Non-specific haustorium-mediated predation in yeasts" and Ian Macreadie and co-workers (CSIRO, Australia) for the poster "Yeast cell killing by the extracellular addition of part of HIV-Vpr: a model for indirect cell killing in AIDS" which were awarded best poster prizes from the ICY.

A discussion group, with comments from G.G. Stewart, H.J. Phaff, M.W. Miller and H. van Dijken considered past, present and future needs on education in yeast biology and technology. It was agreed that the chair of ICY should aim to collate and coordinate information on this subject.

A meeting of 25 commissioners of the ICY was convened during the symposium, where it was decided that the next International Symposium on Yeasts (ISY 10) would be held in Delft, The Netherlands, during the year 2000. Specialised symposia would be held in 1997 and 1998 on the following topics: "Yeast nutrition and natural habitats", Slovenia. Contact, P. Raspor. "Food and beverage yeasts", Portugal. Contact, I. Spencer-Martins. The ICY will also organise a yeast symposium at the Sydney 1999 Conference of the International Union of Microbiological Societies. Many comments have been received about the very successful outcome of ISY9. In this context, the advice from many commissioners of the ICY, the guidance and support of Sally Meyer, immediate past-Chair of ICY, the inputs of the local organising committee, (D. Clark-Walker, I. Dawes, D. Ellis, P. Henschke, I. Jenson, I. Macreadie, N. Pamment and K. Watson), and the support of the host organisation, The Australian Biotechnology Association, are most gratefully acknowledged. ISY9 concluded in a most appropriate manner, with a tasting of fine Australian wines kindly donated by Henschke, Petaluma, Rosemount Estate, MacWilliams, and Penfold companies.

Graham H. Fleet, Chair,
ISY9 Organising Committee and
International Commission for Yeasts.

Dedication of the Herman J. Phaff Culture Collection: Yeasts & Yeast-Like Microorganisms, University of California, Davis, California, U.S.A., October 4, 1996

Opening Remarks. Sharon Shoemaker, CIFAR

Welcome. Dean Barbara Schneeman, College of Agricultural and Environmental Sciences, University of California, Davis

Culture collections and microbial diversity: value unlimited. Jennie Hunter-Cevera, Lawrence Berkeley Laboratory

Yeast taxonomy, past, present and future, and the impact of culture collections. Clete Kurtzman, USDA, Peoria, Illinois

Yeast taxonomy, cruising for yeasts in the Caribbean. Jack Fell, University of Miami

Early days of the yeast collection. Martin Miller, University of California, Davis

Applications in industry for *Phaffia rhodozyma* and other unique cultures. Eric Johnson, University of Wisconsin, Madison

Yeasts in winemaking. Graham Fleet, University of New South Wales, Australia

Between pickles and penicillin: four years with Herman and his yeasts. Arnold Demain, Massachusetts Institute of Technology

Predation among yeasts in nature and in culture collections. Marc-André Lachance University of Western Ontario, Canada

Characterization of yeast communities in coastal ecosystems of southeastern Brazil. Allen Hagler & Leda Christina Mendonça-Hagler, Institute de Microbiologia, Rio de Janeiro, Brazil

Our *Candida* Journey. Sally Meyer, Georgia State University, Atlanta

Less known edges of the best known organisms on earth: *Saccharomyces cerevisiae*. Alessandro Martini & Anne Vaughan Martini, University of Perugia, Italy

Presentation to Professor Phaff. Charles Shoemaker, Chair, Department of Food Science and Technology, College of Agricultural and Environmental Sciences, University of California, Davis

Reflections. Herman Phaff

Forthcoming meetings

EFB Working Party Microbial Physiology Conference: "Microbial Response to Stress: what's new and how can it be applied?" Sesimbra, Portugal, 15-18 March 1997

The conference, organized by the Working Party "Microbial Physiology" of the European Federation of Biotechnology abd by Sociedade Portuguesa de Biotecnologia, will deal writh: (1) Stress Response: sensing and signaling; (2) Stress-Induced Changes in Gene Expression; (3) Cellular Processes Affected by Stress; (4) Acquiisition of Stress Tolerance, and (5) Biotechnological Applications, in Yeast and Bacteria. Many biotechnological industries can only compete with physical or chemical technologies if organisms can be found that can survive in extreme environments. Fundamental studies of how microbes respond to stress therefore provide the foundation on which to build new technologies or to optimize an old one. The aims of this intensive four day residential symposium will be to exchange information about most recent developments in our understanding of yeast and bacteria response to stress.

The conference will include lectures by invited speakers poster sessions and oral presentation of selected posters. We invite all biotechnologists to participate and especially encourage young scientists and PhD students to present their data and exchange ideas with leaders in their field.

Invited speakers: I.R. Booth (Aberdeen); A.M. Chakrabarty (Chicago); J.A. Cole (Birmingham); J.A.M. de Bont (Wageningen); A. Goffeau (Louvain-la-Neuve); R. Hengge-Aronis (Konstanz); R.C. Hockeny (Zeneca Bioproducts); S. Hohmann (Göteborg); P.W. Piper (London); P.W. Postma (Amsterdam); U. Priefer (Aachen); H. Ruis (Wien); H. Santos (Oeiras); R. Serrano (Valencia); J. Thevelein (Leuven); D. Thiele (Michigan).

For further information and to receive the 1st announcement/call for abstracts please contact:

Prof. Isabel Sa-Correia
Secção de Biotecnologia,
Instituto Superior Tecnico,
Av. Rovisco Pais, 1096 Lisboa Codex,
Portugal

Telephone: 351-1-8417233/682
Fax: 351-1-8480072
E-mail: <qisc@beta.ist.utl.pt>
or <qviegas@,beta.ist.utl.pt>

18th International Conference on Yeast Genetics and Molecular Biology, University of Stellenbosch, Stellenbosch, South Africa, March 31 to April 5, 1997.

The XVIII International Conference on Yeast Genetics and Molecular Biology will be held in Stellenbosch, South Africa, March 31-April 5, 1997. The second announcement was mailed to all scientists listed in the Yeast Directory. We have created a home page on the www in Stellenbosch (http://www.sun.ac.za/local/news_eve.htm) and Stanford (http://genome-www.stanford.edu /saccharomyces/icygmb/cygmbhf.htm).

It is with great pleasure that we can announce the names of the following speakers who have accepted invitations to present talks in the platform sessions:

GR FINK, Whitehead Institute for Biomedical Research & MIT, USA. D GALLWITZ, Max Planck Institute, Gottingen, Germany. RA YOUNG, Whitehead Institute for Biomedical Research & MIT, USA. M CARLSON, Columbia University, USA. A SENTENAC, Service de Biochimie et de Genetique Moleculaire, Gif-sur-Yvette, France. M GREEN, University of Massachusetts, USA. P NOVICK, Yale University, USA. H RIEZMAN, University of Bazel, Switzerland. P PHILIPPSEN, University of Bazel, Switzerland. P SLONIMSKI, CNRS, Gif-sur-Yvette, France. RJ PLANTA, Vrije Universiteit Amsterdam, The Netherlands. B DUJON, Institute Pasteur, France. JH HEGEMANN, Justus-Liebig-Universitat Giessen, Germany. M SNYDER, Yale University, USA. V SMITH, Genentech , USA. B HAHN-HAGERDAL, Lund University, Sweden. M VILJOEN, University of Stellenbosch, South Africa. TG COOPER, University of Tennessee, Memphis, USA. K-D ENTIAN, der Johann Wolfgang Goethe-Universitat, Germany. B ERREDE, University of North Carolina, USA. J THEVELEIN, Katholieke Universiteit Leuven, Belgium. J-H HOFMEYR, University of Stellenbosch, South Africa. P MAGER, Vrije Universiteit Amsterdam, The Netherlands. S HOHMANN, Goteborg University, Sweden. IS PRETORIUS, University of Stellenbosch, South Africa. M YANAGIDA, Kyoto University, Japan. C NEWLON, UMDNJ, New Jersey Medical School, USA. J LAZOWSKA, CNRS, Gif-sur-Yvette, France. We hope to announce the names of the remaining six speakers on the web soon.

Hennie J.J. van Vuuren
President XVIII ICYGMB

Contact:

Deidre Cloete
Conferences *et al*
P.O. Box 452
7600 STELLENBOSCH
South Africa

Tel.: 27-21-8864496
Fax: 27-21-88381 77
E-mail: <eikestad@iafrica.com>

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

Scientific topics: Yeasts in conventional, nonconventional and extreme habitats. Modes of nutrition in yeasts. Organic and inorganic nutrition in yeasts. Population dynamics in yeast communities in natural and controlled milieu. Methodology 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, detection of yeasts in simple and complex environments. Yeast pathogenic action in human and animal environment. Yeasts in food production and spoilage. The Symposium will be held on Bled, in beautiful alpine surroundings of the northern part of Slovenia. The 1st announcement and call for abstracts will be delivered in December 1996. 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

Dear Friend and Colleague,

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

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

Change of address: S.B. Rodriguez and R.J. Thornton

We have recently taken up new research positions with the Gallo Winery. Our coordinates are as follows:

Dr. Susan B. Rodriguez,
Senior Research Microbiologist, Basic Research
E & J Gallo Winery
600 Yosemite Boulevard
Modesto, CA 95353, USA

Phone:(209) 579 8090
Email: srodrigu@wac.com
Fax: (209) 579 7067

Dr Roy J. Thornton
Senior Research Microbiologist, Basic Research
E & J Gallo Winery
600 Yosemite Boulevard
Modesto, CA 95353, USA

Phone:(209) 579 7054
Email: rthornto@wac.com
Fax: (209) 579 7067

Publication of Interest

The Yeasts, A Taxonomic Study, 4th revised and enlarged edition, edited by C.P. Kurtzman and J.W. Fell. Approx. 1,400 pages in two volumes

From Elsevier Science, expected publication date: March 1997.

The yeasts are a phylogenetically diverse group of fungi characterized by unicellular growth. Yeasts have been used for bread making and brewing beverages for millennia, and have become increasingly important in biotechnology for production of fuel alcohol, organic acids, enzymes, and various pharmacologically important chemicals. Other species are serous human, animal, and plant pathogens.

Since publication of the 3rd edition in 1984, numerous new species and genera have been described, many because of the application of new molecular biological methods.

Molecular comparisons have now provided a phylogenetic distinction between the yeasts and other fungi, some of which have a unicellular growth phase. This volume provides extensive morphological and biochemical descriptions of all known yeast species, including comprehensive physiological data of importance to biotechnology.

This book is directed to taxonomists, ecologists, mycologists, microbiologists, clinicians, molecular geneticists, and biotechnologists.

This is the most comprehensive work available on yeast systematics. The inclusion of extensive physiological and molecular genetic data extends its use to providing information for various medical and high technology applications.

Purchase price (approx.): Dfl. 775.00 / USD 485.00.
ISBN: 0-444-81312-8. Further details to be announced in the June issue of the YNL.