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Y E A S T

A Newsletter for Persons Interested in Yeast

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Editorials

Important notice on format of communications

Our thanks are extended to the many readers who have sent communications through electronic media, making our task of assembling the Yeast Newsletter much easier. We ask our readers to take note of the following.

General. If the material submitted already exists in the form of a computer file, please send a diskette or an e-mail transmission.

Diskettes. If at all possible, this is the preferred method. You may use any MS-DOS compatible density (3.5" or 5.25"). The text should be in DOS-ASCII or in WP5.1 format. We are also able to convert documents from Macintosh to MS-DOS formats if no other choice is available. In the preparation of text, please adhere as much as possible to the format used in the YNL, especially with respect to the use of capital letters. Please enclose a printout.

E-mail. To avoid truncation of lines, insure that each line does not exceed 78 characters in length. Please mail a printed copy to allow the restoration of correct diacritic or other symbols, italics, superscripts, or subscripts in the final copy. Use only standard ASCII (non-coded) characters or a "uuencoded" file. **Our current e-mail address is <lachance@uwo.ca>.**

Typewritten material. In conformity with our goal of facilitating communications among all yeast researchers, we are happy to receive typewritten materials from readers who may not have access to electronic media. These documents are entered by optical scanning. To facilitate that task, whenever possible, please use clear, evenly spaced, standard characters, left justification only, and wide margins (2.5 cm or more). We have experienced considerable difficulty scanning some proportionally spaced materials, fonts smaller than 12 points or 10 cpi, or very wide text. These difficulties are exacerbated by the use of italics, boldface, or underlining. Please do not send communications by Fax.

Reprints. Readers who prefer to send reprints should forward the originals whenever possible. Alternatively, please send a high quality photocopy of the same size and contrast as the original.

Thank you for your kind consideration of these matters.

Address Update

We regret that several readers in Germany did not receive the usual postcard requesting for communications. This was due to a recent upgrade of German postal codes, which caused several cards to be returned with no address corrections. We ask all our readers to check their mailing labels for accuracy and to inform us of any changes that might expedite delivery of all future correspondence.

I wish all our readers a happy and most prosperous new year!

M. A. Lachance
Editor

I. American Type Culture Collection. 12301 Parklawn Drive, Rockville, Maryland 20852-1776, U.S.A. Communicated by S.C. Jong.

Complete information of the following strains may be obtained upon request from the Mycology and Botany Department at the ATCC.

NAME	ATCC#	DEPOSITOR/STRAIN	SIGNIFICANCE/REFERENCE
Candida kefyr	90902	C. Kurtzman, NRRL Y318	Produces glycerol from whey permeate (Enzyme Microb. Technol. **16**:143-150, 1994)
Cryptococcus humicolus	90770	W. Middelhoven, G29	Degrades benzene compounds (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1993)
Cryptococcus laurentii	90771	W. Middelhoven, G30	Degrades benzene compounds (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1993)
Leucosporidium scottii	90774	W. Middelhoven, G43	Degrades benzene compounds (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1993)
Rhodotorula aurantiaca	90775	W. Middelhoven, G36	Degrades cresols and benzene compounds (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1993)
Rhodotorula glutinis	90781	W. Middelhoven, St41	Degrades benzene compounds (Antonie van Leeuwenhoek **63**:125-144, 1993)
Rhodotorula gracilis	90950	R. Joseph, CFR-1	Produces chitosanase (Lett. Appl. Microbiol. **14**:1-4, 1992)
Saccharomyces cerevisiae	90945	D. Fraenkel, DFY1	Produces chitosanase (Lett. Appl. Microbiol. **14**:1-4, 1994)
	90946	DFY568	
	90947	DFY582	
	90948	DFY583	
	90949	DFY632	
Saccharomyces cerevisiae	90937	B. Ono, OK312-7C	Transformation host (J. Bacteriol. **174**:3339-3347, 1992)
Saccharomyces cerevisiae	90938	B. Ono, IS66-4C	Chromosome standard for control of enzymatic activities (Curr. Genet. **21**:285-289, 1992)
Saccharomyces cerevisiae	90939	B. Ono, OK361-3D	Chromosome standard for transverse alternating field electrophoresis (Curr. Genet. **21**:285-289, 1992)
Saccharomyces cerevisiae	90908	J. Watari, YJW6	Transformation host (Agric. Biol. Chem. **55**:1547-1552, 1991)
Saccharomyces cerevisiae	90894	R. Schiestl, LP27524B	Transformation host (Curr. Genet. **16**:339-346, 1989; Anal. Biochem. **208**:211-212, 1993)
	90895	S35/2-10C	
Saccharomyces cerevisiae	90871	M. Rosenkrantz, BWG1-7a	Transformation host (Mol. Microbiol. **9**:521-532, 1993)
Saccharomyces cerevisiae	90848	J. Boeke, JB503	Transformation host (Science **254**:1808-1810, 1991; Gene **139**:9-18, 1994)
	90849	GRF167	
	90850	YH8	
	90851	YH82	
	90852	AGY9	
Saccharomyces cerevisiae	90847	D. Jenness, DJ676-2-2	Transformation host (Mol. Cell. Biol. **14**:1054-1065, 1994)
Saccharomyces cerevisiae	90833	G. Fink, TD4	Transformation host (Mol. Cell. Biol. **14**:189-199, 1994)
Saccharomyces cerevisiae	96030	T. Kuo, TL154	Transformation host (Curr. Genet. **21**:83-84, 1992; J. Biotechnol. **29**:329-334, 1993)

Saccharomyces cerevisiae	96029	I. Macreadie, DY150	Transformation host (Gene **142**:113-117, 1994; Yeast **9**:565-573, 1993)
Saccharomyces cerevisiae	90752 90753	J. Nikawa, D451-3 D458-5A	Transformation host used for expressing lac Z fusion plasmids (Mol. Microbiol. **10**:955-961, 1993)
Saccharomyces cerevisiae	90741 90742 90743 90744 90745	M. Hampsey, YDW438 T16 YDW383 YDW575 YDW546	Transformation host (Mol. Cell Biol. **14**:226-237, 1994)
Saccharomyces cerevisiae	90732	A. Nieto-Soria, AN1.7D	Transformation host (Yeast **9**:875-887, 1993)
Saccharomyces cerevisiae	90712 90713	J. Bell, JG 113-5R JG 369-3B	Transformation host (Gene **134**:57-65, 1993)
Saccharomyces cerevisiae	90709	C. Ballou, XW471-2D	Gives up to 60% asci in which the spores are lysed by ß-glucanase owing to the absence of a surface layer of chitosan (Proc. Natl. Acad. Sci. **74**:4351-4355, 1977)
Saccharomyces cerevisiae	96099 96100	S. Emr, SEY 6210 SEY 6211	Transformation host (Mol. Cell. Biol. **8**:4936-4938, 1988; Yeast **9**:1057-1063, 1993)
Scedosporium inflatum	90853	R. Pritchard, AMMRL 140.03	Human pathogen (Clin. Inf. Dis. **14**:1027-1033, 1992)
Schizoblastosporion starkeyi-henricii	90776	W. Middelhoven, G28	Degrades hydroxybenzene, hydroxycinnamic acids and 3-hydroxybenzoic acid (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1993)
Schizosaccharomyces pombe	96005 96006 96007 96008 96009 96010 96011 96012	O. Nielsen, Eg545 Eg538 Eg534 Eg489 Eg475 Eg451 Eg432 Eg337	Genetics (EMBO J. **11**:1391-1395, 1992)
Schizosaccharomyces pombe	96115 96116 96117 96118	K. Gould, KGY425 KGY461 KGY553 KGY554	Transformation host (Mol. Gen. Genet. **242**:169-176, 1994)
Stephanoascus ciferrii	90780	W. Middelhoven, 3Ad6	Degrades purines, amines, benzene compounds (Antonie van Leeuwenhoek **63**:125-144, 1993)
Trichosporon dulcitrum	90777	W. Middelhoven, G37	Degrades benzene compounds (Plant Soil **145**:37-43, 1992; Antonie van Leeuwenhoek **63**:125-144, 1992)
Trichosporon laibachii	90778	W. Middelhoven, VOU2515	Degrades uric acid, amines, diamines and benzene compounds (Antonie van Leeuwenhoek **51**:289-301, 1985; Antonie van Leeuwenhoek **63**:125-144, 1993)
Trichosporon moniliforme	90779	W. Middelhoven, G41	Degrades benzene compounds (Antonie van Leeuwenhoek **63**:125-144, 1993)
Yarrowia lipolytica	90903 90904 90905	M. Ogrydziak, DO613 D0625 DX571-57	Transformation host (Yeast **10**:67-69, 1994

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

New acquisitions.

Arthroascus fermentans C.-F. Lee et al.
7830(CCRC 22530); T; ex soil, Taiwan; F.-L. Lee; GPYA, 25C
7831(CCRC 22531); ex soil, Taiwan; F.-L. Lee; GPYA, 25C
7832(CCRC 22532); ex soil, Taiwan; F.-L. Lee; GPYA, 25C

Bulleromyces albus Boekhout & Fonseca
7856(VKM Y-2747); VKM; PDA, 25C; killer

Candida stellimalicola M. Suzuki et al.
7853(JCM 3546); T; ex fruit of *Averrhoa carambola* (star apple), Thailand; M. Suzuki; GPYA, 25C

Cryptococcus laurentii (Kufferath) Skinner
7857(VKM Y-1627); VKM; PDA, 25C; killer

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij var. *hansenii*
7848 ex takuan, Japan; N. Gunge; GPYA + 12% NaCl, 25C, GPYA +10% glycerol, 25C; killer

Filobasidiella depauperata (Petch) Samson et al.
7841 (ATCC 36983); T of *Filobasidiella arachnophila* Malloch et al.; ex dead spider, Canada; D. Malloch (TRTC 48044) > K.J. Kwon-Chung (B3810) > ATCC; ATCC; PDA, 25C
7855 (CCF 2746); ex surface of colony of *Verticillium lecanii,* Czechoslovakia; A. Kubátova ; PDA, 25C, YMA, 25C

Malassezia furfur (Robin) Baillon
7854 ex seborhoeic scalp, Finland; J. Savolainen; LNA, 30-35C

Saccharomyces cerevisiae Meyen ex E.C. Hansen
7833 ex lung of man with immune deficiency syndrome, USA; hist: L.M. Potter & C.J. Papasian > K.V. Clemons; K.V. Clemons; GPYA, 25C; virulent strain
7834 ex strain YJM454, USA; K.V. Clemons; GPYA, 25C; virulent strain
7835 ex peritoneal fluid, USA; K.V. Clemons; GPYA, 25C; virulent strain
7836 ex paracentesis fluid, USA; K.V. Clemons; GPYA, 25C; non-virulent strain
7837 ex blood, USA; K.V. Clemons; GPYA, 25C; virulent strain
7838 ex patient, USA; K.V. Clemons; GPYA, 25C
7839 ex bile tube, USA; K.V. Clemons; cond: GPYA, 25C; intermediate virulent strain
7840 ex ascites fluid, USA; K.V. Clemons; GPYA, 25C; virulent strain

Sporidiobolus pararoseus Fell & Tallman
7858(VKM Y-1632); VKM; PDA, 25C; killer

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

1. A. van Belkum, T. Boekhout & R. Bosboom. 1994. Monitoring spread of *Malassezia* infections in a neonatal intensive care unit by PCR-mediated genetic typing. J. Clin. Microbiol. **32**:2528-2532.

Malssezia furfur and *Malassezia pachydermatis* were isolated from newborn children and incubators in a neonatal intensive care unit. To assess whether persistence or frequent import of the organisms was the cause of the elevated incidence, genetic typing of the strains was performed by PCR-mediated DNA fingerprinting. By using PCR primers aimed at repeat consensus motifs, six different genotypes could be detected in a collection of six *M. furfur* reference strains. In the case of 10 *M. pachydermatis* reference strains, nine different genotypes were detected by three different PCR assays. None of these assays could document genetic differences among the clinical isolates of either *M. furfur* or *M. pachydermatis*. On the basis of these results it is concluded that within the neonatal intensive care unit the longitudinal persistence of both an *M. furfur* and an *M. pachydermatis* strain has occurred and that *Malassezia* species can persist on incubator surfaces for prolonged periods of time. It can be concluded that PCR fingerprinting is a *Malassezia* typing procedure that is to be preferred over the analysis of chromosomal polymorphisms by pulsed-field gel electrophoresis in this genus.

2. T. Boekhout & R.W. Bosboom. 1994. Karyotyping of *Malassezia* yeast: Taxonomic and epidemiological implications. System. & Appl. Microbiol. **17**:146-153. Abstract in Yeast Newsletter **43**:17.

3. T. Boekhout, C.P. Kurtzman, K. O'Donnell & M. Th. Smith. 1994. Phylogeny of the yeast genera *Hanseniaspora* (anamorph *Kloeckera*), *Dekkera* (anamorph *Brettanomyces*), and *Eeniella* as inferred from partial 26S ribosomal DNA nucleotide sequences. Int. J. Syst. Bacteriol. **44**:781-786.

Partial 26S ribosomal DNA sequences of species assigned to the genera *Hanseniaspora*, *Kloeckera, Dekkera, Brettanomyces*, and *Eeniella* were determined. A phylogenetic analysis of the sequences showed that the genus *Eeniella* is derived within the genus *Brettanomyces* and that the genus *Hanseniaspora* (anamorph *Kloeckera*) is not closely related to the genus *Dekkera* (anamorph *Brettanomyces*). As a consequence, the name *Eeniella* is reduced to synonymy with the name *Brettanomyces*. In addition, our data do not support reassignment of certain *Hanseniaspora* species to the recently revived genus *Kloeckeraspora.*

4. Y.S. Cong, D. Yarrow, Y.Y. Li & H. Fukuhara. 1994. Linear DNA plasmids from *Pichia etchellsii*, *Debaryomyces hansenii* and *Wingea robertsiae.* Microbiology **140**:1327-1335.

Linear DNA plasmids were found in the following yeasts: four strains of *Kluyveromyces lactis*, one of *Debaryomyces hansenii*, one of *Wingea robertsiae* and four of *Pichia etchellsii*. In each case, the plasmids were present as a pair of DNA molecules of different sizes. The plasmids of *K. lactis* strains were associated with a killer activity and their structure was similar to the known killer plasmids pGKL1 and 2. The plasmids from the other three species were different from pGKL plasmids and showed no killer activity against the yeast species tested so far. In all case, the linear molecules possessed terminal (probably inverted) repeats and their 5' ends had a protected structure insensitive to λ exonuclease, while the 3' ends were accessible to exonuclease III. All these strains could be efficiently cured of the plasmids by ultraviolet irradiation. The plasmids from *D. hansenii* (pDH1A and B) and from *W. robertsiae* (pWR1A and B) shared related sequences with some of the *K. lactis* killer plasmid genes (encoding the supposed DNA polymerases, RNA polymerase and the chitinase), suggesting related genome organization of these plasmids. The pair of plasmids from *P. etchellsii* (pPE1A and B) appear to be a distantly related member of the group. This pair showed no sequence homology with other plasmids, except weak homology with the putative RNA polymerase gene of pGKL2. None of the plasmids contained the sequences homologous to ORF3 and ORF4 of pGKL1 encoding the toxin resistance determinant and the toxin λ subunit, respectively.

5. M. Th. Smith, J.P. van der Walt & W.H. Batenburg-van der Vegte. 1994. *Babjevia* gen. nov.- a new genus of the Lipomycetaceae. Antonie van Leeuwenhoek (in press).

The species described as *Lipomyces anomalus* Babjeva & Gorin shows significant genetic and phenotypic divergence from the type species *Lipomyces starkeyi* Lodder & kreger-van Rij in terms of rRNA base sequence substitution and ascosporal and septal ultrastructure. The species is consequently reclassified in the new, unispecific genus *Babjevia*, as *Babjevia anomala*.

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

The following are summaries of recently published papers.

1. Grego, J., Šajbidor, J., Malík, F., & Krásny Š. 1994. Influence of immobilization on wine yeast lipid composition in the course of ethanol stress. Kvas. Prum. (Prague) **40**:169-171 (in Slovak).

Comparison of the lipid composition of immobilized and free cells of the wine yeast strain 6C *Saccharomyces cerevisiae* subjected to ethanol stress indicated that the whole impact of ethanol stress on the fatty acid composition is less pronounced with immobilized cells than with non-immobilized ones. The ethanol stress increased in immobilized and free cells the occurrence of palmitoleic acid to the detriment of palmitic acid. The character of lipid composition changes during immobilization may be due to slightly increased stress resistance. Immobilized cells are more resistant to passive membrane fluidization by ethanol.

2. Krásny, Š., Malík, F., Nahálka, J., & Strmisková, G. 1993. Stability of pectate gel used in wine yeast immobilization. Vinohrad (Bratislava) **31**:35-37(in Slovak).

Pectate gel used for wine yeast immobilization showed satisfactory stability in white and rosé wines. In contact with the medium comparatively large quantities of Ca^{2+} ions are disengaged. This unfavourable phenomenon might be easily eliminated if the pectate preparation had been preliminarily "stabilized" for 24 h in wine. A negative influence on sensorial qualities could be observed only in rosé wine. As pectate may not be regarded as a foreign substance for wine and also by its low price, pectate is a prospective carrier of immobilized yeasts for secondary wine fermentation, *e.g.* in sparkling wine production.

3. Vollek, V., Volleková, A., Malík, F., & Krásny, J. 1993. Characterization of yeasts isolated for secondary fermentation of wine. Winohrad (Bratislava) **31**:67-69 (in Slovak).

A number of wine yeast strains had been isolated from wines and yeast biomass sediments. Based on ethanol resistance stress the strains were selected in order to acquire most efficient individuals. The strains FV 3, FV 4 and FV 5 identified as *Saccharomyces cerevisiae* displayed excellent morphological, biochemical, and cultivation properties. For secondary fermentations in sparkling wine production.

4. Krásny, Š., Malík, F,, Tiko, P, 1993. Wine yeast growth in immobilized alginate gel. Vinohrad (Bratislava) **31**:100-101 (in Slovak).

Growth of immobilized wine yeasts in polysaccharide gels caused destruction of the gel particle surface and release of cells from the system. The aim of this contribution was to characterize this process limiting the use of immobilized yeasts in winery practise. The growth of wine yeasts in alginate gel had been examined in aerobic and anaerobic conditions in the process of grape must fermentation.

5. Krásny, Š,, Malík, F,, Tiko, P. 1993. Immobilized yeasts in primary grape must fermentation. Vinohrad (Bratislava) **31**:116-117 (in Slovak).

Three different immobilized yeast preparations in pectate gel carriers had been tested in the primary grape must fermentation process. In the course of fermentation CO_2 formation, decrease of sugar and ethanol formation were studied. The fermentation process was successful only from the point of view of its course, not from the viewpoint of maintaining the stability of the system. Immobilized yeast systems have thus repeatedly proved as not suitable in biological regulation for the primary fermentation process.

IV. Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7. Communicated by M. Celerin <lbach@julian.uwo.ca> and A.W. Day <aday@julian.uwo.ca>.

1. Software: The Virtual Genetics Lab - A simulation of Classical Fungal Genetics Experiments - intended for introductory/intermediate courses.

The Virtual Genetics Laboratory is an integrated series of highly interactive experiments following a set of fungal mutants from isolation to characterization to tests for allelism to mapping via random spore, tetrad analysis and mitotic recombination techniques. Perform the procedures on screen just as you would in the lab - isolate and drag samples from mutant colonies to test genotype, mix haploids in pairs to do complementation tests, score colonies growing on replica plates, etc. Each exercise is preceded by an introduction clearly setting out the purpose and goals of the experiment. Help animations and brief theory summaries are provided wherever necessary.

Exercise 1 Isolation and identification of 3 auxotrophic mutants. You are presented with a pair of replica plates following mutagen treatment. Pick off cells from a mutant colony, inoculate them on minimal medium, then test 8 potential growth factors to identify the requirement. Repeat with remaining two mutants.

Exercise 2. Analysis of mutants with similar phenotypes - Biochemical test to see which are allelic. You are given 10 arginine-requiring mutants. Inoculate each to minimal medium, then test 4 precursors of arginine to see which support growth. Deduce which of the genes in the known arginine pathway are mutant in each of the 10 strains.

Exercise 3. Analysis of mutants with similar phenotypes - Genetical test to see which are allelic (Complementation Test). You are given 10 histidine-requiring mutants. Mate these in all possible combinations to make diploids and record the phenotype of the diploid that is formed. Deduce from this complementation data how many histidine-requiring genes are present and which mutants are allelic. Help files feature an animated-lesson covering the theory of complementation.

Exercise 4. Mapping by random-spore techniques - 3-point cross. You are presented with a sample of 16 individual segregants from a 3-point cross that have been replicated to 5 test media. Deduce the genotype of each in turn, then using the much larger sample of segregants provided calculate the % recombination for each gene pair, make a map and answer questions about map distances, interference etc. 2 Help animations on the principles and practice of mapping included.

Exercise 5. Mapping using Tetrad Analysis - the same 3 mutants as in #4 plus a new orange colony colour marker. You are presented with a sample of 8 asci from a 4-point cross. i) Gene to centromere mapping - first score each ascus for First division and Second division segregation - then using data from a larger sample calculate the gene-to centromere distance for each of the 4 markers. ii) Gene to gene mapping - now using the same sample of 8 asci score them for PD, NPD or T-type tetrads and from the larger sample estimate gene-to gene recombination for each of the 6 possible gene combinations. Using the combined data from exercises 4 and 5 build the best possible map for the 4 genes and their centromeres. Help animation on Tetrad analysis included.

Exercise 6. Mapping via Mitotic Recombination - exploiting haploidization and mitotic crossing- over to obtain further mapping data. You are given 9 segregants from a diploid fungus and asked to score them for i) ploidy ii) different auxotrophic and drug resistance markers. The segregants are then analysed for mapping information. A full length 'help' animation on mitotic recombination is included.

This topic makes students think about what happens during mitosis and what would happen if a rare recombinational event occurred.

Program runs on PC computers running Windows 3.x and are also available as one of the many tools in a very comprehensive animated Genetics text - Visual Genetics - now used by over 100 institutions world-wide.

Further details from Bio-Animate Productions, 28 Askin St., London, Ontario N6C 1E3, Canada. Tel. 519-433-7145. E-mail: <aday@julian.uwo.ca>.

2. Celerin, M, Laudenbach, D.E., Bancroft, J.B., & Day, A.W. 1994. Evidence that fimbriae of the smut fungus *Microbotryum violaceum* contain RNA. Microbiology **140**:2699-2704.

The cells of the fungus *Microbotryum violaceum* produce many long, fine surface hairs that are similar in size and morphology to bacterial pili or fimbriae. These fungal fimbriae are assembled from 74 kDa glycoprotein subunits. We now present evidence that these fimbriae also contain a ribonucleic acid component. Isopycnic centrifugation of fimbriae in cesium chloride produced one band at a density intermediate to that of protein and nucleic acid. The absorbance spectrum of the intact fimbriae was consistent with that of a nucleoprotein. After extraneous RNAs were enzymatically removed from the purified fimbrial preparation, disruption of the fibrils resulted in the release of not only the 74 kDa glycoprotein subunits, but also a 30 base single-stranded RNA species. To our knowledge, this is the first example of extracellular RNA as a component of a surface appendage.

V. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.C. du Preez <preez@wwg3.uovs.ac.za>.

The following papers on yeasts have been published during the past year or are in press:

1. Meyer, P.S. & J.C. du Preez. 1994. Effect of culture conditions on astaxanthin production by a mutant of *Phaffia rhodozyma* in batch and chemostat culture. Appl. Microbiol. Biotechnol. **40**:780-785.
2. Meyer, P.S. & J.C. du Preez. 1994. Astaxanthin production by a *Phaffia rhodozyma* mutant on grape juice. World J. Microbiol. Biotechnol. **10**:178-183.
3. Meyer, P.S., B.D. Wingfield & J.C. du Preez. 1994. Genetic analysis of astaxanthin-overproducing mutants of *Phaffia rhodozyma* using RAPDs. Biotechnol. Tech. **8**:1-6.
4. Meyer, P.S., J.C. du Preez & M.S. & van Dyk. 1994. The effect of monoterpenes on astaxanthin production by a mutant of *Phaffia rhodozyma.* Biotechnol. Lett. **16**:125-128.
5. Meyer, P.S. & J.C. du Preez. 1994. Photo-regulated astaxanthin production by *Phaffia rhodozyma* mutants. System. Appl. Microbiol. **17**:24-31.
6. Du Preez, J.C. 1994. Process parameters and environmental factors affecting D-xylose fermentation by yeasts. Enz. Microb. Technol. **16**: (in press).

Certain environmental factors are of particular significance in the fermentation of the hemicellulose component of lignocellulosic biomass by yeasts; these are discussed in relation to process considerations with a comparative evaluation of the relevant fermentation parameters. The commercial exploitation of the pentose-fermenting yeasts for ethanol production from D-xylose is restricted mainly by their low ethanol tolerance and slow rate of fermentation, as well as by the difficulty in controlling the rate of oxygen supply at the optimal level. Apart from these intrinsic physiological constraints, the use of lignocellulosic hydrolysates as substrate present additional obstacles to an efficient fermentation because of the presence of inhibitors, especially acetic acid, and because the fermentation of sugar mixtures introduce further difficulties. Recent developments in the construction of recombinant microorganisms offer good prospects for the commercial production of ethanol from renewable resources.

7. M.S. Van Dyk, J.L.F. Kock & A. Botha. 1994. Review - Hydroxy long-chain fatty acids in fungi. World J. Microbiol. Biotechnol. **10**:479-504.
8. J.P.J. Van der Westhuizen, J.L.F. Kock, A. Botha & P.J. Botes. 1994. The distribution of the w3- and w6-series of cellular long-chain fatty acids in fungi. System. Appl. Microbiol. **17**:327-345.
9. D. Jansen van Vuuren, J.L.F. Kock, A. Botha & P.J. Botes. 1994. Changes in lipid composition during the life cycle of *Dipodascopsis tothii.* System. Appl. Microbiol. **17**:346-351.
10. A. Botha, J.L.F. Kock, D. Jansen van Vuuren & P.J. Botes. 1994. Arachidonic acid turnover during the life cycle of the yeast *Dipodascopsis tothii.* S.A. J. Sci. (in press).

When labelled arachidonic acid (Aa) was fed to synchronous cultures of *Dipodascopsis tothii* in the ascosporal stage, it was rapidly taken up by the cells. The uptake was followed by incorporation of labelled Aa into the cellular triacylglycerols (TAGs) and phospholipids (PLs) during the subsequent vegetative growth stages. Highest incorporation of labelled Aa occurred during ascosporogenesis. During this stage the amount of labelled Aa in the TAGs increased abruptly. However, the concomitant increase of labelled Aa in the PLs reached its peak with the maturation of the asci and was followed by a definite decline. It was found that polar labelled Aa metabolites (complex X), which may include the products of the prostaglandin endoperoxide synthase and lipoxygenase enzymes, are released into the medium, especially during ascosporogenesis. It was found that although 1mM aspirin had no effect on the uptake of labelled Aa by the cells, the incorporation of this fatty acid into the TAGs and PLs was severely inhibited, especially during ascosporogenesis. The production of labelled complex X was not inhibited by 1mM aspirin.

VI. Instituto de Microbiologia Bioquimica, Departamento de Microbiologia y Genetica, Universidad de Salamanca, Avda. Campo Charro s/n, 37007 Salamanca, Spain. Communicated by J.R. Villanueva.

The following doctoral theses have been defended in the department recently.

1. Rodriguez Cousiño, M.N. 1992. Clonación y caracterización molecular del RNA bicatenario W de *Saccharomyces cerevisiae* y estudio de su relación con 20S RNA (Dir: Dra. Rosa M. Esteban Cañibano).
2. Puente Lanzarote, P. 1992. Cambios globales en la composición proteica de *Aspergillus nidulans* durante la conidiación (Dir: Dr. Fernando Leal Sánchez).
3. Diaz Martinez, M. 1992. Aislamiento y caracterización del gen *cwg2+* de *Schizosaccharomyces pombe* (Dir: Dra. Pilar Pérez González y Dr. Angel Durán Bravo).
4. Coll Fresno, P.M. 1993. Caracterización bioquimica y genética de la actividad fenoloxidásica del basidiomyceto PM1 (CECT 2971) (Dir: Dra. Pilar Pérez González y Dr. Ramon Santamaria Sánchez.
5. Correa Bordes, J. 1993. Caracterización del gen *EXG2* de *Saccharomyces cerevisiae* (Dir: Dr. Francisco J. del Rey Iglesias).
6. Fernandez Abalos, J.M. 1993 Caracterización molecular del gen *celA1* de *Streptomyces halstedii* JM8 (Dir: Dr. Ramon Santamaria Sánchez y Prof. Julio R. Villanueva).
7. Esteban Cañibano, L.M. 1993. Caracterización molecular de los RNA virales T y 23S RNA de *Saccharomyces cerevisiae* (Dir: Dra. Rosa Esteban Cañibano).
8. Tabernero Holgado, C. 1993. Aislamiento y caracterización de los genes *bgaA* y *xyaA* de *Bacillus* sp. no 137 (Dir: Dra. Pilar Perez González y Ramon Santamaria Sánchez).
9. Ovejero Lopez Santa Cruz, M.C. 1993. Aislamiento y caracterización de antigenos importantes en el inmunodiagnostico de las aspergilosis (Dir: Dr. Fernando Leal Sánchez).
10. Castro Pichel, C. 1993. Clonación y caracterización del gen *PBR1* de *Saccharomyces cerevisiae* (Dir: Dr. Angel Durán Bravo y Francisco del Rey).
11. San Segundo y Nieto, P.A. 1994. Caracterización de *SSG1,* gen que codifica una 1,3-β-glucanasa especifica de esporulación en *Saccharomyces cerevisiae* (Dir: Dr. Francisco del Rey Iglesias).
12. Fernandez Garcia, N. 1994. Caracterización y purificación de polipeptidos de *Nocardia uniformis* detectados en fase de producción activa de Nocardicinas (Dir: Dr. Fernando Leal Sánchez).
13. Lopez Medrano, R. 1994. Serodiagnóstico de las aspergillosis por inmunoblotting: caracterización de un antígeno de 90 KDa de *A. fumigatus* con actividad catalasa (Dir: Dr. Fernando Leal Sánchez).
14. Prado Gonzalez, M. 1994. Análisis de la sensibilidad al cobre de *Yarrowia lipolytica*. Clonación y caracterización estructural de los genes *CRFI, MTPI* y *MTP2* (Dir: Dr. Angel Dominguez Olavarri).
15. Sanchez Torres, J. 1994. Aislamiento y caracterización del gen *CELB1* de *Bacillus* sp. 186-1, que codifica una celulasa alcalina (Dir: Dr. Pilar Pérez Gonzalez y Ramon Santamaria Sánchez).
16. Torres Guzman, J.C. 1994. Aislamiento y caracterización del gen *HOY1* involucrado en la transición levadura-micelio de *Yarrowia lipolytica* (Dir: Dr. Angel Dominguez Olavarri).

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35. Garcia-Cantalejo, J., Baladron, V., Esteban, P.F., Santos,m.A., Bou, G., Remacha, M.A., Revuelta, J.L., Ballesta, J.P.G., Jimenez, A. & Rey, F.del. 1994. The complete sequence of a 18002 bp segment of *Saccharomyces cerevisiae* chromosome XI contains the HBSI, MRP-L20 and PRPI 6 genes, and six new open reading frames. Yeast **10**:231-245.

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40. Fujimura, T., Esteban, R., Ribas, J.C., Wickner, R.B. Transcription, replication and encapsidation of L-A double-stranded RNA virus of *Saccharomyces cerevisiae.* In: The Proceedings of the International Workshop on Viruses of Fungi and Simple Eukaryotes (in press).

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42. Esteban, R., Rodriguez-Cousiño, N., Esteban, L.M. Genomic organization of the T and W double-stranded RNAs from *Saccharomyces cerevisiae.* In "The Proceedings of the International Workshop on Viruses on Fungi and Simple Eucaryotes". Ed. M. Leibowitz, Y. Koltin and V. Rubio (in press).

VII. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nuβdorfer Läende 11, A-1190 Vienna, Austria. Communicated by R. Messner & H. Prillinger <messner@star3.boku.ac.at>.

The following papers have been published or accepted recently.

1. R. Messner, H. Prillinger, F. Altmann,[1] K. Lopandic, K. Wimmer, O. Molnar & F. Weigang.[2] 1994. Molecular characterization and application of RAPD-analysis of *Mrakia* and *Sterigmatomyces* species. Int. J. Syst. Bacteriol., **44**:694-703.

[1]Institut für Chemie, Universität für Bodenkultur, Gregor Mendel Str. 33, A-1180 Wien.
[2]Analytische Messtechnik, Hewlett-Packard Ges.m.b.H., Lieblgasse 1, A-1222 Wien.

The qualitative and quantitative monosaccharide spectra of purified yeast cell walls indicated three phylogenetically distinct lineages of sterigma forming basidiomycetous yeasts: 1. *Kurtzmanomyces* and *Sterigmatomyces* with dominant proportions of mannose; 2. *Tilletiopsis* with glucose, galactose and small proportions of mannose; 3. *Fellomyces, Kockovaella, Sterigmatosporidium,* and *Tsuchiyaea* appeared to be closely related, based on a dominance of glucose and the presence of xylose. Yeast cell wall neutral sugars of *Sporobolomyces antarcticus* and *Sterigmatomyces aphidis* were close to those of members of the genus *Tilletiopsis.* Conspecificity, however, was excluded by Random Amplified Polymorphic DNA (RAPD)-analysis. The conspecificity of *Mrakia frigida* and *M. nivalis,* of *M. gelida* and *M. stokesii,* and of *Sterigmatomyces halophilus* and *S. indicus* was confirmed by RAPD-analysis. RAPD-analysis was found to be a simple and highly sensitive method, which can be used to differentiate species at the DNA level. It can replace n-DNA/n-DNA hybridization experiments in species identification, characterization, and delimitation.

2. Halmschlager,E.,[1] R. Messner, T. Kowalski,[2] & H. Prillinger. 1994. Syst. Appl. Microbiol. In press.

[1]Institute of Forest Entomology, Forest Pathology and Forest Protection, UNI-BOKU, Vienna. Austria.
[2]Institute of Forest Pathology, Academy of Agriculture, Krakow, Poland.

"Ophiostoma piceae" was found to comprise of two distinct species by the means of morphological investigation by synnemata size as well as by the PCR based RAPD technique for genomic analysis. Both species are distinguished undoubtedly at the species level. The differentiation power of the morphological approach is certified upon application of the statistical Kolmogorov-Smirnov Test to the synnemata size data (SPSS statistics package). The species-forming result of the RAPD analysis is ensured by phylogenetic computation of the discrete state matrix arising from the arbitrary PCR fragment patterns (PHYLIP package). The two *Ophiostoma* species separated harbour isolates predominantly grown on spruce on the one hand *(O. piceae)* and gather isolates from oak and probably other hardwoods on the other hand *(O. querci)*. For the latter species the name *O. querci* (Gregorevitch) Nannf. must be reinstated. Both species could be cultivated as yeasts.

3. H. Prillinger, R. Messner, M. Breitenbach,[1] P. Briza,[1] E. Staudacher,[2] O. Molnar, F. Weigang,[3] M. Ibl[4] & G. Himmler. 1994. Phytopathogenic filamentous fungi with falcate ascospores *(Eremothecium, Ashbya)* as new members within the Saccharomycetaceae. Centenarium MUCL, In press.

[1]Institut für Allgemeine Biologie, Universität Salzburg, Salzburg, Austria.
[2]Institut für Chemie, Universität für Bodenkultur, Vienna, Austria.
[3]Analytische Messtechnik, Hewlett-Packard Ges.m.b.H., Vienna, Austria.
[4]Codon Genetic Systems, Colloredogaβe 29/13 A-1180 Vienna, Austria.

Based on the qualitative and quantitative cell wall monosaccharide pattern the presence of dityrosine in ascospore walls, the ubiquinone system, and partial ribosomal DNA sequences the genera *Ashbya, Eremothecium, Nematospora,* and *Kluyveromyces* have to be included in the family of the Saccharomycetaceae. Mannose and glucose appeared to be the characteristic cell wall neutral sugars of all investigated species. Dityrosine was detected in the ascospores of *Ashbya, Eremothecium, Nematospora,* and *Saccharomyces* strains. The strain of *N. coryli* did not form ascospores. A ubiquinone Q-6 was found in all species except *N. coryli,* where ubiquinone Q-5 was detected. Segments homologous to positions 619 to 1035 and 1205 to 1617 of the 18S rDNA and positions 470 to 890 and 1535 to 1984 of the 25S rDNA of *Saccharomyces cerevisiae* were sequenced. The maximal resolving power of rDNA sequence analysis is provided by including the rapidly evolving ITS1 and ITS2 regions. The investigated molecular characters suggest that morphology and ornamentation of ascospores, the persistence of ascus walls as well as mycelium formation and saprophytism or parasitism should not be used as differentiating characters in yeast taxonomy, especially in family delineation.

VIII. Departamento de Microbiologia, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de México. A.P. 70-242, México D.F., 04510 México. Communicated by A. Peña <apd@ifcsun1.ifisiol.unam.mx>.

The following communication has been sent for publication:

1. Peña, A., Ramírez, J., Rosas, G., & Calahorra, M. Proton pumping and the internal pH of yeast cells, measured with pyranine introduced by electroporation.

The internal pH of yeast was determined by measuring the fluorescence changes of pyranine, introduced into the cells by electroporation. This may be a suitable procedure because: a) only minor changes in the physiological status of the cells seemed to be produced; b) the dye does not seem to leak at a significant rate from the cells; c) different incubation conditions produced large fluorescent changes of the dye, which in general agree with the current knowledge of the proton movements of the yeast cells under different conditions; d) pyranine introduced by electroporation seemed to be located in the cytoplasm, avoiding the vacuole, probably measuring actual cytoplasmic pH; e) correction factors to obtain a more precise estimation of the internal pH are not difficult to apply, and the procedure may be useful for other yeasts and microorganisms, as well as for the introduction of other substances into cells. Values for the cytoplasmic pH of yeast higher than those reported previously were obtained, probably because this fluorescent indicator did not seem to penetrate into the cell vacuole.

IX. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via Nazario Sauro, 85, 85100 Potenza, Italy. Communicated by P. Romano and G. Suzzi.

The following papers are recently published or in press.

1. Romano P., Suzzi G., Turbanti L., & Polsinelli M. 1994. Acetaldehyde production in *Saccharomyces cerevisiae* wine yeasts. FEMS Microbiol. Lett., **118**:213-218.

Eighty-six strains of *Saccharomyces cerevisiae* were investigated for their ability to produce acetaldehyde in synthetic medium and in grape must. Acetaldehyde production did not differ significantly between the two media, ranging from a few mg/l to a bout 60 mg/l, and was found to be a strain characteristic. The fermentation temperature of 30°C increased considerably the acetaldehyde produced. This study allowed to differentiate the strains in different phenotypes: "low, medium and high acetaldehyde producers". The low and high phenotypes differed considerably also in the production of acetic acid, acetoin and higher alcohols and can be useful for studying acetaldehyde production in *S. cerevisiae,* both from the technological and genetic point of view.

2. Vannini L., De Simone G., Norscia P., Romano P., & Suzzi G. 1994. Acetaldehyde production as a selective character in *Saccharomyces cerevisiae.* Industrie delle Bevande, in press.

A total of 100 strains of *Saccharomyces cerevisiae,* isolated from different grape musts of different Italian regions, were examined for the capacity to produce acetaldehyde in synthetic medium. A considerable strain variation in acetaldehyde product ion was observed and it has been possible to distinguish four groups: low-producers, medium-producers, medium-high producers and high-producers. Acetaldehyde production resulted a strain characteristic. The low acetaldehyde producers had traces of acetoin, lower amounts of acetic acid and higher amounts of isobutanol and isoamyl alcohol, whereas the medium and high producers produced higher amounts of acetoin and acetic acid. In grape must and in wort, each strain kept its characteristic to be low or medium or high acetaldehyde producer. As differing amounts of secondary products can influence the taste of alcoholic beverages, the differences found in acetaldehyde production confirm the importance of strain selection for technological applications.

3. De Simone G., Norscia P., Suzzi G., & Romano P. 1994. Relationship between selected strains of *Saccharomyces cerevisiae* and must composition variability. Industrie delle Bevande, in press.

The behaviour of 30 selected strains of *Saccharomyces cerevisiae* was studied in 9 different grape musts. The must composition affects the strain vigour in the early stages of fermentation, whereas it has not effect on the strain ability to complete the fermentation process. SO_2 produced in the different wines depends on the metabolic characteristics of the yeast strain, and seems not to be affected by the must differences. Finally, secondary products formed by high and low SO_2 producing strains during the fermentation of 9 grape musts emphasized the importance of the "strain metabolic characteristics" in determining the wine bouquet.

4. De Simone G., Romano P., Suzzi G., & Norscia P. 1994. Fermentation by-products in high SO_2 producing strains of *Saccharomyces cerevisiae.* Industrie delle Bevande, in press.

Twenty-eight high SO_2 producing strains of *Saccharomyces cerevisiae* were isolated from sulfited musts. The strains were characterized for the production of SO_2, acetaldehyde, acetic acid and higher alcohols in synthetic and complex media. A great variability in the formation of by-products was observed in the complex medium, with the exception of isoamyl alcohol. In this medium a direct correlation between acetaldehyde and SO_2 produced was not always found. These strains could be useful for the production of low alcohol beers, which need an adequate SO_2 content.

5. Suzzi G., Romano P., Westall F., Vannini L. 1994. Flocculent phenotypes in apiculate wine yeasts. Proceedings 7th Intern. Congr. of Bacteriol. Appl. Microbiol. Div., Prague, p. 254-255.

Flocculation of yeast cells is the reversible and spontaneous aggregation of cells. This phenomenon is well known in brewer's yeast *Saccharomyces cerevisiae* and *S. uvarum.* Among wine yeasts four flocculent phenotypes were found in *S. cerevisiae* (Suzzi and Romano, 1991) and two in *Zygosaccharomyces* (Suzzi et al. 1992). In this paper, different aspects of apiculate flocculation are investigated by studying the influence of proteolytic enzyme digestion and the inhibition by various saccharides with the aim to know the pattern of cell-cell interaction. Ten flocculent *Kloeckera* strains isolated from Italian musts were used. The yeasts were cultured in Sabouraud liquid medium at 25°C without shaking. Proteinase treatments have been performed according to the method of Hodgson et al. (1985) and sugar treatments according to Suzzi et al. (1992). Preparation methods for SEM and TEM followed procedures described in Westall and Rincé (1994). Three different flocculation phenotypes were discriminated by protease digestion. The first phenotype was characterized by high resistance to all the proteolytic enzymes and the second by resistance only to trypsin and chymotrypsin. In the third phenotype, the floc-forming ability was irreversibly lost during incubation with pronase, proteinase K, chymotrypsin and trypsin. As regards the inhibition of flocculation by sugars, the apiculate cells flocculated normally in presence of fructose, glucose, maltose and mannose, whereas flocculation was greatly or completely inhibited by 1-M galactose. The EM studies showed that some apiculate strains were characterized by thick mucous links between the cells, whereas other strains were predominantly linked by fine "fimbriae". The mechanisms of apiculate wine yeast flocculation involve a galactose-specific protein, lectin like, differently from the mannose-specific protein found in flocculation of *Saccharomyces cerevisiae.*

6. Mortimer R.K., Romano P., Suzzi G., & Polsinelli M. 1994. Genome renewal: a phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from naturale fermentation of grape musts. Yeast, **10**:000-000.

We have analyzed by genetic means 43 strains of *Saccharomyces* that had been isolated from fermenting grape musts in Italy. Twenty eight of these strains were isolated from 28 cellars in the Region of Emilia Romagna. The other 15 strains came from 5 fermentations at four cellars near the city of Arpino, which is located south and east of Rome. We found that 20 of the 28 strains from Emilia Romagna were heterozygous at from one to seven loci. The balance were, within the limits of our detection, completely homozygous. All these strains appeared to be diploid and most were homozygous for the homothallism gene (HO/HO). Spore viability varied greatly between the different strains and showed an inverse relation with the degree of heterozygosity. Several of the strains, and in particular those from Arpino, yielded asci that came from genetically different cells. These different cells could be interpreted to have arisen from a heterozygote that had sporulated and, because of the HO gene, yielded homozygous diploid spore clones. We propose that natural wine yeast strains can undergo such changes and thereby change a multiple heterozygote into completely homozygous diploids, some of which may replace the original heterozygous diploid. We call this process "genome renewal".

X. Yeast Division of the All-Russian Collection of Microorganisms (BKM, VKM), Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142292, Russia. Communicated by W.I. Golubev.

The following papers have appeared since the last issue of the Yeast Newsletter.

1. Shubakov, A.A., Okunev, O.N., Golubev, W.I. & Mikhaleva, N.I. 1994. Occurrence of xylanase activity among basidiomycetous yeasts. Mikrobiologiya **63**:217-220.

Out of 165 cultures (50 species of 20 genera) of basidiomycetous yeast-like fungi tested 80% of the strains belonging to genera *Apiotrichum, Atractogloea, Cryptococcus, Endophyllum, Filobasidium, Gymnosporangium, Holtermania, Microstroma, Tremella* and *Trichosporon* were able to utilize xylan as sole carbon source in a medium. The production of extracelluar xylanases was frequently found in *Cryptococcus* spp. Out of 4 *Cr. podzolicus* strains studied the strain VKM Y-2249 revealed the highest level of xylanase activity.

2. Golubev, W.I. & Shabalin, Y. 1994. Microcin production by the yeast *Cryptococcus humicola.* FEMS Microbiol. Lett. **119**:105-110.

Cryptococcus humicolus strains secrete killer toxins inhibitory (at pH values from 3 to 5.5) to many ascomycetous and basidiomycetous yeast-like fungi RNA or DNA plasmids were not detected in the killers. The amino acid-containing toxins were of low M_r soluble in methanol, resistant to proteolysis, thermostable, cellophane-diffusible and were specified as microcins. These findings show that the killer phenomenon in yeasts, as in bacteriocinogeny, may be due to excretion of two types of killer toxins: mycocins and microcins.

3. Golubev, W.I. and Blagodatskaya, V.M. 1994. Intra- and intergeneric killing patterns of mycocins produced by *Pichia punctispora* (Melard 1910) Dekker 1931. Mikrobiologiya **63**:637-642.

P. punctispora mycocins (killer toxins) manifest their activity at different taxonomic levels. From five groups of killers the mycocins produced by the group I act against strains of the same species and *P. membranaefaciens.* The mycocins of the groups III, IV and V are active against some more 9 species and 1 variety of *Pichia,* 2 *Williopsis* species, and *Metschnikowia bicuspidata* (weakly). Killing pattern of the mycocins produced by the group II of killers includes the members of the Spermophthoraceae, the species of monospecific or small genera *(Arxula, Clavispora, Gulliermondella, Pachysolen, Pachytichospora, Trigonopsis, Zygoascus),* and also *Kluyveromyces africanus* and *Williopsis pratensis.* All basidiomycetous yeasts and other ascomycetous ones, including the *Pichia* spp. classified in the genera *Hyphopichia, Issatchenkia, Saturnospora* and *Yamadazyma,* are insensitive to *P. punctispora* mycocins.

4. Golubev, W.I. 1994. Reidentification of the yeast strains used for SCP production from wood hydrolysates. Biotechnologiya **6**:6-9.

In accordance with their morphological, physiological and biochemical characteristics the industrial yeast strains used in production of SCP from wood hydrolysates and known as *Candida scottii* were identified as *C. blankii.*

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

The following papers are in preparation.

1. L.M. Santopietro Ducrey, J.F.T. Spencer, D.M. Spencer and F. Siñeriz. Effects of oxidative stress on production of carotenoid pigments (astaxanthin) by *Phaffia rhodozyma*.

The resistance to killing by $H_2O_2 + Fe^{2+}$ solutions, which are generators of free radicals, by two mutants of *Phaffia rhodozyma,* was determined. One of these (Mutant 5-7) did not produce astaxanthin but produced ß-carotene, and the other (Mutant 3-4) produced neither astaxanthin nor ß-carotene, or other carotenoid pigments. The resistances were compared with a wild-type strain of *P. rhodozyma* which produced astaxanthin in normal yields. The resistance of mutant 5-7 was approximately the same as that of the wild-type, but mutant 3-4 was killed much more rapidly than either mutant 5-7 or the wild-type, and its numbers in mixed continuous cultures decreased greatly. Possession of either carotenoid pigment increased the resistance to killing by free radicals in the culture medium considerably.

2. L.M. Santopietro Ducrey, J.F.T. Spencer, D.M. Spencer and F. Siñeriz. Formation of protoplasts from *Phaffia rhodozyma.*

The classic protocol used for making protoplasts from *Saccharomyces cerevisiae* yields spheroplasts when used for obtaining protoplasts from *Phaffia rhodozyma.* These can be used in intergeneric fusion. However, when the isolation of total DNA from them is attempted, the yield is very low. The same difficulty is encountered when attempting to prepare blocks for separation of chromosomes by pulsed field gel electrophoresis. The object of this investigation was to develop an efficient protocol for preparing and regeneration of protoplasts and spheroplasts from *Phaffia rhodozyma,* for use in studies of the genetics of this yeast species.

3. Loray, M.A., Spencer, J.F.T., Spencer, D.M., and de Figueroa, L.I.C. Hybrids obtained by protoplast fusion with a salt-tolerant yeast.

An industrial strain of *Saccharomyces cerevisiae* was fused with an osmotolerant yeast, *Debaryomyces hansenii,* to obtain hybrids having increased tolerance to elevated salt concentrations in the media. Fusion products constructed using as one parent an osmotolerant yeast have better performance in consumption of glucose in media having high salt concentrations.

4. D.M. Spencer, J.F.T. Spencer, L.I. de Figueroa and E. Fengler. Yeasts associated with algarrobo trees (*Prosopis* spp.) and species of giant cacti in Northwest Argentina.

Yeasts were isolated from slime fluxes from three sites in northwest Argentina, two between the towns of Amaicha del Valle and Cafayate and one in the Quebrada de Cafayate, a deep river valley north of Cafayate. Trees at Las Breñas, Quimili and Añatuya, in the Chaco region, were also sampled. Samples from rot pockets of decaying cacti were obtained near the ruins at Quilmes, a pre-Columbian Indian town south of Colalao del Valle, and in the Parc Nacional de Cardons east of Chachi. The majority of the yeasts were identified as *Candida famata* and *Rhodotorula graminis,* though isolates of other species of *Rhodotorula, Candida boidinii, Pichia membranaefaciens,* and occasional isolates of other species were obtained. None of the species was the same as those isolated in Crete, from pods of the carob (European algarrobo). Of about 700 cultures investigated, 26 utilized methanol as sole carbon source, and about 75 fermented xylose. Another 100 were classified as *Candida famata* (perfect stage *Debaryomyces hansenii).* The frequency of isolation of methylotrophic yeasts and osmotolerant yeasts from this habitat may prove to be of considerable scientific and technological interest.

5. D.M. Spencer, J.F.T. Spencer, L.I. de Figueroa and E. Fengler. Yeasts associated with algarrobo trees (*Prosopis* spp.) in north-west Argentina (preliminary report).

Yeasts were isolated from slime fluxes from three sites in northwest Argentina, two between the towns of Amaicha del Valle and Cafayate and one in the quebrada de Cafayate, a deep river valley north of Cafayate. The majority of the yeasts were identified as *Candida famata* and *Rhodotorula graminis,* though isolates of other species of *Rhodotorula, Candida boidinii, Pichia membranaefaciens,* and occasional isolates of other species were obtained. None of the species was the same as those isolated in Crete, from pods of the carob (European algarrobo). Of 96 cultures investigated, 26 utilized methanol as sole carbon source. The frequency of isolation of these yeasts such a habitat may be of interest.

6. C. Colin, J.F.T. Spencer, C.M. Abate, and D.M. Spencer. Intergeneric protoplast fusion using *Saccharomyces cerevisiae* and *Hansenula polymorpha.*

Protoplasts can be used in numerous studies, other than for hybridization, including investigations involving isolation of nuclear and mitochondrial DNA, and especially for isolation and separation of relatively intact chromosomes or chromosome-sized sections of DNA. In addition, fusion of yeast protoplasts has become a recognized technique for the production of hybrids which cannot be

obtained by any other methods. In our laboratory we investigated intergeneric protoplast fusion with the objective of using this technique for the genetic improvement of industrial microorganisms. The microorganisms used were *Saccharomyces cerevisiae* ade$^-$, his$^-$, trp$^-$, and *Hansenula polymorpha* met$^-$. The fusion products were selected by a selection system based on nutritional complementation of auxotrophic markers. In the majority of crosses the fusion products recovered were diploid, as indicated by the segregation of the markers. We obtained 7 fusion products from 400 colonies analyzed, 5 were morphologically similar to *Saccharomyces cerevisiae,* and 2 similar to *Hansenula polymorpha.* All the fusion products sporulated, and when the asci were dissected by micromanipulation there was a range of genetic segregation: 2:2, 3:1, 1:3 and 0:4.

7. F. Vasquez,[1] H. Heluane,[2,3] D. Spencer,[3] F. Spencer,[3] & L.C.de Figueroa.[2,3] Construction of yeast strains able to hydrolyze xylans, by fusion of yeast protoplasts with nuclei of filamentous fungi.

[1]Universidad Nacional San Juan, Argentina.
[2]Universidad Nacional Tucuman.
[3]PROIMI.

Protoplasts obtained from a yeast hybrid constructed by fusion of protoplasts of *Saccharomyces cerevisiae* and the xylose-fermenting yeast *Pachysolen tannophilus,* were fused with nuclei extracted from the filamentous, xylan-hydrolyzing and xylose-fermenting plant pathogenic fungus *Fusarium moniliforme.* The yeast-like hybrids obtained were able to hydrolyze xylans and convert the resulting xylose to biomass. Xylanase activity obtained, in 14 hybrids, reached 9.55-20.22 kat/ml after 24 h and decreased to 7.27-16.43 nkat/ml after 48 h, in a medium initially containing 2% xylan. From 35-50% of the xylan was consumed. Ethanol was not detected in the cultures.

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

The following are summaries of articles accepted for publication or recently published.

1. Mercado J.J., Smith R., Sagliocco F.A., Brown A.J.P. & Gancedo J.M. 1994. The levels of yeast gluconeogenic mRNAs respond to environmental factors. Eur. J. Biochem. **224**:473-481.

The *FBP1* and *PCK1* genes encode the gluconeogenic enzymes fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase, respectively. In the yeast, *Saccharomyces cerevisiae,* the corresponding mRNAs are present at low levels during growth on glucose, but are present at elevated levels during growth on gluconeogenic carbon sources. We demonstrate that the levels of the *FBP1* and *PCK1* mRNAs are acutely sensitive to the addition of glucose to the medium and that the levels of these mRNAs decrease rapidly when glucose is added to the medium at a concentration of only 0.005%. At this concentration, glucose blocks *FBP1* and *PCK1* transcription, but has no effect on iso-1 cytochrome c *(CYC1)* mRNA levels. Glucose also increases the rate of degradation of the *PCK1* mRNA approximately twofold, but only has a slight effect upon *FBP1* turnover. We show that the levels of the *FBP1* and *PCK1* mRNAs are also sensitive to other environmental factors. The levels of these mRNAs decrease transiently in response to a decrease of the pH from pH 7.5 to pH 6.5 in the medium, or to a mild temperature shock (from 24°C to 36°C). The latter response appears to be mediated by accelerated mRNA decay.

2. Blázquez M.A., Gancedo J.M. & Gancedo C. 1994. Use of *Yarrowia lipolytica* hexokinase for the quantitative determination of trehalose 6-phosphate. FEMS Microbiol. Lett. **121**:223-228.

The paper describes a procedure for the quantitative determination of trehalose 6-phosphate (T6P) based on its ability to inhibit hexokinase from *Yarrowia lipolytica.* The assay is linear between 1 nmol and at least 8nmol. The concentration of T6P in wild-type *Saccharomyces cerevisiae* (0.15mM) and in *ras2* mutants (0.25mM) remained unchanged in the exponential or stationary phase of growth or after heat-shock. A *tps1* mutant affected in T6P synthase did not show detectable T6P. Heat shock increased the concentration of T6P in *Schizosaccharomyces pombe* from 0.43 to 0.75 mM.

3. Vincent O. & Gancedo J.M. Expression of a yeast gene can be blocked by insertion of short DNA fragments between an UAS and the TATA box. Current Genet. In press.

We have constructed a plasmid, pOV10, which facilitates the introduction of putative upstream activating sequences (UAS) or upstream repressing sequences (URS) from yeast genes into plasmids containing *CYC1-lacZ* fusions. We have observed that the insertion of yeast sequences from 155 to 195 bp between the UAS and the TATA box of a *CYC-lacZ* fusion gene can block beta-galactosidase expression. It is suggested that this block is related with the formation of nucleosomes on the DNA.

4. Gamo F.-J., Lafuente M.J. & Gancedo C. 1994. The mutation DGT1-1 decreases glucose transport and alleviates carbon catabolite repression in *Saccharomyces cerevisiae.* J. Bacteriol. **176**: (in press).

Glucose in ethanol-glycerol mixtures inhibits growth of *Saccharomyces cerevisiae* mutants lacking phosphoglycerate mutase. A suppressor mutation that relieved glucose inhibition was isolated. This mutation, DGT1-1 (decreasing glucose transport), was dominant and produced pleiotropic effects even in an otherwise wild-type background. Growth of the DGT1-1 mutant in glucose was dependent on respiration, and no ethanol was detected in the medium within 7h of glucose addition. When grown on glucose, the mutant had a reduced glucose uptake and both the low- and high-affinity transport systems were affected. In galactose-grown cells, only the high-affinity glucose transport system was detected. This system had similar kinetic characteristics in the wild-type and in the mutant. Catabolite repression of several enzymes was absent in the mutant during growth in glucose but not during growth in galactose. In contrast with the wild type, the mutant grown in glucose had high transcription of the glucose transporter gene *SNF3* and no transcription of *HXT1* and *HXT3.* Expression of multicopy plasmids carrying the *HXT1, HXT2,* or *HXT3* genes allowed partial recovery of both fermentative capacity and catabolite repression in the mutant. The results suggest that *DGT1-1* codes for a regulator of the expression of the glucose transport genes. They also suggest that glucose flux might determine the levels of molecules implicated as signals in catabolite repression.

5. Riballo E., Mazón M.J. & Lagunas R. 1994. cAMP-dependent protein kinase is not involved in catabolite inactivation of the transport of sugars in *Saccharomyces cerevisiae.* Biochim. Biophys. Acta **1192**:143-146.

It has been reported that catabolite inactivation of the sugar transport systems in *S. cerevisiae* requires cAMP-dependent protein kinase activity (cAPK) and that the levels of these transport systems are decreased in the absence of a functional cAPK regulatory subunit. We have re-examined these possibilities and have found that catabolite inactivation does not require cAPK activity and that normal levels of the transports occur independently from the presence of the regulatory subunit. With the available information, it is difficult to ascertain the reasons for the discrepancy between our results and the ones previously reported. The inadequacy of the method used to measure the sugar transport activities might contribute to this discrepancy.

6. Riballo E. & Lagunas R. 1994. Involvement of endocytosis in catabolite inactivation of the K^+ and glucose transport systems in *Saccharomyces cerevisiae.* FEMS Microbiol. Letters **121**:77-80.

The possible relationship between endocytosis and catabolite inactivation of plasma membrane proteins in *S. cerevisiae* has been investigated. Using mutants with an increased rate of endocytosis we have shown that there is a positive correlation between the rate of endocytosis and the rate of inactivation of the K^+ and glucose transport systems. It is concluded that endocytosis is involved in catabolite inactivation of these two transport systems.

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

The following works have appeared recently.

1. Weusthuis, R.A. Disaccharide fermentation by yeasts. PhD Thesis, Delft University of Technology, 85 pp.
2. Weusthuis, R.A., M.A.H. Luttik, W.A. Scheffers, J.P. van Dijken & J.T. Pronk. 1994. Is the Kluyver effect in yeasts caused by product inhibition? Microbiol. **140**:1723-1729.
3. Heus, J.J., K.S. Bloom, B.J.M. Zonneveld, H.Y. Steensma & J.A. van den Berg. 1993. Chromatin structures of *Kluyveromyces lactis* centromeres in *K. lactis* and *Saccharomyces cerevisiae.* Chromosoma **102**:660-667.
4. Heus, J.J., B.J.M. Zonneveld, H.Y. Steensma & J.A. van den Berg. 1993. The consensus sequence of *Kluyveromyces lactis* centromeres shows homology to functional centromeric DNA from *Saccharomyces cerevisiae.* Mol. Gen. Genet. **236**:355-362.
5. Van der Aart, Q.J.M., C. Barthe, F. Doignon, M. Aigle, M. Crouzet & H.Y. Steensma. 1994. Sequence analysis of a 31 kb DNA fragment from the right arm of *Saccharomyces cerevisiae* chromosome II. Yeast **10**:959-964.
6. Wenzel, T.J., A.M. Zuurmond, A. Bergmans, J.A. van den Berg & H.Y. Steensma. 1994. Promoter analysis of the *PDA1* gene encoding the E1a subunit of the pyruvate dehydrogenase complex from *Saccharomyces cerevisiae.* Yeast **10**:297-308.
7. Heikoop, J.C., H.Y. Steensma, G.J.B. van Ommen & J.T. den Dunnen. 1994. A simple and rapid method for segregation of cocloned YACs by retrofitting and negative selection. Trends Genet. **10**:40.

8. Den Dunnen, J.T., P.M. Grootscholten, H.Y. Steensma & G.-J.B. van Ommen. YAC breeding to reconstitute large loci. In: D.L. Nelson and B.H. Brownstein (eds.), YAC Libraries, a User's Guide, W.H. Freeman & Co., New York, pp.181-202.

XIV. Center for Biological Research, Northwest Region (CIBNOR), Division of Experimental Biology, Marine Pathology Department, P.O. Box 128, La Paz, 23000 B.C.S., México. Communicated by N.Y. Hernández-Saavedra <nhernan@cibnor.conacyt.mx>.

The following are summaries of papers published recently or in press.

1. J.L. Ochoa, Hernández-Saavedra N.Y., Hernández-Saavedra, D. , Tovar-Ramírez, D. & Ramírez-Orozco, M. 1994. Biotechnological utilization of marine yeast: SOD extraction from *Debaryomyces hansenii* cultured on glucose enriched sea water. XX Congreso Nacional. Sociedad Mexicana de Bioquímica. Zacatecas, Zac. México. October-November, 1994.

A cheap and easy procedure has been designed for the culture of the marine yeast *Debaryomyces hansenii* strain C-11, as a source of SOD enzyme. The yeast strain was isolated from a oceanographic cruise performed on 1986 along the west coast of Baja California peninsula with the oceanographic ship EL PUMA. Cellular biomass was obtained with a glucose medium in sea water reaching yields higher than 33% (on basis of carbon source) on 60 l plastic bottles. The incubation was carried out during 48 h at room temperature with shaking (850 rpm) and aeration (3.3 l/min.). Contamination risks were controlled by means of chlorine dioxide (Halox E- 100 ™ 1:500 v/v); the cell biomass was collected by continuous centrifugation. Several methods for SOD enzyme extraction showed yields between 2 and 3% on dry weight basis, with a total enzyme activity between 103,000 and 327,000 units/100 g of cell biomass. These results are compared with those obtained from other yeasts and sources of the enzyme.

2. Hernández-Saavedra, N.Y., Ochoa, J.L. & Vázquez-Duhalt, R. In press. Effect of salinity in the growth of the marine yeast *Rhodotorula rubra*. Microbios.

The marine yeast *Rh. rubra* was studied to determine its response to saline stress in batch cultures at different temperatures. An increase in the salt concentration (4, 8 and 12%) resulted in a decrease in the specific cell growth rate from 0.27 to 0.05 h^{-1} (at 20°C). At higher temperatures (25°C and 30°C), the effect was less noticeable (from 0.32 to 0.14 $h^{-1)}$. Intracellular solute concentration (carbohydrates and free amino acid) decreased with increasing salinity. Therefore, the participation of these two kinds of metabolites in the osmoregulation of *Rh. rubra* was excluded. While the total lipid content of the cells increased with salinity, a close correlation between intracellular glycerol concentration and medium salinity was observed, suggesting its participation in osmoregulation. The glycerol pool concentration (157.08, 302.65 and 650.63 μg/mg dry $weight^{-1}$) may balance the osmotic pressure in culture media of 4, 8 and 12% NaCl.

3. Hernández-Saavedra, N.Y., Ochoa, J.L. & Vázquez-Duhalt, R. Osmotic adjustment in marine yeast. In press. J. Plankton Res.

The effect of environmental salinity on cell growth, and on the composition and accumulation of compatible solutes, or osmotica, of five yeast strains (*Aureobasidium pullulans, Candida* sp., *Cryptococcus albidus* var. *albidus*, *Debaryomyces hansenii* and *Rhodotorula rubra*) was compared. All these yeast were isolated from marine environments however they were also able to grow in the absence of salt, therefore should be considered as halotolerant strains. According to their specific cell growth rates at different salt concentrations, these strains vary in their capacity to osmotically adjust to modifications in external salinity. *Candida* sp. appears to be the most sensitive since the maximum salt concentration at which it can grow is 1.54 mol l^{-1} NaCl, however, showed the highest specific cell growth in the range of zero to 1.54 mol l^{-1} NaCl. *Aureobasidium pullulans* on the other hand, showed the lowest specific growth rate but the highest halotolerance range from zero to 5.13 mol l^{-1}NaCl, *Debaryomyces hansenii* in contrast, showed higher specific growth at this salinity range. *Cryptococcus albidus* var. *albidus* and *Rhodotorula rubra* showed similar specific cell growth rates values and halotolerance between zero and 2.47 mol l^{-1} NaCl. The protein and carbohydrate content of the different cell yeast biomass, as a result of external salinity variation, remained practically constant. The most important effects of the increase in the salt concentration in the medium culture were the reduction of cell volume and the accumulation of low molecular weight metabolites (LMWM), which appear to act as osmoregulators. Glycerol was found as the major compatible solute in the different marine yeast herein studied with a total contribution of 64-96% of the internal cell osmolarity. Other LMWM, like carbohydrates and aminoacids, contributed in a lesser extent to compensate the rise in osmotic pressure promoted by the salinity of the external environment.

The following papers have been submitted for publication.

4. Ochoa, J.L., Ramírez-Orozco, M., Hernández-Saavedra, N.Y., & Hernández-Saavedra, D. The halotolerant yeast *Debaryomyces hansenii* as an alternative source of Cu/Zn-SOD enzyme. Proceedings of IMBC'94 in collaboration between J. Mar. Biotechnol. and Mol. Mar. Biol. Biotechnol.

Considerable amounts of cell biomass of the halotolerant yeast *Debaryomyces hansenii* were produced with a simple fermentation process using a culture medium formulated with sea water. The culture was carried out in 60 l plastic bottles under non axenic conditions in the presence of chlorine dioxide (Halox^{TM}) to repress the growth of any contaminant microorganism. The cell biomass was treated with different physical and chemical methods to extract the enzyme superoxide dismutase (SOD). The combination of cell disruption mechanical methods, using glass beads, and the chemical extraction with chloroform/ethanol yielded best results. Approximately, 0.6% of protein (on dry matter basis) with a SOD activity of 1500 Units/mg were obtained in one single step, which compares well with other SOD sources such as Brewer's yeast, bovine liver and human erythrocyte. We conclude that *Deb. hansenii* may be an excellent alternative for producing SOD in an economically attractive manner.

5. Hernández-Saavedra, N.Y., Hernández-Saavedra, D., & Ochoa J.L. Factors affecting the distribution of the genus *Candida* (Berkhout) along the west coast of B.C.S., México. System. Appl. Microbiol.

Of 141 yeast strains isolated from 98 samples of sea water off the Pacific coast of Baja California Sur, Mexico, 22 % were of the genus *Candida*. Five different groups of *Candida* were found based on physiological characteristics. A distribution pattern was established which correlated these group to physicochemical parameters of the sea water samples. We found that temperature and dissolved oxygen influenced the distribution of this genus, while the amount and kind of nutrients influenced both distribution and population density.

The following are summaries of work in progress as part of the major project "Biotechnological use of Marine Yeasts".

6. Sánchez-Paz, A., Ochoa J.L. & Hernández-Saavedra N.Y. Identification and classification of marine yeasts using electrophoretic techniques.

Traditionally the identification and differentiation of yeast relies on a number of criteria which suffer from a lack of sensitivity and specificity. The need of new techniques for yeast identification and classification is particularly significant for the selection of organisms of biotechnological and industrial importance, the treatment of infections, and to understand how these species interact in nature. 79 Whole-cell protein extracts and mitochondrial DNA analysis with restriction endonucleases on PAGE of the same number of yeast strains, isolated from the West coast of Baja California peninsula, were studied. The results obtained suggest that this approach may be useful for routine diagnosis and for the classification and identification of marine yeasts.

7. Ramírez-Orozco, M. & Ochoa, J.L. Evaluation of the cell yield and superoxide dismutase activity in *Debaryomyces hansenii* under different culture conditions.

We are presently working in the design and optimization of a semi-artisanal method for biomass production of the marine yeast *Debaryomyces hansenii* strain C-11, from CIBNOR Marine Yeast Collection. The method avoids the need of a sterilization step by using sea water, low pH value, and a powerful oxidant agent, in the culture of *Deb. hansenii* strain C-11. Optimization studies include aeration, agitation, temperature and media composition. In addition, we are also looking at the effect of the various culture conditions in the yield of Superoxide Dismutase in order to determine whether this organisms could be employed as a novel commercial source for this enzyme.

8. Hernández-Saavedra, N.Y. and Ochoa J.L. Isolation and characterization of a Cu/Zn-SOD from the marine yeast *Debaryomyces hansenii*.

Superoxide dismutase (SOD, EC 1.15.1.1) is the enzyme that helps to eliminate the toxic $\circ O_2^-$ species in respiring cells through a disproportionation mechanism: $\circ O_2^- + \circ O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. It is now considered to be ubiquitous to all aerobic organisms, but absent in obligate anaerobes. Different superoxide dismutase enzymes compose a family of metalloproteins which have iron (Fe-SOD), manganese (Mn-SOD) or copper (Cu/Zn-SOD) in the active site. The cytosolic Cu/Zn-SOD enzyme has a dimmer structure but its extracellular form (EC-SOD), found in mammalian body fluids and also in some tissues, is tetrameric and always glycosylated. The mass of the subunit in both kinds of Cu/Zn-SOD is 15.0 kDa and is believed to derive from a common ancestor which is different from that of Mn- and Fe-SOD. We have isolated the cytosolic form of Cu/Zn-SOD from *Deb. hansenii.* This enzyme has a dimmer structure (30.91 kDa) with a subunit mass of 15.6 kDa. The preparation was found to be heterogenous by IF electrophoresis with two pI's ranges: 5.14 to 4.0 and 1.6 to 1.8. It is worth mentioning that the enzyme preparation showed a strong stability at pH 7.0 surviving boiling periods of 10 minutes without loosing activity. By means of the western blot technique, a strong Ag-Ab reaction was observed for this enzyme with antibodies raised in rabbits against *Deb. hansenii* extracts, while only a light crossed-reaction could be

detected using antibodies generated against either *Saccharomyces cerevisiae* and/or bovine erythrocyte Cu/Zn-SOD's. In sequencing analysis, a peptide obtained by trypsin digestion was found to correlate 100 % in homology with the carboxyl terminal end of the human EC-SOD. Considering all these properties, we may conclude that the cytosolic SOD of *Deb. hansenii* could be an interesting alternative in biomedical applications. Future studies include cloning and regulation of the Cu/Zn-SOD gene from this particular marine yeast strain.

9. García-González A., Hernández-Saavedra N.Y., Sierra A., & Ochoa J.L. Clinical use of a Cu/Zn-SOD from a marine yeast strain of *Debaryomyces hansenii*.

Free radicals participate in a great variety of normal processes like aging, cell differentiation, energy production and xenobiotic detoxification. They also participate in some pathologic entities like ischemia-reperfusion injury and chronic arthritis. Oxygen free-radicals scavengers have been used to treat these situations with good results. As reported elsewhere, we have isolated a Cu/Zn-SOD from a marine yeast strain of *Deb. hansenii*. The purpose of this work is to evaluate the potential clinical use of this novel SOD source. Two experimental models using lab animals were designed: a) the chronic arthritis adjuvant induced on Lewis rats, and 2) the ischemia-reperfusion injury in rabbit heart. On the first model, arthritis is induced by injecting Freund's complete adjuvant in the left hind foot of the Lewis rat. SOD enzyme is injected intraperitoneally in a daily basis during specific periods of time. Arthritis is measured using both Vernier and volume displacement techniques periodically. At the end of the experiment, the rats are sacrificed and the hind foot is separated for pathologic analysis. Two different groups of animals are being used: 1) negative control group, animals on which only adjuvant is administered, and 2) group, animals injected with adjuvant and treated with SOD. The second model is carried out by surgical exposure of the heart of the rabbit and ligation of the main coronary artery for a 90 minutes period. Reperfusion initiates when ligation is removed. Evidence of heart damage is measured by cardiac enzymes determination at different time intervals, direct blood pressure and surface electrocardiogram. After 3 hours, the animals are sacrificed and the heart removed for pathologic evaluation. Two groups of animals in this study are included: 1) control animals on which ischemia-reperfusion is induced without SOD treatment, and 2) animals treated with marine yeast SOD (ischemia-reperfusion is induced and a continuous infusion of enzyme is ensured).

XV. Chemical Engineering, University of Sydney, NSW, Australia. Communicated by J.P. Barford.

The following papers have been published by our group.

1. Barford, J.P., Mwesigye, P.K., Phillips, P.J., Jayasuriya, D. & Blom, I. 1993. Further evidence of direct uptake of sucrose by *Saccharomyces cerevisiae* in batch culture. J. Gen. Appl. Microbiol. **39**:389-394.
2. Mwesigye, P.K. & Barford, J.P. 1994. Transport of sucrose by *Saccharomyces cerevisiae.* J. Ferment. Bioeng. **77**:687-690.
3. Mwesigye, P.K. & Barford, J.P. 1994. Combined fermentation and radiometric studies to elucidate the mechanism of sucrose uptake in *S. cerevisiae* I. Chem. E. Symposium Vol **137** (in press).
4. Barford, J.P., Johnston, J.H. & Mwesigye, P.K. 1994. Continuous culture study of transient behaviour of *S. cerevisiae* on sucrose and fructose. J. Ferment. Bioeng. (in press).
5. Mwesigye, P.K. & Barford, J.P. Regulation of sucrose utilisation in strains of *S. cerevisiae.* J. Ferment. Bioeng. (submitted for publication).

XVI. Department of Biology, Faculty of Medicine, Masaryk University, 66243 Brno, Czech Republic. Communicated by M. Kopecká <mkopecka@med.muni.cz>.

The following paper has recently been sent to the publisher.

1. M. Gabriel & M. Kopecká. 1994. Disruption of the actin cytoskeleton in budding yeast results in the formation of an aberrant cell wall. Microbiology (UK).

Cells of the temperature-sensitive, conditionally lethal actin mutants of *Saccharomyces cerevisiae* DBY 1693 were used to study the reflection of dysfunction on the actin cytoskeleton in the morphogenesis of the cell wall using light and electron microscopy. The cells of the mutant strain which survived at least 24 h at the restrictive temperature (37°C) were selected as model objects. These cells showed isodiametric growth. The mutant cells accumulated vesicles, probably as a consequence of chaotic secretory transport resulting from the loss of polarity. A conspicuous morphological response to the dysfunction of actin was the formation of an aberrant wall all over the surface of the isodiametrically growing cell. This wall was of loose texture with protruding glucan microfibrils, incompletely masked with amorphous matrix. It resembled the regenerating cell wall on the surface of yeast protoplasts. The

localization of wall synthesis over the whole surface of ts actin mutant cells was in accordance with the even distribution of submembraneous actin in the form of patches (similarly to regenerating protoplasts). A further interesting finding was the delocalization of finger-like invaginations of the plasma membrane from the bud region to the whole surface of the growing cell.

The following theses have been presented.

2. M. Gabriel. 1994. The protoplast state of a cell: a model for the study of karyokinesis and cytokinesis. Associate Professor Dissertation, 123 pp (in Czech).

Contents: I.Introduction. II. Protoplast state of the cell. III. Protoplasts, karyokinesis and localization of the septum. IV. Cell wall as a morphogenic factor. V. Renewal of polarity and the actin cytoskeleton. VI. Actin cytoskeleton and wall synthesis. VII. Conclusions. VIII. References. IX. Papers of the author. X. Science Citation Index of the papers of the author (138). XI. Reprints of papers of authors (1-79). XII. List of author's papers and textbooks.

3. M. Kopecká. 1994. Study of three-dimensional architecture of the yeast cell wall and of molecular mechanisms of its assembly. Associate Professor Dissertation, 96 pp (in Czech).

Contents: I. Introduction. II. The term "cell wall". III. Model objects. IV. Methods. V.A study of three-dimensional architecture of the cell wall of budding yeasts, *Saccharomyces cerevisiae.* VI. A study of three-dimensional architecture of the cell wall of the fission yeast, *Schizosaccharomyces pombe*. VII. A study of molecular mechanisms of assembly of three-dimensional wall architecture. VIII. The use of experience from the wall ultrastructural study in applied research. IX. Conclusions. X. References. XI. References of authors papers. XII. Science Citation Index (418). XIII. Reprints of papers of the author. XIV. List of author's papers and textbooks.

The following lectures have recently been delivered.

4. M. Gabriel. A role of the cytoskeleton in cytokinesis. Meeting of the Czechoslovak Biological Society, Brno, and Scientific Council of the Faculty of Medicine, Brno, October 1994.
5. M. Gabriel. A role of the actin cytoskeleton in cell wall morphogenesis in yeast. Scientific Council of the Faculty of Medicine, Masaryk University, Brno, October 1994.
6. M. Kopecká. A study of three-dimensional architecture of the yeast cell wall. Meeting of Czechoslovak Biological Society and Scientific Council of the Faculty of Medicine, Brno, November 1994.

The following is a list of presentations at symposia and congresses.

7th International Congress of Bacteriology and Applied Microbiology Division, 7th International Congress of Mycology Division. Prague, Czech Republic, July 3rd-8th, 1994.

7. A. Svoboda. Morphogenesis during protoplast regeneration in *Sporotrix schenckii.* Plenary Lecture. Abstract Book, p. 439.
8. I. Pokorná, A. Svoboda & V. Farkaš. Changes in the cell wall and cytoskeleton in cells under osmotic stress. Poster. Abstract Book, p. 338.
9. J. Šandula, M. Kopecká, Z. Kossaczka & A. Bronišová. Structural and electron microscopic studies of Candida cell wall mannans. Their role in adherence, pathogenicity and immunological activity. Poster. Abstract Book, p. 350.

Fourth European Congress of Cell Biology. Prague, Czech Republic, June 23-July 1, 1994.

10. O. Nečas, R. Janisch, I. Pokorná, M. Gabriel, M. Kopecká & A. Svoboda. Spectrin in fungi and protozoa. Poster. Cell Biol. Int. 18, 1994, p.481.
11. M. Havelková, I. Hones, K. Augsten, E. Unger & A. Svoboda. The Effect of isopropyl N-(3-chlorophenyl)-carbamate on yeast cells of *Yarrowia lipolytica.*
12. I. Pokorná & A. Svoboda. Mating behaviour of yeast cells and protoplasts. Poster. Cell Biol. Int. 18, 1994, p. 561.
13. M. Gabriel & M. Kopecká. A role for actin patches in the synthesis of wall components in yeasts. Poster. Cell Biol. Int., 18, 1994, p. 561.

XVII. Institut Universitaire de la Vigne et du Vin, Laboratoire d'Oenologie, Université de Bourgogne, 21004 Dijon. Communicated by C. Charpentier.

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

1. H. Alexandre, I. Rousseaux & C. Charpentier. 1993. Ethanol adaptation mechanisms in *Saccharomyces cerevisiae*. Biotechnol. Appl. Biochem. **20**:173-183.

Determination of the membrane lipid composition of *Saccharomyces cerevisiae* revealed an increase in the unsaturation index, qualitative and quantitative changes in sterol content and an alteration of the activity of the plasma membrane when cells were pre-adapted to ethanol. All these changes may constitute different adaptation mechanisms which allow the cell to cope with ethanol stress. The importance of the lipid environment on the plasma membrane ATPase activity is also discussed.

2. H. Alexandre, J.P. Berlot & C. Charpentier. 1994. Effect of ethanol on membrane fluidity of protoplasts from *Saccharomyces cerevisiae* and *Kloeckera apiculata* grown with or without ethanol, measured by fluorescence anisotropy. Biotechnol. Techniques **8**:295-300.

Direct measurements of membrane fluidity by fluorescence anisotropy of protoplasts from *Kloeckera apiculata* and *Saccharomyces cerevisiae*, a low and a high ethanol tolerant strain respectively, are presented. The comparison of the behaviour of the two strains grown with or without ethanol enabled us to demonstrate the existing relationship between ethanol tolerance and membrane fluidity.

3. H. Alexandre, I. Rousseaux, & C. Charpentier. 1994. Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae* and *Kloeckera apiculata.* FEMS Microbiol. Lett. (in press).

The lipid composition of a strain of each of two yeasts, *Saccharomyces cerevisiae* and *Kloeckera apiculata,* with different ethanol tolerances, was determined for cells grown with or without added ethanol. An increase in the proportion of ergosterol, unsaturated fatty acid levels and the maintenance of phospholipid biosynthesis seemed to be responsible for ethanol tolerance. The association of ethanol tolerance of yeast cells with plasma membrane fluidity, measured by fluorescence anisotropy, is discussed. We proposed that an increase in plasma membrane fluidity may be correlated with a decrease in the sterol:phospholipid and sterol:protein ratios and an increase in unsaturation index.

4. H. Alexandre & C. Charpentier. 1994. The plasma membrane ATPase of *Kloeckera apiculata*: purification, characterization and effect of ethanol on activity. World J. Microbiol. (in press).

Partially (6-fold) purified plasma membrane ATPase from a ethanol sensitive yeast *Kloeckera apiculata,* had an optimum pH of 6.0, an optimum temperature of 35°C, a K_m of 3.6 mM ATP and a V_{max} of 11 µmol Pi/min.mg protein. SDS-PAGE of the semi-purified plasma membrane showed a major band of 106,000 Da. No in vivo activation of the ATPase by glucose was observed. 4% (v/v) ethanol decreased growth rate by 50%. The ATPase was not affected by growth in the presence of 4% (v/v) ethanol. Concentrations of ethanol ≤2 % (v/v) inhibited the enzyme in vitro. The characteristics of the enzyme from *Kloeckera apiculata* did not change during growth in the presence of ethanol.

5. H. Alexandre, T. Nguyen Van Long, M. Feuillat & C. Charpentier. 1994. Contribution à l'étude des bourbes: Influence sur la fermentescibilité des moûts. Rev. Fr. Oenol. (Cahiers scientifiques) **146**:11-20.

This study emphasizes the role of insoluble materials on the ability to favour the fermentation. Several fermentations have been carried out covering a large scale of turbidity from 3 to 260 NTU which corresponds to the addition of different quantities of insoluble materials. During the course of the fermentation, analysis of "colloïds", medium chain fatty acids and acetic acid have been realized. In order to determine the nutrition value of the insoluble materials, they have been analysed to determine the different constituents. It appears that the presence of insoluble materials in the fermentation medium limits the production of caprylic acid, capric acid and acetic acid.

XVIII. École Nationale Supérieure Agronomique de Montpellier, Chaire de Microbiologie Industrielle et de Génétique des Microorganismes. Communicated by P. Galzy.

Recent publications.

1. Vivier D., Rivemale M., Reverbelj P., Ratomahenina R. and P. Galzy. 1994. Study of the growth of yeasts from feta cheese. Int. Food Microbiol. **22**:207-215.

2. Janbon G., Arnaud A., and Galzy P. 1994. Selection and study of a *Candida molischiana* mutant derepressed for ß-glucosidase production. FEMS Microbiol. Lett. (GBR) **118**:207-211.

3. Gueguen Y., Chemardin P., Arnaud A. and Galzy P. 1994. Purification and characterization of the endocellular ß-glucosidase of a new strain of *Candida entomophila* isolated from fermenting agave (*Agave* sp.) juice. Biotechnol. Appl. Biochem. (USA) **2**:185-198.

4. Lomascolo A., Dubreucq E., Perrier V., and Galzy P. 1994. Study of lipids in *Lipomyces* and *Waltomyces.* Can. J. Microbiol. **40**:724-729.

5. Briand D., Dubreucq E. and Galzy P. 1994. Enzymatic fatty esters synthesis in aqueous medium with lipase from *Candida parapsilosis* (Ashford) Langeron and Talice. Biotechnol. Lett. **16**:813-818.

6. Boutur O., Dubreucq E. and Galzy P. In press. Methyl esters production, in aqueous medium, by an enzymatic extract from *Candida deformans* (Zach) Langeron and Guerra. Biotechnol. Lett.

XIX. Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary. Communicated by A. Halász.

Our recent publications dealing with yeast research follow.

1. A. Halász. & M. Szakács-Dobozi. 1993. Proteolytic enzyme activity of *S. cerevisiae* baker's yeast and *S. carlsbergensis* brewer's yeast. Acta Alimentaria **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 baker'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 the case of *S. carlsbergensis.* Temperature optimum and heat resistance of cell wall bound proteinases were different from those of the soluble ones.

2. Halász A., Szakács-Dobozi, M., Szalma-Pfeiffer, I., & Greskovits, E. 1994. Distribution of proteolytic enzyme activity between the main protein fractions of *Saccharomyces* yeast strains. Lecture, Hungarian Food Scientists Meeting, Budapest.

Saccharomyces pastorianus-CBS 1503, *S. cerevisiae* CBS 1395 and two auxotrophic mutants of *S. cerevisiae,* CB 67 and CB 89, were investigated for the effect of glucose concentration and aeration intensity on the distribution of proteinase activities. Enzyme activities were determined on casein and azocasein substrates resp., protein fractionation was done according to Osborne. *S. pastorianus* had the highest caseinase activity when grown in fermentative conditions; increased aeration resulted in a decrease of enzyme activity. Azocaseinase activity was distributed among fractions 1, 2, 4, 5, and 6. Fractions 1 + 2 represented 40-60 % of the enzyme activity. *S. cerevisiae* showed highest the caseinase value in fermentative growth conditions. Azocaseinase activity was maximum at 1% glucose and 500 $LL^{-1}h$-1 aeration intensity. The activity was distributed among fractions 1, 2, 4, 5, and 6. Fractions 1 + 2 represented 68-100%. At high aeration intensity only fractions 1 + 2 possessed any proteinase activity. *S. cerevisiae* CB 67 was significantly different from the original strain as this yeast had haemoglobin hydrolyzing activity and azocaseinase activity was also increased. Fractions 1 + 2 contained 90-100 % of the activity. Glucose concentration influenced activity distribution between fraction 1 and 2. On both substrates enzyme activities were highest at oxidative growth conditions. *S. cerevisiae* CB 89 showed similarity to CB 67, however activity maximum was at lower aeration intensity and fractions 1 + 2 represented a somewhat smaller ratio of the enzyme activity (70-95%). Our findings showed that proteinase activity value and its distribution is different at oxidative and fermentative metabolism of the investigated yeasts.

3. Halász. A., Szakács-Dobozi, M., Bruschi, C., Szalma-Pfeiffer, I. & Greskovits E. 1994. Methionine-rich protein subfractions in yeasts. Lecture, Annual Meeting of German Food Chemists, Dresden, Sept 27-30.

Glucose-sensitive (*S. carlsbergensis, S. cerevisiae* CBS 1395, *S. cerevisiae* CB 67, *S. cerevisiae* CB 89, and polymet DNA hybrid CB 89/2) and glucose-non-sensitive yeasts *(Rhodotorula glutinis* CBS 803 and *Candida guilliermondii* CBS 812) were investigated. Yeasts were grown in batch culture at two glucose concentrations (100 g and 1000 g per litre) and two aeration intensities (200 $LL^{-1}h^{-1}$ and 1,000 $LL^{-1}h^{-1}$). After centrifugation cell wall was disrupted by ultrasonication and

protein content fractionated according to Osborne. In both glucose-non-sensitive yeasts, fractions 1 + 2 and residue were dominant for protein distribution and fraction 5 showed extremely high methionine contents (33% methionine on protein content of the fraction for *R. glutinis)*. In the case of *C. guilliermondii* fractions 2 and 6 resp. dependent on aeration intensity had similar methionine concentrations as fraction 5 (7-8 %). *S. carlsbergensis* had two methionine-rich protein subfractions 4 and 5 (8 and 17%), in *S. cerevisiae* CBS 1395 the residue showed the best values which varied with aeration intensity (3-9, 7%). In the *S. cerevisiae* mutant yeasts CB 67 and CB 89 also residue fractions where most enriched in methionine (5-11%). CB 89/2 polymet DNA hybrid yeast showed increased methionine contents in fractions 4, 5, 6 and residue at both aeration intensities.

4. Amal Mohamed, Tóth, Á. & Halász. A. 1994. Characterization of *Saccharomyces* yeast by their NIR spectra. Lecture, Colloquium of Food Scientists, Budapest, Nov. 25. 1994.

Saccharomyces pastorianus CBS 1503, *Saccharomyces cerevisiae* CB 1395 and two auxotrophic *S. cerevisiae* mutants CB 67 and CB 89 were investigated. The yeast showed morphological differences in the microscope: CB trains are round shaped whereas the CBS strain was ellipsoid. SDS-PAGE protein prints of water-salt soluble protein fractions were significantly different and varied also by pretreatment like growth phase or previous heat-shock treatment. The NIR spectra were determined from freeze-dried yeast powder. The four *Saccharomyces* strains showed significantly different spectra which were influenced by previous pretreatments. The NIR spectra enabled to detect even 10% contamination of one yeast strain in an other population.

XX. Escola Superior de Agricultura Luuz de Queiroz, Universidade de São Paulo and Fermentec s/c Ltda. R. Treze de Maio, 768 - Sala 44, Ed. Sisal Center, 13400-900 Piracicaba, S.P. Brasil. Communicated by H.V. Amorim.

The following is a summary of a recently completed research project.

1. Basso, L.C., Oliveira, A.J., & Amorim, H.V. 1994. Yeast selection for fuel ethanol from Brazilian distilleries monitored by the karyotyping technique.

In a previous work it was observed during industrial fermentation, that wild yeast strains of *Saccharomyces* dominate over the traditional starter strains (baker's yeast or others selected and even improved strains). In the present work the yeast populations from more than 20 distilleries were subjected, during the last two seasons (93/94 and 94/95), to the karyotyping analysis and around 30 *Saccharomyces* strains exhibiting dominance and persistence during industrial processes were isolated. Such strains were submitted to laboratory fermentation tests in comparison with traditional starter yeasts, and again selected for good fermentation capabilities, evaluated by means of ethanol yield, glycerol and biomass production and maintenance of high viability. The selected strains showed better fermentation performance, higher ethanol tolerance, thermotolerance, osmotolerance and better tolerance to the acidic treatment of the industrial process. Some selected strains, when used as starter yeast were able to survive the stressing conditions of the industrial process. Because of their capabilities, these selected yeasts should be an interesting material for yeast genetic improvement.

XXI. Alko Ltd., The Finnish State Alcohol Company, POB 350, SF-00101 Helsinki, Finland. Communicated by M. Korhola.

The following are abstracts of our recent publications.

1. G.I. Naumov[1], E.S. Naumova[1], C. Gaillardin[2], H. Turakainen[3] & M. Korhola. 1994. Identification of new chromosomes of *Saccharomyces bayanus* using gene probes from *S. cerevisiae.* Hereditas **120**:121-126.

[1]State Institute for Genetics and Selection of Industrial Microorganisms, 1 Dorozhnyi 1, Moscow 113545, Russia.
[2]Laboratoire de Génétique des Microorganisms INRA-CNRS, Centre de Biotechnologie Agro-Industrielles, Institut National Agronomique, F- 78850 Thiverval-Grignon, France.
[3]Department of Genetics, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki, Finland.

The *Saccharomyces cerevisiae* genes *HIS3* (chr. XV), *LYS2* (chr. II), *ARG4* (chr. VIII), *MFαI* (chr. XVI) and *MEL1* (chr. II) and the *S. pastorianus* gene *MELx* were used for identification of the homeologous chromosomes in *S. bayanus* by using pulsed-field gel electrophoresis and Southern blot hybridization. The results showed differences in hybridization intensities and mobilities between the homeologous chromosomes of the two sibling yeasts *S. cerevisiae* and *S. bayanus.* The probes *HIS3, LYS2, MEL1,* and *MFα1* identified four new homeologous chromosomes of *S. bayanus.* The *ARG4* probe confirmed the previously identified location of chr. VIII (Naumov et al. 1992b). Using the *MFα1* gene, another chromosome besides chr. XVI was identified in some *S. bayanus* strains suggesting translocation of this sequence to the *S. bayanus* chromosome that normally migrates to the position of chr. XI of *S. cerevisiae.*

2. A.E.I. Vainio. 1994. Effect of upstream sequences of the *ADH1* promoter on the expression of *Hormoconis resinae* glucoamylase P by *Saccharomyces cerevisiae.* FEMS Microbiol. Lett. **121**:229-236.

The effect of the upstream sequences of the yeast *ADH1* promoter on the expression of *Hormoconis resinae* glucoamylase P by *Saccharomyces cerevisiae* was studied. Sequence analysis of the 5'-terminal region of the promoter revealed sequence patterns resembling a transcription start point and the binding site for the regulatory protein *ADR1*. A short promoter was constructed by deleting all the promoter sequences upstream of nucleotide - 409, including the upstream activating sequence UASRpG. A medium-length promoter was constructed by deleting a fragment of 558 bp containing the putative upstream transcription start point but not the UAS. The short promoter increased the glucoamylase expression level 1.6-fold compared with the long promoter, but the beginning of secretion was delayed by about 10 h probably because of the absence of the UAS. The medium-length promoter directed expression of the glucoamylase without an initial delay, with the enzyme activity lying between the activities produced under the long and short promoters. Northern blot analysis confirmed the secretion patterns of the strains with different promoters but failed to reveal any transcripts starting at the putative upstream start point.

3. H. Turakainen[1], M. Hankaanpää, M. Korhola, & S. Aho. 1994. Characterization of *MEL* genes in the genus *Zygosaccharomyces*. Yeast **10**:733-745.

[1]Department of Genetics, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki, Finland.

We cloned and sequenced a *Zygosaccharomyces cidri MEL* gene with a view to investigating the structure and regulation of yeast *MEL* genes. The amino acid sequence deduced from the nucleotide sequence showed 78.6% and 78.2% similarity to *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* α-galactosidases, respectively. The expression of the *MEL* gene in several *Zygosaccharomyces* strains was induced by galactose. An electrophoretic karyotype of several *Zygosaccharomyces* species was obtained using contour-clamped electric field gel electrophoresis. The minimum number of chromosomes was five for *Z. cidri,* six for *Z. fermentati,* three for *Z. florentinus,* and four for *Z. microellipsoides.* The sizes of the chromosomes were generally larger than those of *S. cerevisiae,* the smallest containing approximately 0.4 megabase. The *MEL* gene was located, using the *Z. cidri MEL* gene as a probe, on the largest chromosome of the *Z. cidri* strains. In addition, a smaller chromosome (600 kb) in *Z. cidri* strain CBS4575 showed hybridization to the homologous MEL probe. This chromosome was absent in *Z. cidri* strain CBS5666. The probe hybridized to the largest chromosome of Mel+ *Z. fermentati* strains but failed to hybridize to any chromosome of Mel+ *Z. mrakii* or *Z. florentinus* strains. These results suggest the existence of a polymorphic *MEL* gene family in the yeast *Zygosaccharomyces.* The sequence has been deposited in the EMBL Data Library under Accession Number L24957.

4. H. Turakainen[1], P. Kristo & M. Korhola. 1994. Consideration of the evolution of the *Saccharomyces cerevisiae MEL* gene family on the basis of the nucleotide sequences of the genes and their flanking regions. Yeast **10**: (in press).

Analysis of the DNA sequences of new members of the *Saccharomyces cerevisiae MEL1-MEL10* gene family showed high homology between the members. The *MEL* gene family, α-galactosidase-coding sequences, have diverged into two groups; one consisting of *MEL1* and *MEL2* and the other of *MEL3-MEL10.* In two *S. cerevisiae* strains containing five or seven *MEL* genes each, all the genes are nearly identical suggesting very rapid distribution of the gene to separate chromosomes. The sequence homology and the abrupt change to sequence heterogeneity at the centromere-proximal 3' end of the *MEL* genes suggest that the distribution of the genes to new chromosomal locations has occurred partly by reciprocal recombination at solo delta sequences. We identified a new open reading frame sufficient to code for a 554 amino acid long protein of unknown function. The new open reading frame is located in 3 non-coding region of *MEL3-MEL10* genes in opposite orientation to the *MEL* genes (Accession number). Northern analysis of total RNA showed no hybridization to a homologous probe, suggesting that the gene is not expressed efficiently if at all.

XXII. Department of Bioscience and Biotechnology, University of Strathclyde, Royal College Bldg, 204 George Street, Glasgow G1 1XW, Scotland. Communicated by J.R. Johnston.

The following book has appeared recently.

1. J.R. Johnston, editor. 1993. Molecular Genetics of Yeast; A Practical Approach. Oxford University Press (The Practical Approach Series), Oxford, New York, Toronto. ISBN 019 963430 0 (Hbd), 019 963419 7 (Pbk).

XXIII. Institut Technique de la Vigne et du Vin, 14, rue Étienne Pallu, 37033 Tours Cedex, France. Communicated by C. Cuinier.

Recent publication.

1. A. Bruetschy, M. Laurent[1] & R. Jacquet.[2] 1994. Use of flow cytometry in oenology to analyse yeasts. Lett. Appl. Microbiol. **18**:343-345.
   [1]Comité Interprofessionnel du Vin de Champagne, Épernay.
   [2]Chemunex, Maisons Alfort, France.

Flow cytometry is a rapid method with many microbiological applications. This technique can be used to obtain counts of viable yeasts in 30 min, whereas a 48 h incubation is necessary with plate counts. This rapid method was tested for its suitability to analyse wine yeasts in a multicentre study in our three laboratories. The study compares measurements obtained by flow cytometry and the usual method, in order to test the reliability of the new method. The results obtained were very similar in terms of both the micro-organisms detected and the precision of measurements.

XXIV. Institute for Molecular and Cell Biology, Faculty of Biology, University of Amsterdam, BioCentrum Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands. Communicated by H. Kapteyn.

Recent publication.

1. J.C. Kapteyn, R.C. Montijn, G.J.P. Dijkpraaf & F.M. Klis. In press. Identification of ß-1,6-glucosylated cell wall protein in yeast and hyphal forms of *Candida albicans.*

Several cell wall proteins released from yeast and hyphal cells of *Candida albicans* by laminarinase reacted with an affinity-purified antiserum raised against ß-1,6-glucan. Binding of the antiserum was competitively inhibited by ß-1,6-glucan, but not by ß-1,3-glucan or isolated N-chains. Immunodetection was completely abolished when the proteins were treated with periodate. These results demonstrate that the laminarinase-released wall proteins of *C. albicans* possess an epitope consisting of ß-1,6-linked glucose residues. The yeast form of *C. albicans* contained four ß-1,6-glucosylated wall proteins, an Endo H-resistant protein of 125 kDa and three glycoproteins which became only detectable after Endo H digestion and had a molecular mass of 320, 170 and 44 kDa, respectively. As for the hyphal form, a different set of ß-1,6-glucosylated wall proteins was found cons¡sting of two Endo H-resistant glycoproteins of 125 and 80 kDa, respectively, and two glycoproteins that after Endo H digestion had a molecular mass of 320 and 38 kDa, respectively. Sodium dodecyl sulfate-extractable wall proteins and medium proteins did not react with the ß-1,6-glucan antiserum. The ß-1,6-glucan epitope could be removed by aqueous hydrofluoric acid indicating that the epitope is phosphodiester- linked to the cell wall proteins. It is speculated that the epitope forms part of a GPI-anchor and might be involved in the anchoring of mannoproteins into the cell wall.

XXV. Technology Department, Sapporo Wines Ltd., Katsunuma, Yamanashi 409-13, Japan. Communicated by M. Kishimoto.

The following articles have been published or will appear in the near future.

1. Kishimoto, M. 1994. Fermentation characteristics of hybrids between the cryophilic wine yeast *Saccharomyces bayanus* and the mesophilic wine yeast *Saccharomyces cerevisiae.* J. Ferment. Bioeng. **77**:432-435.

The cryophilic wine yeasts *Saccharomyces bayanus* YM-84 and YM-126 were used for hybridization with the mesophilic wine yeast *S. cerevisiae* OC-2. All six hybrids were stable in tetrad analysis and pulsed field gel electrophoresis, even after twenty subcultures over two years. The fermentabilities of these hybrids at a low temperature of 7°C were superior to the mesophilic wine yeast and the same as the cryophilic wine yeasts. Conversely, their fermentabilities at the intermediate temperatures of 28 and 35°C were similar to the mesophilic wine yeast. For laboratory-scale wine-making using Koshu grape juice at 1O°C, the fermentability of these hybrids was superior to the mesophilic wine yeast. They also produced higher amounts of malic acid and flavor compounds such as higher alcohols, ß-phenylethyl alcohol, isoamyl acetate and ß-phenylethyl acetate, and lower amounts of acetic acid than those of OC-2. These results suggest that the cryophilic wine yeast *S. bayanus* is useful for improving the low temperature fermentation ability of wine yeast strains.

2. Oshida, A., Kishimoto, M., Yanagida, F., Shinohara, T., & Goto, S. In press. Isolation and some properties of mutants for higher yield of ethanol from the cryophilic wine yeast *Saccharomyces bayanus.* J. Brew. Soc. Japan.

Two mutants, that were able to grow at 35°C and had high ethanol-resistance at 25°C with increased yield of ethanol at the intermediate temperatures of 25 and 30°C, were isolated from the cryophilic wine yeast *Saccharomyces bayanus* YM-84M(Arg^-), and their genetic properties were studied. Hybrids obtained from the mutants and the cryophilic wine yeast YM-84A(His^-) were unable to grow at 35°C, and showed inferior ability for ethanol-resistance and yield of ethanol at the intermediate temperatures. In tetrad analysis of the hybrids, the each properties were segregated irregularly. Phenotypes of spore-clones isolated from the hybrids suggested that the properties of growth at 35°C and increased yield of ethanol at 30°C were related with ethanol-resistance. In the laboratory-scale wine-making using Koshu grape juice, the mutants showed good fermentability at a low temperature of 1O°C and high yield of ethanol at an intermediate temperature of 25°C.

3. Kishimoto, M & Goto, S. Growth temperatures and electrophoretic karyotyping as tools for identification of *Saccharomyces bayanus* and *Saccharomyces cerevisiae.* Submitted to J. Gen. Appl. Microbiol.

Growth temperature, fermentation characteristics and electrophoretic karyotype of sixteen strains of *Saccharomyces bayanus* and nine strains of *S. cerevisiae* were examined. Growth temperatures at 1 and 2°C or 35°C, clearly distinguished these two species, and also fermentation characteristics, such as fermentation velocity at a low temperature (7°C) and ethanol yield for fermentation at an intermediate temperature (28°C), supported this distinction. Additionally, in pulsed field gel electrophoresis under the conditions for separating large DNA molecules, specific DNA bands were observed in each of the two species. From these results, it was concluded that growth temperatures and electrophoretic karyotyping were convenient tools for identification of the two species.

XXVI. Laboratoire de Biologie végétale et Biotechnologie, Faculté des Sciences et des Techniques, 2, rue de la Houssinière, Université de Nantes, F-44072 Nantes Cedex 03. Communicated by L. Simon.

The following Ph.D. thesis has been defended recently.

1. A. Versavaud. 1994. Ecological survey of the yeast microflora of the Charentes vineyard: analysis of the biodiversity and application to strain selection. Université de Nantes (France).

A study of the inter and intraspecific biodiversity of the indigenous yeast populations of the Charentes vineyard (France) has been carried out in order to select strains representative of thus area and adapted to brandy (Cognac) production. Several methods based on the genetic polymorphism were used for strains differentiation: Transverse alternating field electrophoresis karyotypes, restriction profiles of the mitochondrial DNA, Polymerase chain reaction amplification products of interspersed genomic sequences. Some recent data on the polyclonality of the spontaneous populations of *Saccharomyces cerevisiae* have been confirmed: a small number of dominant strains (representing together more than 60% of the monospecific population) is responsible for the fermentation process. They are associated to a variable but larger number of minority strains. Using rare cutting endonucleases in association with pulsed field restriction fragments separation we have been able to differentiate also, within the species, some asporogenous yeasts as *Kloeckera apiculata* or *Candida famata:* several strains were shown to develop simultaneously during the prefermentative and fermentative phases. The geographical repartition of the 70 strains of *S. cerevisiae* isolated during this work has been investigated. Only one strain was found to be widely distributed in the area: it was a dominant strain of killer type. The genetic relatedness between strains has been calculated using Euclidean distances and Jaccard index. Several of the strains collected were tested for their technological performances before industrial production. Using gas chromatography analyses, a significant effect of the strain used for fermentation on the production of aromatic compounds has been evidenced. Fermentation assays using seeding by pure or mixed strains of *S. cerevisiae* and *Kl. apiculata* have also been undertaken.

The following paper has been accepted recently.

2. L. Simon, B. Bouchet,[2] C. Chow-Vaugien[1] & D.J. Gallant.[1] In press. Pullulan elaboration and differentiation of the resting forms in *Aureobasidium pullulans*. Can. J. Microbiol.

[1]Laboratoire de Biochimie I, UFR Sciences de la Nature, Faculté des Sciences et des Techniques, Université de Nantes.
[2]Laboratoire de Technologie appliquée à la nutrition - INRA, BP 527, F 44026, Nantes Cedex 03.

In order to identify the cellular forms which are responsible for the synthesis of pullulan produced by *Aureobasidium pullulans,* we performed cytochemical and ultrastructural glucan localizations in the cellular forms of this microorganism (blastospores and resting forms). Concurrently growth conditions cell populations and pullulan production were studied. Our results are consistent with a model in which the resting forms (swollen cells and chlamydospores) might be primarily involved in this extracellular polysaccharide elaboration. At the cellular level pullulan production could be the result of 3 main stages: (i) cell wall thickening and extracellular polysaccharide synthesis by the swollen cell (ii) fibrillar arrangement of this polysaccharide into pullulan along a capsular network around the chlamydospore (iii) subcellular hydrolysis separating the capsule from the periplasmic zone and permitting consequently the solubilization of pullulan in the culture medium. A melanization process in the outer layer of the cell wall and the capsule accompanies these patterns.

XXVII. National Institutes of Health, Bldg 10 - Room 9 N 115, Bethesda Maryland 20892, U.S.A. Communicated by E. Cabib.

1. Nomenclature of genes related to chitin synthesis

A new system of nomenclature is proposed for genes related to chitin synthesis in *Saccharomyces cerevisiae.* This proposal has been endorsed by most of the investigators involved in the discovery of those genes, *i.e.* M. Breitenbach (Salzburg University, Austria), E. Cabib (National Institutes of Health, Bethesda, MD), A. Duran (University of Salamanca, Spain), P. Orlean (University of Illinois at Urbana-Champaign), P.W. Robbins (Massachusetts Institute of Technology), and S. Silverman (American Cyanamid, Princeton).

The present nomenclature is confusing. It includes *CHS1*[1] and *CHS2*[2] plus the partially overlapping *CAL*[3,4] and *CSD*[5] series. *CAL1/CSD2* has also been isolated as *D1T1O1*[6] and *KT12*.[7] It is proposed that all genes discovered by screens that relate them to chitin synthesis will be called *CHS* (for chitin synthesis, not chitin synthase) and numbered in the order of their discovery. Thus the well established *CHS1* and *CHS2* will be maintained, but *CAL1/CSD2, CAL2/CSD4* and *CAL3* will become *CHS3, CHS4,* and *CHS5,* whereas *CSD3* will be *CHS6.* Any gene of the series found in the future will have a higher number. This simple system will easily accommodate future findings. A similar system may also be used for other fungi, with the proviso that in each organism the numbers of the *CHS* genes will be in the order in which they are discovered. Therefore, there will be in general no correspondence between the function of a *CHS* gene with a certain number in one species and a *CHS* gene with the same number in another species.

1. Bulawa, C.E., Slater, M., Cabib, E., Au-Young, J., Sburlati, A., Adair, W.L., & Robbins, P.W. 1986. Cell **46**:213-225.
2. Silverman, S.J., Sburlati, A., Slater, M.L., & Cabib, E. 1988. Proc. Natl. Acad. Sci. USA **85**:4735-4739.
3. Roncero, C., Valdivieso, M.H., Ribas, J.C., & Duran, A. 1988. J. Bacteriol. **170**:1950-1954.
4. Valdivieso, M.H., Mol, P.C., Shaw, J.A., Cabib, E., & Duran, A. (1991) J. Cell Biol. **114**:101-109.
5. Bulawa, C. E. 1992. Mol. Cell. Biol. **12**:1764-1776.
6. Pammer, M., Briza, P., Ellinger, A., Schuster, T., Stucka, R., Feldmann, H. & Breitenbach, M. 1992. Yeast **8**:1089-1099.
7. Butler, A.R., Martin, V.J., White, J.H., & Stark, M.J.R. 1992. Yeast **8**:S319.

XXVIII. 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[1], B.Y. Yoo[2] & G.B. Calleja.[1,3] In press. Smashed fission yeast walls: structural discontinuities related to wall growth. Cell Biophys.

[1]Institute for Biological Sciences, National Research Council, Ottawa/ Ontario K1A 0R6.
[2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick.
[3]Diliman Institute, P-9 Dalan Roces, Area 14, 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 non-extensile 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 cross-linking 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 non-extensile (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.

XXIX. Laboratorio de Ecologia Microbiana e Taxonomia, and Laboratorio de Leveduras, Coleção de Culturas, Dept. Microbiol. Geral, Inst. Microbiol, CCS, Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, 21941, Brasil. Communicated by A.N. Hagler & L.C. Mendonça-Hagler.

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

1. Morais, P.B., C.A. Rosa, & A. N. Hagler. 1994. The use and diet partitioning by larval and adult stages of *Drosophila serido* feeding on yeast communities of the cactus *Pilosocereus arrabidae.* Antonie van Leeuwenhoek **65**: (in press).

2. Rosa, C.A., P.B. Morais, S. P. Reis, L.C. Mendonça-Hagler, & A.N. Hagler. 1994. Yeast communities associated with different plant substrates in sandy coastal plains of Southeastern Brazil. Mycol. Res. (in press).
3. Morais, P.B., C.A. Rosa, A. N. Hagler & L.C. Mendonça-Hagler. 1994. The yeast communities associated with the *Drosophila fasciola* subgoup (*repleta* group) in tropical forests. Oecologia (submitted for publication).
4. Morais, P.B., C.A. Rosa, S.A. Meyer, A.N. Hagler, & L.C. Mendonça-Hagler. 1995. *Candida amapae* sp. nov., an amino acid-requiring yeast from the Amazon fruit *Parahancornia amapa.* J. Industrial Microbiol. (submitted for publication).
5. Araujo, F., C.A. Soares, A.N. Hagler, & L.C. Mendonça-Hagler. 1995. Ascomycetous yeast communities of invertebrates in a southeast Brazilian mangrove ecosystem. Antonie van Leeuwenhoek (submitted for publication).

The following graduate theses were recently defended.

6. F.V. de Araujo. 1994. M.Sc. Comunidades de leveduras associadas a caranguejos e sedimentos no manguezal de Coroa Grande, Baia de Sepetiba, RJ. (Yeast communities associated with crabs in the Coroa Grande Mangrove, Sepetiba Bay, Rio de Janeiro), programa de Pós-Graduação em Ecologia, Inst. Biol., UFRJ.

The ascomycetous yeast communities associated with sediments, and 4 crab species were studied in the mangroves at Coroa Grande on Sepetiba Bay in Rio de Janeiro, Brazil. The ascomycetous yeast communities were made up mostly of diverse but sparse and apparently allochthonous populations. The striking exception was a prevalent population of the species *Kluyveromyces aestuarii.* It was found in all samples of the dark mud sediments and sand under vegetation of the mangrove area. *K. aestuarii* also predominated in the yeast communities of 2 detritus feeding crabs, *Sesarma rectum* and a *Uca* sp. However, it was absent from the herbivorous crab *Aratus pisonii* and omnivore *Goniopsis cruentata.* The 143 yeasts isolated were classified as 52 species of which 24 fit standard descriptions well, 19 had minor differences from species descriptions, and 9 could only be identified to the genus level.

7. C.A. Soares. 1994. M.Sc. Comunidades de leveduras associadas a bivalves e sedimentos no manguezal de Coroa Grande, Baia de Sepetiba, RJ. (Yeast communities associated with bivalves and sediment in the Corora Grande Mangrove, Sepetiba Bay, Rio de Janeiro.), programa de Pós-Graduação em Ecologia, Inst. Biol., UFRJ.

The ascomycetous yeast communities associated with sediments and 3 bivalve mollusc were studied in the mangrove at Coroa Grande on Sepetiba Bay in Rio de Janeiro, Brazil. The ascomycetous yeast communities were made up mostly of diverse but sparse and apparently allochtonous populations. *Kluyveromyces aestuarii* predominated in the yeast communities of the shipworm *Neoteredo reynei* and sediments under mangrove vegetation, but was absent in the clam *Anamalocardia brasiliana* and rare in *Tagelus plebeius* from mostly submerged more sandy sediments in front of the mangrove area. *Candida valida, Pichia membranaefaciens, Candida famata*-like, and the methylotrophic yeast *Candida boidinii* were isolated with modest but significant frequency from bivalves. *Kloeckera* spp, *Candida guilliermondii, Candida silvae* and *Geotrichum* spp. were notable in *T. plebeius* collected from near a polluted stream. The ascomycetous yeast communities of the mangrove ecosystem include many new biotypes needing better taxonomic definition.

8. P.B. Morais. 1994. D.Sc. Comunidades de leveduras associadas a *Drosophila* em ecossistemas de floresta tropical umida e restingas brasileiros (Yeast communities associated with *Drosophila* in tropical moist forests and sandy coastal plains of Brazil). Instituto de Microbiologia, UFRJ.

The yeast communities associated with Drosophila were studied in Atlantic Rain Forest, Amazon Forest, and a Restinga ecosystems. Specificity of substrate colonization by the yeasts was suggested as an important factor for niche occupation by the flies. The cactophilic yeast community was closely associated with the *Drosophila repleta* group and *Drosophila serido* was the main vector of these yeasts in necrotic tissues of the cactus *Pilosocereus arrabidae* in restingas. Cactophilic yeasts were isolated for the first time from forests in association with the *fasciola* subgroup (repleta group). These yeast communities were distinct from those of other forest inhabiting flies indicating niche separation of *fasciola* and other *Drosophila* groups in the forest. The yeast community associated with the *fasciola* subgroup suggested that these flies are the closest related to the ancestor of the *repleta* group in South America. Yeast colonization of fallen *Parahancornia amapa* fruit in the Amazon Forest was an important factor for the community structure of drosophilids that use amapa fruit as a feeding and breeding site.

The yeast succession was defined by the substrate changes during the colonization, by production of killer toxins by *Pichia kluyveri* and *Candida fructus,* and by the drosophilids visiting the fruits. *Drosophila* and yeasts formed an independent system that promoted community organization of both yeasts and vectors allowing the colonization of ephemeral substrates. *Candida amapae* sp. nov. isolated from the amapa fruit but not other fruits of the region was described.

The following communications have been presented in recent Brazilian or international meetings.

9. Hagler, A.N., F. Araujo, C.A. Soares, & L.C. Mendonça-Hagler. 1994. Yeasts associated with bivalves and crabs in a Brazilian mangrove area. Am. Soc. Microbiol. An. Meeting, p. 324.
10. Santos, E.A., R.B. Oliveira, L.C. Mendonça-Hagler, & A.N. Hagler. 1994. Yeasts associated with flowers and fruits from the semi-arid region of the Brazilian Northeast. IUMS, Prague CH, p.
11. Hagler, A.N. 1994. Biodiversity of yeasts from some Brazilian ecosystems. XVI Reuniao Anual Sobre Evolução, Sistematica e Ecologia Micromoleculares, e III Jornada de Inicação Cientifica em Biodiversidade, Inst. Biol., UFF, Niterói, RJ. p. 26.
12. Araujo, F., C.A. Soares, A.N. Hagler, & L.C. Mendonça-Hagler. 1994. Ascomycetous yeast communities of invertebrates in a southeast Brazilian mangrove ecosystem. Fifth International Mycology Congress, Vancouver, B.C., Canada p. 80.

XXX. Department of Food Science, University of Arkansas, 272 Young Avenue, Fayetteville, Arkansas 72704, U.S.A. Communicated by J.E. Morris.

The following paper has appeared recently.

1. F.R. Terrell, J.R. Morris, M.G. Johnson, E.E. Gbur, & D.J. Makus. 1993. Yeast inhibition in grape juice containing sulfur dioxide, sorbic acid, and dimethyldidicarbonate. J. Food Sci. **58**:1132-1154.

Sulfur dioxide ($S0_2$), sorbic acid (SB), dimethyldicarbonate (DMDC), and their combinations were studied for suppression of fermentation at 21°C or 31°C in grape juice inoculated with 2, 200, or 20,000 colony forming units (CFU)/mL of yeast. The other preservatives did not suppress as well as DMDC. The DMDC (0.8 mM) prevented fermentation at all inoculation levels at both temperatures, except that inoculated with 20,000 CFU/mL at 21°C. The 0.8-mM level of $S0_2$ + DMDC, and SB + DMDC prevented fermentation in samples inoculated with 2 or 200 CFU/mL at 31°C. Storage at 31°C decreased effectiveness of $S0_2$, SB, and S02 + SB but increased effectiveness of DMDC, S02+DMDC, and SB+DMDC (0.8 mM level).

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

The following Ph.D. thesis was defended recently. Prof. S.A. Meyer, Georgia State University, served as external examiner.

1. J.E. Kaden. 1994. Molecular taxonomy of the *Sporopachydermia cereana* complex. Department of Plant Sciences, University of Western Ontario, London, Ontario Canada.

The delimitation of phenotypically similar species is often problematic. Increasingly, molecular methods of taxonomy are called upon to resolve closely related taxa. DNA reassociation is a method capable of distinguishing between sibling species. This method has been used to clarify the genetic group boundaries that exist within the *Sporopachydermia cereana* species complex. The yeast genus *Sporopachydermia* currently comprises three species, *Sp. lactativora, Sp. quercuum,* and *Sp. cereana.* These yeasts are unusual ascomycetes that assimilate inositol, lack the ability to ferment sugars, and may give a positive diazonium blue B reaction. Preliminary studies indicated that *Sp. cereana* contained 4 or 5 genetically distinct species by mol% G+C and DNA reassociation experiments (Phaff, unpublished). Standard taxonomic methods, such as assimilation and resistance profiles, were not sufficient to define the apparent genetic groups. The elucidation of the genetic groups that comprise the *Sp. cereana* species complex was achieved by DNA reassociation. Six distinct genetic groups can now be identified within the *Sp. cereana* complex. Of the six groups, 3 are possible new species, 2 are probable varieties, and the sixth is *Sp. cereana sensu stricto.* The DNA reassociation data are supported by Phaff's (unpublished) preliminary results. Electrokaryotyping was investigated as a method of studying the genetic variation that exists in the species complex. Chromosome sizes and numbers were shown to vary throughout the species complex. These characters were not sufficient to clarify the genetic groups independent of the DNA reassociation method. Electrokaryotypic patterns were characteristic for some genetic groups, but variable within others. Assimilation profiles, growth characteristics, mating and killing patterns were investigated as potential taxonomic characters. Mating ability was found to be limited within the *Sp. cereana* complex. Extensive mating studies throughout the complex established that self-fertile and asexual strains are common. Definition of reproductively isolated populations was not possible for this reason. Killing experiments were conducted to investigate intra- and inter-group interactions. These experiments uncovered a unique system in which mating type and killing ability are

associated. However, neither mating nor killing results were found to correlate to the genetic boundaries indicated by the DNA reassociation data. Informative assimilation and resistance tests, growth characteristics, and electrokaryotypes are summarized for each genetic group, and provide the basis for future descriptions of the new species and varieties in this intriguing genus.

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

2. M.A. Lachance, P. Nair, & P. Lo. 1994. Mating in the heterothallic haploid yeast *Clavispora opuntiae*, with special reference to mating type imbalances in local populations. Yeast **10**:895-906.

3. H.J. Phaff, W.T. Starmer, M.A. Lachance, & P.F. Ganter. 1994. *Candida caseinolytica* sp. nov. a new species of yeast occurring in necrotic tissue of *Opuntia* and *Stenocereus* in the Southwestern United States and Baja California, Mexico. Int. J. Syst. Bacteriol. **44**:641-645.

Recent meeting

International Commission on Yeasts

ICY Commissioners in attendance at the IUMS Division of Mycology meeting in Prague, Czech Republic, July 3-8, 1994 met to discuss future ISSY meetings. Those present included James du Preez, South Africa; W.A. Scheffers, The Netherlands; Karen Øxenboll, Denmark; Maria Loureiro-Dias, Portugal; Leda Mendonça-Hagler, Brazil; Inge Russell, Canada; Graham Stewart, Canada/UK; Frank Spencer, Argentina; and Sally Meyer, USA. Presently, no meeting are planned beyond 1996. We hope to encourage interested individuals to plan ISSY for 1997, 1998 and 1999. ISY is scheduled for 2000. Anyone willing to take on the organization of an ISSY should contact Sally Meyer, Biology Department, Georgia State University, Atlanta, GA, USA.

In 1995, ISSY-17 "Yeast Growth and Differentiation: Biotechnological, Biochemical and Genetic Aspects" will be held in Edinburgh, Scotland, August 27-Sept.1. For information contact Dr. David Berry (see announcement below).

S. A. Meyer, Chair,
International Commission on Yeasts

Forthcoming meetings

Second International Congress on Vitamins and Biofactors in Life Science (ICVB), February 16-19, 1995, San Diego, California

A principal aim of this Congress is to encourage transfer of basic information concerning the chemistry, biochemistry, and function of vitamins and biofactors to health-oriented professionals and clinicians. The co-organizers, B. Babior and F.C. Stadtman, are arranging a scientific program covering the following topics: Biological oxidants and antioxidants; Lipoprotein oxidation and atherogenesis; Copper oxidases and their cofactors (PRR and TOPA); Iron responsive elements and iron-sulfur proteins; Oxygen radicals in phagocytosis; Nitric oxide; Prostaglandin D2 and sleep; Enzyme mechanisms involving radical intermediates; Vitamin B12; Vitamin D functions; Retinoic acid responsive transcription elements; Vitamin K dependent carboxylations; Vitamin C; Mammalian selenoproteins. **To receive additional information and the 2nd circular contact:**

Susan J. Buntjer, C.M.P., Conference Coordinator
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, CA 92037, USA Fax: (619)554-6310

Seventeenth International Conference on Yeast Genetics and Molecular Biology, June 10-16, 1995, Lisbon, Portugal

The second announcement and call for abstracts is now available. The deadline for submission and early registration is January 31 1995.

Scientific program: 1. Cell Growth; 2. Protein Traffic in Organelle Biogenesis; 3. Nuclear Dynamics; 4. Signal Transduction; 5. Regulation of Gene Expression I; 6. Regulation of Gene Expression II; 7. Metabolic Regulation; 8. Biotechnology; 9. Morphogenesis, Cell Wall, and Membranes. Workshops: 1. Stress-responses; 2. *Schizosaccharomyces pombe*; 3, 4. Biochemical and Genetic Analysis of Transcription; 5. Recombination and DNA Repair; 6. Yeast Genome; 7. Catabolite Repression; 8. Yeast Biotechnology; 9. Post-transcriptional Processes; 10. Replication; 11. Transport Systems; 12. Nutrient Signalling. Posters: 1. Cell Cycle; 2. Replication; 3. Meiosis,

Sporulation; 4. Recombination/Repair; 5. Regulation of Gene Expression; 6. RNA Processing; 7. Translation; 8. Organelles; 9. Cytoskeleton; 10. Signalling; 11. Protein Traffic; 12. Membranes; 13. Metabolic Regulation; 14. Yeast Biotechnology.

The conference Centre is a modern building, overlooking the river Tagus in a famous area of the city of Lisbon, in the Junqueira/Belem-Jerónimos Monastery, historical quarter. The registration fee (800 DM regular, 650 Dm students) includes participation in all scientific sessions, congress documentation, free buffet lunches and dinner, tea/coffee between sessions and official social program. Hotels of different categories (75-230 DM single) have been reserved. Lisbon has frequent flight connections with all major cities. **Contact:**

TOP-TOURS
Att.: Mr. Victor Alves
International Division
Rua Luciano Cordeiro, 116
1000 Lisboa - Portugal

Phone: 351 1 3520028
Fax: 351 1 3555231
351 1 3523227

17th ISSY. Yeast Growth and Differentiation: Biotechnological, Biochemical, and Genetic Aspects. 27 August - 1st September 1995, Heriot-Watt University, Edinburgh, Scotland

The first announcement and call for papers is now available. Morning and afternoon sessions will consist of invited lectures and offered papers to be selected from those submitted. It is anticipated that individual sessions will be dedicated to product formation, aerobic growth and anaerobic growth, dimorphism & differentiation, cell wall metabolism and diversity of the physiology of growth in yeasts. Poster sessions will also be held. **Contact:**

Prof. D.R. Berry
Department of Bioscience and Biotechnology
University of Strathclyde
204 George Street, Glasgow G1 1XW
Scotland, U.K.

Tel. 041 552 4400 ext 2092
Fax. 041 552 6524

6th International Symposium on the Microbiology of Aerial Plant Surfaces, 11-15 September 1995, Island of Bendor, Bandol, France

Since 1970 scientists from diverse disciplines have met every five years to discuss issues related to the biology and ecology of microorganisms associated with aerial surfaces of plants. These symposia have helped advance our understanding of a habitat that harbors plant pathogens as well as microorganisms beneficial for plant health. Furthermore, this tremendously complex habitat harbors microorganisms that may influence global weather, that have an impact on food technology, and that may be noxious to animals or a part of their normal intestinal flora. The Symposium in 1995 will strive to bring together researchers in the areas of microbiology, plant pathology, physiology, biochemistry, ecology, micrometeorology, microscopy, statistics, food science and genetics. It will provide a forum to explore recent research on the nature of the environment at plant surfaces, biotic and abiotic factors that influence colonization of plant surfaces by microorganisms, the response of plants to microorganisms associated with their aerial surfaces, and the impact of these microorganisms on agricultural practices and food quality. The major topics will include: the physical and chemical environment of aerial plant surfaces; interactions between microbial epiphytes and plants; interactions among microorganisms in epiphytic communities; quantification and prediction of spatial and temporal dynamics of epiphytic microorganisms; the impact of epiphytic microorganisms on agricultural practices and food quality. The Symposium will be held on the magnificent Island of Bendor, in the Mediterranean Sea just off the coast of Bandol, France. This island is a 5-minute ferry ride from the city of Bandol and is equipped with an auditorium and several hotels with multiple conference rooms and diverse recreational facilities. The site, which is readily accessible through domestic and international means of transportation, was chosen for its ambiance. It will be extremely conducive to informal discussion among participants during leisure time. The date of the conference, 11 - 15 September 1995, was chosen to avoid the high tourist season on the French Riviera while maximizing the probability of excellent weather. **For additional information please contact:**

Cindy E. Morris
INRA - Station de Pathologie Végétale
B.P. 94
84143 Montfavet Cedex, France

Phone: (33)-90-31-63-84
Fax: (33)-90-31-63-35
E-mail: <cornic@jouy.inra.fr>
Telex: INRAAVI 432.870 F

Biotecnologia Habana '95. New Opportunities in Plant, Animal, and Industrial Biotechnology. Center of Genetic Engineering and Biotechnology (CIGB), Havana. November 12-17, 1995.

Three parallel events will take place, based on the following subjects: (1) Biotechnology applied to animal production and health. (2) Biotechnology applied to plant production. (3)Biotechnology applied to industry. Registration: USD$325 (private sector), USD$250 (academic and government), USD$150 (students). **Contact:**

Organization Committee, Biotecnologia Habana '95
Apartado Postal 6162, C.P. 10600
Havana, Cuba

Tel. (53-7) 218008, 216008, 218164, 216832.
E-mail: <biot95@ingen.cigb.edu.cu>

Beijerinck Centennial Symposium. Microbial Physiology and Gene Regulation: Emerging Principles and Applications. The Hague, The Netherlands, 10-14 December 1995.

The symposium will be held at The Hague, The Netherlands, 10-14 December 1995, and will include a visit to Delft. This Beijerinck Symposium celebrates 100 years of the Delft School of Microbiology, and honours Martinus Willem Beijerinck and his successors Albert Jan Kluyver and Cornelis Bernardus van Niel. The teachings of"The Delft School" have had a lasting influence on the study of microbial biochemistry, biodiversity and biotechnology. Beijerinck and his successors each addressed basic questions in microbial physiology: How does the intact organism interact with its abiotic and biotic environment? How can fundamental principles be brought to bear on applied problems? What is the place of micro-organisms in the natural world?

Modern tools of molecular biology bring us deeper understanding of these enduring problems and allow us to formulate new principles of metabolic control and microbial gene regulation. There are new opportunities for the application of microbes to human benefit such as optimization of primary and secondary metabolite production, yield improvement, agricultural productivity, understanding of virulence mechanisms, production of enzymes and heterologous proteins, and novel pathways for biodegradation. The biochemical virtuosity of micro-organisms from extreme environments can now be harnessed in novel ways; traditionally exploited fermentation systems can be engineered to higher productivity.

The Beijerinck Centennial will bring together an international group of scientists in a Symposium that addresses cutting-edge principles of microbial physiology and gene regulation as observed in important model and production organisms. It will highlight the microbial diversity that provides the raw materials for this research, and will honour the grand tradition of the Delft School, teaching that microbes can be the basis of whole new ways of thinking in biology, and whole new commercial processes within industry.

The Beijerinck Centennial will be organized jointly by the American Society for Microbiology, the Netherlands Biotechnological Society, and the Netherlands Society for Microbiology, under the auspices of the Netherlands Foundation for Biotechnology. **For further information please contact:**

Beijerinck Centennial, Symposium Office,
W.A. Scheffers,
Kluyver Laboratory of Biotechnology,
Julianalaan 67, 2628 BC Delft, The Netherlands

Tel.: (31) 15782411
Fax: (31) 15 782355 or (31) 15133141

10th International Biotechnology Symposium, August 25-30, 1996, Sydney, Australia

In recognition of biotechnology's growth and its impact on the country, the Australian Biotechnology Association is proud to be hosting the 10th International Biotechnology Symposium in Sydney between August 25-30, 1996. The Symposium will be held right in the heart of Sydney at the Sydney Convention and Exhibition Centre, Darling Harbour. Not only will it be a showcase for Australian biotechnology but also your opportunity to come and see the industry firsthand. Professor Peter Gray is Chairman of the Organising Committee. **To join the mailing list for the Symposium, contact:**

Australian Biotechnology Association,
PO Box 4, Gardenvale Victoria 3185,
Australia.

Telephone: 61 3 596 8879
Facsimile: 61 3 596 8874

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

This symposium will be held in conjunction with the 10th INTERNATIONAL BIOTECHNOLOGY CONGRESS. The venue is the Darling Harbour Convention Centre which is a prime location in Sydney, adjacent to its magnificent harbour, bridge and Opera House. Planning of the scientific programme has commenced with proposals for mini symposia covering topics within Yeast Ecology, Yeast Systematics and Taxonomy, Yeast Culture Collections, Medically Significant Yeasts, Yeasts in the Food and Beverage Industries, Yeast Cell Envelopes, Molecular Biology of Yeasts, Yeasts and Fermentation Technology, Yeast Physiology and Biochemistry, Modern Methods for Study of Yeasts, etc. Suggestions for any other topics and suggestions for outstanding speakers in these areas would be most appreciated. Please direct these to Graham Fleet at the address below.

Strong interaction between sessions within the Yeast and Biotechnology symposia is anticipated. A First Announcement of the Symposium will be circulated early in 1995. **Contact:**

Associate Professor Graham Fleet,
Department of Food Science and Technology,
The University of New South Wales,
Sydney, New South Wales 2052,
Australia.

Telephone 61 2 385 5664
Fax: 61 2 385 5931