ISSN 0513-5222

Y E A S T

A Newsletter for Persons Interested in Yeast

Official Publication of the
International Commission on Yeasts and Yeast-like
Microorganisms of the International Union
of Microbiological Societies (IUMS)

JUNE 1989 Volume XXXVIII, Number I

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EDITORIAL

Communication technology is undergoing quite a number of changes, some of which are useful in assembling the Yeast Newsletter. Several readers have taken advantage of FAX facilities to send their communications. Since phone signals can be noisy, it is advisable to insure that FAX be used only for material that has been typewritten with large, clear characters. For smaller, dot-matrix printouts, regular mail still remains the preferred alternative. Readers are encouraged to use electronic mail (A1146@UWOCC1.BITNET), or to send a 5 1/2 inch DS-DD diskette containing material in MS-DOS compatible ASCII files. Your diskette will be returned with your next issue of the YNL.

Some overseas readers have enquired about advance payment as a means of avoiding excessive bank charges. Advance payment will be credited in dollars and not in years. I shall continue to make every effort to maintain the subscription rates as low as possible. The current rate of US$4.00 (US$8.00 airmail) will continue to apply in 1990.

Last, readers may find it useful to be made aware of recent review papers or monographs of general interest to yeast researchers, but which have not been communicated to the YNL by readers. Space allowing, I shall list such titles that come to my attention, under the heading "Titles of interest".

M.A. Lachance
Editor

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

a. Recent acquisitions

Candida ishiwadae Sugiyama & Goto: 7401 = IGC 4503, ex skins of pressed black grapes, Portugal, Dois Portos-Torres Vedras, N. van Uden

Candida mucifera Kocková-Kratochvílová & Sláviková see Stephanoascus ciferrii

Candida populi Hagler et al.: 7351 = ATCC 64933 = UCD 68-675B, T, ex exudate of Populus tremuloides USA, Alaska, Delta Junction, 7352 = UCD 68-775A, ex exudate of Betula sp. Canada, British Columbia, Liard Hot Springs, 7353 = UCD 68-791B, ex exudate of Populus trichocarpa Canada, Yukon Territory, Alcan highway, H.J. Phaff (Hagler et al., Int. J. Syst. Bacteriol. 39:97-99, 1989.)

Candida sp.: 7375 = CDC 86 041135, ex cellulitis of foot of 75-year-old woman USA, Missouri, D.G. Ahearn

Leucosporidium fellii Giménez-Jurado & van Uden 7287, T = IGC 4403, ex soil, Portugal, near Oeiras, G. Giménez-Jurado (Giménez-Jurado & van Uden, A. van Leeuwenhoek 55:133-141, 1989)

Saccharomyces cerevisiae Meyen ex Hansen: 7413 = YNN27, single-spore isolate from hybrid YNN6 x YNN34, trp1-289 ura3-52 gal2, is transformed by YRp12 at a high frequency (2000-10000 col./µg DNA), T. Stinchcomb (Stinchcomb et al., Proc. Nat. Acad. Sci. USA 77:4559-4563, 1980.)

Saccharomyces douglasii Hawthorne: 7400, Douglas' strain 279 ex spoiled mayonnaise, D.C. Hawthorne (Hawthorne, Abstracts 7th International Symposium on Yeast, Perugia 1988, p. 114)

Sporobolomyces phylladus van der Walt & Yamada: 7169, T, ex underside of leaf of Sclerocarya caffra South Africa, Transvaal, Groblersdal District, J.P. van der Walt (van der Walt et al., A.V. Leeuwenhoek 55:189-195, 1989)

Stephanoascus ciferrii M.T. Smith et al.: 7409 = CCY 29-170-1 (T of Candida mucifera Kocková-Kratochvílová & Sláviková), ex liver of frog Bufo granulosus, Brazil, Manaus, MT a, CCY (Kocková-Kratochvílová & Sláviková, J. Basic Microbiol. 28:613-618, 1988)

Trichosporon adeninovorans Middelhoven et al.: 7350, ex ensiled whole-crop chopped maize, Netherlands, Wageningen, W.J. Middelhoven; 7377, ex garden soil South Africa, Pretoria, J.P. van der Walt

Zygosaccharomyces rouxii (Boutroux) Yarrow: 7412 = IFO 1053 = NCTC 813 = NCYC 170, ex syrup containing ginger root.

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b. Publications

1. Golubev, V.I., M.T. Smith, G.A. Poot and J.L.H. Kock. 1989. Species delineation in the genus Nadsonia. Antonie van Leeuwenhoek 55:369-382.

* * *

2. Saëz, H. and L. Rodrigues de Miranda. 1988. Deux nouvelles espèces de levure, d'origine animale, isolées au parc zoologique de Paris: Cryptococcus feraegula et Candida nanaspora. Bull. Soc. Myc. Fr. 104:213-215.

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3. Weijman, A.C.M. and L. Rodrigues de Miranda. 1988. Carbohydrate patterns of Candida, Cryptococcus and Rhodotorula species. Antonie van Leeuwenhoek 54:535-543.

Within the genus Candida three distinct groups are recognized on the basis of carbohydrate patterns of intact whole cell hydrolyzates. In the first, ascomycetous, group mannose is dominant, while rhamnose, fucose and xylose are absent; this is indicative of an affinity with endomycetous families. Among the basidiomycetous representatives, two groups can be recognized. One group is usually characterized by the presence of xylose and has a low mannose content. The pattern is typical for Cryptococcales and Tremellales (e.g. Cryptococcus, Trichosporon, Bullera and Tremella). The other basidiomycetous group is characterized by the presence of fucose and/or rhamnose with significant amounts of mannose. This pattern is characteristic for Sporobolomycetaceae.

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4. Weijman, A.C.M., L. Rodrigues de Miranda and J.P. van der Walt. 1988. Redefinition of Candida Berkhout and the consequent emendation of Cryptococcus Kützing and Rhodotorula Harrison. Antonie van Leeuwenhoek 54:545-553.

On the basis of affinitive characters Candida has been restricted to anamorphs related to the Endomycetales. The excluded basidiomycetous anamorphs have been transferred to the emended genera Rhodotorula and Cryptococcus.

* * *

5. Nakase, T.[1] and T. Boekhout. 1988. Emendation of the genus Bensingtonia Ingold. J. Gen. Appl. Microbiol. 34:433-437. [1]Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama 351-01, Japan)

An emendation of the diagnosis of the ballistospore-forming yeast genus Bensingtonia Ingold is proposed to permit the inclusion of yeasts forming pigmented colonies and curved ballistospores. This genus is characterized by Q-9 as the major ubiquinone component and the lack of xylose in the cells walls. The following six new combinations are proposed: Bensingtonia intermedia (Nakase et Suzuki) Nakase et Boekhout, Bensingtonia miscanthi (Nakase et Suzuki) Nakase et Boekhout, Bensingtonia naganoensis (Nakase et Suzuki) Nakase et Boekhout, Bensingtonia subrosea (Nakase et Suzuki) Nakase et Boekhout, Bensingtonia yamatoana (Nakase, Suzuki et Itoh) Nakase et Boekhout and Bensingtonia yuccicola (Nakase et Suzuki) Nakase et Boekhout. A key to the species is given.

* * *

c. The following has been submitted:

6. Roeijmans, H.J. et al. 1989. Some name changes necessitated by the redefinition of the genus Candida.

In a recent publication Weijman et al. (1988) further amended the description of the genus Candida and removed some basidiomycetous species to the genera Rhodotorula and Cryptococcus. However they did not completely purge Candida of all its basidiomycetous species, i.e. all those which give a positive result in the diazonium blue B and urea tests, have a fine structure characteristics of basidiomycetous yeasts and whose polysaccharides contain either xylose, fucose or rhamnose. Four species were overlooked, namely: Candida buffonii, C. dulciaminis, C. lignophila, and C. huempii. Moreover, although Weijman et al. (1988) placed Apiotrichum in synonymy with Cryptococcus they failed to rename the species A. futronensis and A. nothofagi. The purpose of this short communication is to remedy these oversights.

The new combinations are:

Cryptococcus huempii (Ramirez & Gonzalez) Roeijmans et al.
Rhodotorula buffonii (Ramirez) Roeijman et al.
Rhodotorula dulciaminis (Tokuoka et al.) Roeijmans et al.
Rhodotorula lignophila (Dill et al.) Roeijmans et al.
Rhodotorula futronensis (Ramirez & Gonzalez) Roeijmans et al.
Rhodotorula nothofagi (Ramirez & Gonzalez) Roeijmans et al.

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

The strains listed have been added to the ATCC. Complete information on these strains may be obtained upon request from the Mycology and Botany Department of ATCC.

Name	ATCC No.	Depositor & Strain	Significance & Reference
Candida pintolopesii	66058	J. Artwohl	Animal pathogen (Appl. & Environ. Microbiology 54: 2371-2374, 1988)
Candida utilis	64882	J.M. Becker	Human pathogen (J. Clin. Microbiol Apr. 621-624, 1988)
Cryptococcus fuscescens	64839	W.I. Golubev, BKM-Y-2600	Type strain (J. Gen. Appl. Microbiol. 30:427, 1984)
Cryptococcus neoformans	64941	R.A. Fromtling, MY1364	Human pathogen (Mycopathologia 102:79-86, 1988)
Hanseniaspora guilliermondii	66166	J. Barcenilla, 910	Fermentation tests (Biotech. Lett. 7:229, 1985)
Hansenula polymorpha	66057	H.Y. Fang, p-5	For production of secondary alcohol dehydrogenase (J. Gen. Appl. Microbiol. 31:125-134, 1985)
Kloeckera apiculata	66165	J. Barcenilla, 1098	Fermentation tests (Biotech. Lett. 7:229, 1985)
Kluyveromyces marxianus	64884	C.P. Kurtzman, NRRL Y-1174	Ethanol production (Biotech. Lett. 8:593-596, 1986; Enzyme Microb. Technol. 9:5-15, 1987; U.S. Patent 4,687,741)
Kluyveromyces marxianus	64885	C.P. Kurtzman, NRRL Y-1175	Ethanol production (Biotech. Lett. 8:593-596, 1986)
Pachysolen tannophilus	64886-64889	C.P. Kurtzman, NRRL Y-2461, NRRL Y-2462, NRRL Y-2463, NRRL Y-6704	Ethanol production (Enzyme Microb. Technol. 9:1987)

Saccharomyces beticus	66164	J. Barcenilla, 19663	Fermentation tests (Biotech. Lett. 7:229, 1985)
Saccharomyces cerevisiae	64944-	A. Duran, CR-4, HV-25, CR-1, HV-25 HV-23	Resistant to Calcofluor white (J. Bacteriol. Apr. 1950-1954, 1988)
Saccharomyces cerevisiae	66005-66006	F. A. Loewus, G-25, tetraploid strain	Production of ascorbic acid (Plant Science 38:65-69, 1985)
Saccharomyces cerevisiae	66070-66090	Bun-ichiro Ono	Study on strain sensitive to inorganic mercury (Curr. Genet. 10:187-195, 1985)
Saccharomyces cerevisiae	66102-66104	M.E. Parish, KCSU 58	Wine production research (Am. J. Enol. Vitic. 38(1) 1987)
Saccharomyces cerevisiae	66163	J. Barcenilla, 87	Fermentation tests (Biotech. Lett. 7:229, 1985)
Saccharomyces pastorianus	66162	J. Barcenilla, 556	Fermentation tests (Biotech. Lett. 7:229, 1985)
Saccharomyces veronae	66161	J. Barcenilla, 615	Fermentation tests (Biotech. Lett. 7:229, 1985)
Torulaspora rosei	66160	J. Barcenilla, 742	Fermentation tests (Biotech. Lett. 7:229, 1985)
Zygosaccharomyces rouxii	66069	Y. Oshima, ME-3	Study on the holding stability of a yeast plasmid (Mol. Gen. Genet. 206:88-94, 1987)

* * *

III. All-Union Collection of Microorganisms, Institute for Biochemistry and Physiology of Microorganisms, Pushchino 142292, USSR. Communicated by W.I. Golubev.

The following papers have been recently published.

1. Smith, M.Th., G.A. Poot, and W.I. Golubev. 1987. Taxonomic examination of yeast genus Nadsonia Sydow. III. DNA-DNA homology studies. Abstr. Inst. Symp. "The expanding realm of yeast-like fungi", Amersfoort, The Netherlands, 3-7 August 1987, 53.

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2. Golubev, W.I. 1987. Differentiation of the imperfect ascomycetous and basidiomycetous yeast. In "Problems of identification of micromycetes and other microorganisms". Vilnius, 10-12.

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3. Weijman, A.C.M. and W.I. Golubev. 1987. Carbohydrate patterns and taxonomy of yeasts and yeast-like fungi. Studies in Mycology, N30:361-371.

* * *

4. Golubev, W.I., L.G. Churkina, and L.B. Kuznetsova. 1988. Killer phenomenon in yeast taxonomy. Abstr. VII Int. Symp. on Yeasts. Perugia, Italy, August 1-5, 1988, 124.

* * *

5. Semenova, S.A. and W.I. Golubev. 1988. Characterization and localization of pigment in ascospores of Nadsonia commutata. Mikrobiologiya 57:664-668.

* * *

6. Golubev, W.I. and V.M. Blagodatskaya. 1988. Reidentification of some strains of mycelial and yeast fungi of All-Union Collection of Microorganisms. Mykologiya i Phytopathologiya 22:374-375.

* * *

7. Golubev, W.I., A.B. Tsiomenko and L.P. Tickomirova. 1988. Plasmid-free killer strains of the yeast Sporidiobolus pararoseus. Mikrobiologiya 57:805-809.

Killer strains producing protease-resistant toxins with molecular masses of about 10 kDa have been found in the basidiomycetous yeast Sporidiobolus pararoseus. The toxins were most active at low incubation temperatures and pH 5. The killer strains were not curable by treating to high temperature and chemicals known to eliminate plasmids, and plasmids were not detected by electrophoresis. The strains sensitive to Sporidiobolus pararoseus toxins occur in the species of Sporidiobolus, Sporobolomyces, Rhodosporidium and Rhodotorula. The taxonomic significance of killer-sensitive relationships is discussed.

* * *

8. Golubev, W.I. and S.A. Semenova. 1988. The determination of taxonomic affinity of asporogenous yeasts. Mykologiya i Phytopathologiya 22:396-399.

According to the ultrastructure of cell walls the species Candida blankii, C. edax, C. hydrocarbofumarica, C. peltata, C. valdiviana and Mastigomyces philippovii have ascomycetous affinity, and the species Cryptococcus amylolenthus, Cr. humicolus, Cr. hungaricus, Cr. podzolicus, Rhodotorula acheniorum, Rh. araucariae and Rh. lactosa have basidiomycetous affinity.

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9. Golubev, W.I. 1989. Action spectrum of killer toxins produced by Rhodotorula glutinis and its taxonomic significance. Mikrobiologiya 58:99-103.

Two Rhodotorula glutinis strains were found that showed killer activity. The action spectra of their toxins were identical. These mycocins had an effect on all other strains of Rh. glutinis as well as on some species of the genera Rhodotorula, Rhodosporidium, Sporobolomyces, Sphacelotheca and Ustilago. Rh. glutinis killers had no effect on ascomycetous and basidiomycetous tremelloid yeasts. The yeasts Torulopsis fujisanensis, Candida ingeniosa, C. javanica and Bullera punicea are sensitive to these killers, which confirmed the reclassification of these species in the genera Rhodotorula and Sporobolomyces. Red yeasts are abundant on aerial plant surfaces and probably their killer systems may play an important part in biological control of smut fungi.

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10. Golubev, W.I. 1989. Catabolism of i-inositol and taxonomic value of D-glucuronate assimilation in yeasts. Mikrobiologiya 58:276-283.

Inositol oxygenase, glucuronate reductase and xylitol dehydrogenase were present in cell-free extracts of yeasts grown on i-inositol. After incubation of cells with C^{14}-inositol the main radiolabelled compounds were glucuronic acid and gulonic acid. Thus, both ascomycetous and basidiomycetous yeasts catabolize i-inositol as sole source of carbon and energy through a glucuronate-gulonate pathway. Some organisms lost the first step of this pathway but they keep the ability to utilize glucuronate. The majority (76%) of basidiomycetous yeasts are able to assimilate it while the majority (more than 90%) of ascomycetous ones are glucuronate-negative. Teleomorphs of glucuronate-positive basidiomycetous yeasts belong to Filobasidiales and supposedly to Tremellales while glucuronate-negative basidiomycetous yeasts are close to Ustilaginales. The heterogeneity of taxons with respect to ability to utilize D-glucuronate is indicative of their taxonomic heterogeneity.

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IV. Bulgarian Academy of Sciences, Institute of Molecular Biology, Sofia, Bulgaria. Communicated by P. Venkov.

Below follow the abstracts of articles from my laboratory. The papers will be submitted for publication in Yeast.

1. Waltchewa, L., D. Philipova and P. Venkov. Increased extracellular secretion in fragile mutants of Saccharomyces cerevisiae.

Evidence is presented for a generally increased extracellular secretion capability of the fragile mutants of S. cerevisiae. Proteins secreted in wild type yeasts to the periplasmic space can not be retained by the defective cell wall of the fragile cells and are released into the medium. Changes in the structure of non-mannan components of the cell wall might be the reason for the extracellular release of the periplasmic proteins.

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2. Philipova, D. and P. Venkov. Cell fusion of Saccharomyces cerevisiae fragile mutants.

Fragile mutants of S. cerevisiae are defective in the structure of the cell wall and plasma membrane. The cells lyse in hypotonic solutions, but grow exponentially when an osmotic stabilizer is included in the medium. The growing fragile cells display a general increase in the permeability of the plasma membrane. We have shown here that fragile yeast cells of the same mating type can fuse without protoplast formation and treatment with polyethyleneglycol (PEG) or electric forces. The frequency of cell fusion is 1-2 orders of magnitude lower than that obtained by protoplast fusion and can be significantly increased if cells of one partner are converted to protoplasts. Microscopic observations and genetic analysis demonstrate that the hybrids obtained are fusion products. The increased level of fusion between fragile cells is explained in terms of the existence of local changes on their surface where the cell wall is thinner (or even missing) thus allowing a direct contact of cells by means of their plasma membranes.

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V. Research Department, Labatt Brewing Company Limited, 150 Simcoe Street, London, Ontario, Canada, N6A 4M3. Communicated by T.M. Dowhanick.

The following is an abstract of a recently published paper.

1. Dowhanick, T.M., S.W. Scherer, G. Willick, I. Russell, G.G. Stewart and V.L. Seligy. 1988. Differential glucoamylase expression in Schwanniomyces castellii induced by maltose. Can. J. Microbiol. 34:262-270.

Different levels of glucoamylase expression occurred when Schwanniomyces castellii strain 1402 was shifted during growth on glucose to either glucose, maltose, or soluble starch medium. Extracellular glucoamylase activity was greatest from cells grown on maltose (22x), slightly less on soluble starch (16x), and least on glucose (1x). Glucoamylase biosynthesis was further studied by labelling of total protein in vivo with [^{35}S] methionine and immunoprobing with a polyclonal anti-glucoamylase antibody. Mature active glucoamylase is 146 kDa. Maltose cultures expressed four cellular (75,78,138, and 146 kDa) and two extracellular (78 and 146 kDa) polypeptides. Neither the 138-nor the 146-kDa products were detected in cells in the presence of glucose; the 78-kDa product is expressed at approximately 5% the level obtained from cells in maltose. The 146-kDa glucoamylase is expressed within 30 min after transfer of cells from glucose- to maltose-containing medium. This expression appears to be cell growth and concentration independent and is therefore similar to galactose-inducible enzyme expression in Saccharomyces cerevisiae.

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The following Ph.D. thesis was completed recently.

2. Dowhanick, T.M. 1988. Molecular Studies on the Induction of Amylolytic Expression in the Yeast Schwanniomyces castellii. Carleton University, Ottawa, Ontario.

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VI. Akademie der Wissenschaften der DDR, Zentralinstitut für Genetik und Kulturpflanzenforschung, Corrensstraße 3, Gatersleben 4325, DDR. Communicated by J. Hofemeister.

The following is an abstract of a recently published paper.

1. Kunze, G.,[1] M. Meixner,[1] G. Steinborn, M. Hecker,[1] R. Bode,[1] I.A. Samsonova,[1] D. Birnbaum,[1] and J. Hofemeister. 1988. Expression in yeast of a Bacillus alpha-amylase gene by the ADH1 promoter. Journal of Biotechnology 7:33-48.
[1]Sektion Biologie der Ernst-Moritz-Arndt-Universität, Grefswald, GDR.

A DNA fragment of Bacillus amyloliquefaciens encoding alpha-amylase was cloned and combined with the yeast ADH1 promoter on Escherichia coli/Saccharomyces cerevisiae shuttle vectors and transformed into S. cerevisiae laboratory strains. The Bacillus gene expression in yeast was found essentially to depend on activity of the yeast ADH1 promoter in plasmid pAAH5, which also contained the ADH1 terminator sequence. The yeast transformants were characterized as to the presence of the recombinant plasmid, identity of the cloned Bacillus DNA fragment, mitotic stability and significance of the alpha-amylase activity. By Northern blotting of total RNA isolated either from E. coli or yeast transformants, 1 or 3 transcripts, respectively, were identified by hybridization with the ClaI/BamHI Bacillus DNA fragment containing the amylase gene. Variable length of amylase gene transcripts is suggested to be due to not strictly determined transcription (probably termination) with Bacillus DNA. Alpha-amylase was determined by enzymographic techniques after isoelectric focusing of yeast cellular proteins and found to differ in its isoelectric point from alpha-amylase of B. amyloliquefaciens. Incubation of pAAH5-amy transformed yeast cells on starch media showed, after a prolonged period of time, hydrolytic activities as well as cell division, thus indicating substrate utilization.

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VII. Yeast Consulting Services, 23930 Los Codona #115, Torrance, CA 90505-5839, USA. Communicated by M. Crandall.

The following is a recent press release.

HOW TO PREVENT YEAST INFECTIONS

Torrance, Calif. -- May 12, 1989 -- A booklet on "How to Prevent Yeast Infections" is now available from Marjorie Crandall, Ph.D., Candida Specialist and Director of Yeast Consulting Services.

"How to Prevent Yeast Infections" contains new information about medical and self-care for candidiasis. This booklet explains:

how to avoid the risk factors that cause yeast infections,
what to do if some risk factors are unavoidable,
how to work in partnership with physicians,
how Candida infections are diagnosed,
what antifungal treatments are available.

The booklet describes a four step program to a yeast-free future which offers hopefulness in place of helplessness to patients trying to cope with chronic yeast infections.

About 6% of women of childbearing age and 14% of pregnant women suffer from Candida vaginitis. After an initial infection, recurrences occur in 50% of pregnant women, and about every 5 months in 22% of nonpregnant women. In addition, men, women and children can develop chronic oral, esophageal and intestinal yeast infections.

The information in "How to Prevent Yeast Infections" is based on 10 years of federally-funded research. While a UCLA Research Mycologist, Dr. Crandall studied Candida albicans, the yeast that is normally found in our bodies, but which causes infections when we have decreased immunity.

Personal experience with recurrent vaginal yeast infections for 25 years motivated Dr. Crandall to read everything she could about the causes and cures for this miserable infection. Then she switched the focus to her research from yeast genetics to studies of Candida vaginitis.

Dr. Crandall discovered from the medical literature that recurrent candidiasis is both a yeast infection and a yeast allergy. Hence, both these diseases must be treated. Dr. Crandall received her doctorate in Microbiology from Indiana University in 1968. She gives public lectures and consults with patients, physicians, attorneys, and industrial firms. She states that: "Physicians and researchers should have predicted that yeasts would be major allergens since they are found in our bodies and in our foods. Now that the yeast syndrome has been identified, health care professional should be more open-minded to these new ideas."

Copies of the booklet can be obtained by sending $4.00 to P.O. Box 11157, Torrance, CA 90510.

* * *

VIII. Institut Curie, Section de Biologie, Orsay 91405, France. Communicated by H. Fukuhara.

The following abstracts are from recent publications.

1. Fukuhara, H. 1987. The RF1 gene of the killer DNA of yeast may encode a DNA polymerase. Nucl. Acids Res. 15:10046.

The killer plasmid k1 of the yeast Kluyveromyces lactis is a linear double-stranded DNA whole 5' ends are blocked by attached proteins like some animal viruses (1,2). The genome contains four tightly packed genes of which three (RF2, 3 and 4) code for the killer toxin and the immunity determinant (3,4,5,6). Only the function of the RF1 gene remains unknown. The amino acid sequence of the RF1 product, deduced from its nucleotide sequence (4), has been compared with the gene products of other linear DNAs including animal viruses, a bacteriophage and a plant mitochondrial plasmid. We found, as shown in Fig. 1, a significant homology with all of them at the three positions characteristic of viral DNA polymerase sequences (7,11). Therefore, we suggest that (i) RF1 codes for a viral type DNA polymerase, (ii) the k1 genome does not code for its terminal protein (which may then be encoded by the companion plasmid k2 known to be necessary for the maintenance of k1), and (iii) k2 may also code for its own DNA polymerase, since the replication of k2 is independent of k1. The sequence of k2 still remains to be established.

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2. Bianchi, M.M., C. Falcone,[1] C.X. Jie, M. Wéslowski-Louvel, L. Frontali,[1] and H. Fukuhara. 1987. Transformation of the yeast Kluyveromyces lactis by new vectors derived from the 1.6 μm circular plasmid pKD1. Curr. Genet. 12:185-192. [1]Department of Cellular and Developmental Biology, University of Rome, I-00185, Italy.

The circular plasmid pKD1 (or 1.6 μm DNA) has recently been isolated from Kluyveromyces drosophilarum. This plasmid appears to have a functional organization analogous to that of the 2 μ DNA of Saccharomyces cerevisiae, although the respective nucleotide sequences show little homology. pKD1 can be transferred to Kluyveromyces lactis where it is replicated stably. Using recombinant molecules derived from pKD1, a practical transformation system has been developed for Kluyveromyces lactis, with an efficiency and stability comparable to the 2 μ-based Saccharomyces cerevisiae transformation system.

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3. Chen, X.A., M. Wésolowski-Louvel, C. Tanguy-Rougeau, M.M. Bianchi,[1] L. Fabiani,[1] M. Saliola,[1] C. Falcone,[1] L. Frontali,[1] and H. Fukuhara. 1988. A gene-cloning system for Kluyveromyces lactis and isolation of a chromosomal gene required for killer toxin production. J. Basic Microbiol. 28:211-220. [1]Department of Cellular and Developmental Biology, University of Rome, I-00185, Italy.

A transformation system derived from the circular plasmid pKD1 has been developed for Kluyveromyces lactis. The principle is essentially equivalent to that of the 2 μ/Saccharomyces cerevisiae transformation system. The main features of the system are presented. Using a pKD1-based DNA bank of K. lactis, the KEX1 gene involved in the killer system was isolated by complementation.

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4. Tanguy-Rougeau, C., M. Wésolowski-Louvel, and H. Fukuhara. 1988. The Kluyveromyces lactis KEX1 gene encodes a subtilisin-type serine proteinase. FEBS Letters 234:464-470.

KEX1 is a chromosomal gene required for the production of the killer toxin encoded by the linear DNA plasmid pGKL-1 of Kluyveromyces lactis. The nucleotide sequence of the cloned KEX1 gene has been determined. The deduced structure of the KEX1 protein, 700 amino acids long, indicated that it contained an internal domain with a striking homology to the sequences of the subtilisin-type proteinases, and a probable transmembrane domain near the carboxyl terminus. The results confirm the hypothesis that the product of the gene KEX1 of K. lactis is a proteinase involved in the processing of the toxin precursor.

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5. Chen, X.J. and H. Fukuhara. 1988. A gene fusion system using the aminoglycoside 3'-phosphotransferase gene of the kanamycin-resistance transposon Tn903: use in the yeasts Kluyveromyces lactis and Saccharomyces cerevisiae. Gene 69:181-192.

The aminoglycoside 3'-phosphotransferase type I (APHI)-coding gene of the bacterial transposon Tn903 confers resistance to kanamycin on bacteria and resistance of geneticin (G418) on many eukaryotes. We developed an APHI fusion system that can be used in the study of gene expression in these organisms, particularly in yeasts. The first 19 codons of the Km^R (APHI) gene can be deleted, and replaced by other genes in a continuous reading frame, without loss of APH activity. Examples of vector constructions are given which are adapted to the yeast Kluyveromyces lactis transformation system. Their derivatives containing the 2 μ origin of replication can also be used in Saccharomyces cerevisiae.

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6. Ragnini, A. and H. Fukuhara. 1988. Mitochondrial DNA of the yeast Kluyveromyces: guanine-cytosine rich sequence clusters. Nucleic Acids Research 16:8433-8442.

Mitochondrial DNA from the yeast Kluyveromyces marxianus var. lactis (K. lactis) is a circular molecule of 39 kilobase-pairs. A genetic and physical map was constructed. We found that this genome contained a large number of guanine-cytosine(GC)-rich sequence clusters, many of which are characterized by the presence of SacII restriction sites (CCGCGG). The primary sequence of the GC clusters often showed a palindromic structure. These GC clusters were present in several varieties of K. marxianus, but not in others. The presence of these clusters is a major feature that distinguishes K. lactis strains from those of K. marxianus var. marxianus (including K. fragilis).

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7. Wésolowski-Louvel, M., C. Tanguy-Rougeau, and H. Fukuhara. 1988. A nuclear gene required for the expression of the linear DNA-associated killer system in the yeast Kluyveromyces lactis. Yeast 4:71-81.

The killer system of Kluyveromyces lactis is associated with two linear DNA plasmids, pGKL1 and pGKL2. The killer toxin and the immunity determinant are coded for by pGKL1. Mutations which block the expression of the killer character have been isolated. These mutants reside in a single chromosomal gene which we have named KEX1. The KEX1 gene of K. lactis has been cloned by complementation of kex1 mutations by using a recombinant plasmid pool containing the entire Kluyveromyces lactis genome, on a multicopy plasmid KEp6, which contains the Saccharomyces cerevisiae URA3 gene as a marker. Genetic analyses of strains carrying a disrupted kex1 allele demonstrated that the cloned DNA corresponded to the KEX1 gene. The cloned KEX1 gene of K. lactis has low but significant sequence homology with the KEX2 gene of Saccharomyces cerevisiae. In vivo complementation of the kex1 mutation of K. lactis by the KEX2 gene of S. cerevisiae, and complementation of the kex2 mutation of S. cerevisiae by the KEX1 gene of K. lactis, demonstrated that KEX1 of K. lactis is functionally related to the KEX2 gene of S. cerevisiae. K. lactis diploids homozygous for kex1 are deficient for sporulation.

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8. Sor, F. and H. Fukuhara. 1989. Analysis of Chromosomal DNA patterns of the genus Kluyveromyces. Yeast 5:1-10.

Using an improved procedure of pulsed field gel electrophoresis, yeast chromosomes were separated over a wide range of molecular size (250-4000 kbp) on single gels. The chromosomal DNA patterns of all the species belonging to the genus Kluyveromyces were examined. Within the species K. marxianus, the varieties lactis, drosophilarum and vanudenii showed closely related patterns; very different from them, the varieties bulgaricus and marxianus were related to each other, forming a distinct group; the strains commonly called 'K. lactis' and 'K. fragilis were unambiguously different from each other in chromosome patterns. These differences were correlated with the presence of characteristic repetitive sequence elements in the mitochondrial DNA of the former group and not in the latter. Analysis of Candida macedoniensis, which had been considered to be an anamorph of K. marxianus var. marxianus, showed that these two yeast species were indeed similar in chromosome patterns and in mitochondrial DNA restriction patterns.

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IX. Department of Genetics, University of California, Davis, California 9561. Communicated by A. Bakalinsky.

The following is the abstract from my Ph.D. dissertation entitled: Conversion of Wine Yeast Strains of Saccharomyces cerevisiae to Heterothallism and Determination of their Chromosomal Constitution. Correspondence should be addressed to: Department of Food Science and Technology, Oregon State University, Corvallis, Or 97331, USA.

The first chapter of this thesis describes a general method to convert homothallic wine yeast strains of S. cerevisiae to heterothallism which is applicable to strains that sporulate poorly or produce an abundance of inviable and few mating-competent spores. The heterothallic (ho) allele was introduced into the wine strains by hybridization and the resultant hybrids sporulated and heterothallic segregants of the proper genotype isolated for use in successive backcrosses. A helpful prerequisite to the introduction of ho was a genetic purification of the parental strains based on repeated cycles of sporulation, ascus dissection and clonal selection. A positive selection to isolate laboratory x wine strain hybrids requiring no prior genetic alteration of the industrial strain coupled with a partial selection to reduce the number of spore progeny needed to be screened in order to detect heterothallic segregants made the procedure valuable for genetically intractable strains. Trial grape juice fermentation indicated that the introduction of ho had no deleterious effect on fermentation behavior.

The second chapter describes a genetic determination of the chromosomal constitution of three wine strains and one derivative strain. The procedure is based on an analysis of segregation frequencies for input markers among random spore progeny of wine x laboratory strain hybrids. The multiply-auxotrophic haploid tester strains constructed for use in study were also erythromycin-resistant allowing hybrids to be selected in crosses with unaltered wildtype industrial strains. Results of the analysis indicate that UCD Enology 522 (Montrachet) is diploid and possibly trisomic for chromosome VII; 522X is diploid; UCD Enology 505 (California Champagne) is disomic for chromosome XVI, trisomic for chromosomes I, II, III, VI, VIII, IX, X, XII, XV, tetrasomic for chromosomes IV, XI, XIII, XIV and either trisomic or tetrasomic for chromosomes V and VII; and that UCD Enology 595 (Pasteur Champagne) is disomic for chromosomes I, II, III, IX, XVI, trisomic for chromosomes IV, VI, X, XII, XIV, XV, tetrasomic for chromosomes V, VIII, XI, XIII and either disomic or tetrasomic for chromosome VII.

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X. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Communicated by W.M. Ingledew.

The following papers are in press or have been published since the last issue of the Yeast Newsletter.

1. Munoz, E. and W.M. Ingledew. 1989. An additional explanation for the promotion of more rapid, complete fermentation by yeast hulls. Am. J. Enol. Vitic. 40:61-64.

Fermentations were carried out using three different yeast hull preparations at 1 g/L concentration in a nitrogen-deficient Chardonnay must under limited oxygen supply. The yeast hulls enhanced fermentation rate and stimulated the growth of more yeast cells and cell mass when compared with unsupplemented controls. Ergosterol and Tween 80 (a source of oleic acid) supplements in musts had the same enhancing effects as the hulls. Yeast hulls were analyzed for their usable nitrogen, total sterol, and fatty acid contents. Their actions as fermentation promoters are postulated to be due to their role as providers of oxygen substitutes (sterols and unsaturated fatty acids), as well as through the previously reported adsorption of medium chain length fatty acids.

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2. Munoz, E. and W.M. Ingledew. 1989. The effects of yeast hulls on stuck and sluggish wine fermentations: importance of the lipid component. Appl. Envir. Microbiol. 55(6):in press.

The effect of yeast hulls (yeast ghosts) on sluggish or stuck white wine fermentations was studied. The enhancing effect on yeast growth and fermentation rate displayed by the hulls was shown to be caused by the lipid component. Unsaturated fatty acids and sterols were incorporated into the yeast from lipid extracts of yeast hulls during fermentation carried out under oxygen limited conditions. Adsorption of toxic medium chain-length fatty acid (decanoic acid) onto the yeast hulls took place through a dialysis membrane. However, when the hulls were placed inside a dialysis bag, the increase in yeast growth and fermentation rate seen when freely suspended hulls were used did not occur. Accordingly, the effect of yeast hulls in preventing stuck fermentations cannot be attributed to the adsorption and consequent removal of medium chain length fatty acids from the juice.

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XI. Allied Breweries Limited, 107 Station Street, Burton-on-Trent DE14 1BZ, England. Communicated by R.G. Anderson.

The following is an abstract of a paper recently accepted for publication.

1. Anderson, R.G. 1989. Yeast and the Victorian Brewers: incidents and personalities in the search for the true ferment. J. Inst. Brew. (September/October).

Events in the elucidation of the role of yeast in fermentation up to the end of the nineteenth century are described. It is concluded that, contrary to the popular view, the more enlightened brewers had deduced the true nature of yeast prior to the time of Pasteur and were in advance of the dogma of the chemical establishment as represented by Liebig. The activities of scientists of the Victorian period employed in the Burton-on-Trent breweries are placed within this context.

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XII. Parc Zoologique du Muséum, Laboratorie d'Ethologie et Conservation des Espèces Animales, 53, avenue de Staint-Maurice, 75012 Paris, France. Communicated by H. Saëz.

The following are recent publications.

1. Saëz, H. et L. Rodrigues de Miranda. 1988. Deux nouvelles espèces de levure, d'origine animale, isolées au parc zoologique de Paris: Cryptococcus feraegula et Candida nanaspora. Bull. Soc. Myc. Fr. 104:213-215.

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2. Saëz, H. 1987. Distinction biomorphologique de Trichosporon cutaneum (de Beurmann, Gougerot et Vaucher) OTA 1909 et Trichosporon lutetiae Saëz 1977. Feuillets de biologie 28:56-57.

The isolation of 5 new strains of T. lutetiae, previously represented only by the type culture, allows a better separation of that yeast from T. cutaneum. The two Trichosporon species diverge principally by the assimilation of galactose and lactose (positive in T. cutaneum). The maximum growth temperature, which never exceeds 35°C in T. lutetiae, the production of starch, which is always positive in T. lutetiae and cycloheximide resistance, which is variable in T. cutaneum.

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XIII. Institute of Physiological Chemistry, Ruhr-University, Postfach 10 2148 D-463 Bochum, FRG. Communicated by W. Duntze.

The following is the abstract of a paper which was recently submitted for publication.

1. Steden, M., R. Betz, and W. Duntze. 1989. Isolation and characterization of Saccharomyces cerevisiae mutants supersensitive to G1 arrest by the mating hormone a factor.

Ten independent mutants which are supersensitive (ssl^-) to G1 arrest by the mating hormone a factor were isolated by screening mutagenized Saccharomyces cerevisiae MATα cells on solid medium for increased growth inhibition with a-factor. These mutants carried lesions in two complementation groups, ssl1 and ssl2. Mutations at the ssl1 locus were mating-type specific: Matα ssl1 cells were supersensitive to a-factor but MATa ssl1 were not supersensitive to α-factor. In contrast mutations at the ssl1 locus conferred supersensitivity to the mating hormone of the opposite mating type on both MATα and MATa cells. The α-cell specific capacity to inactivate externally added a-factor was shown to be lacking in ssl1 mutants but only slightly reduced in ssl2 cells. Complementation analysis showed that ssl2 and ssl2, a mutation originally defined as conferring supersensitivity to α-factor to MATa cells, are lesions in the same gene. The ssl1 gene was mapped 30.5 centimorgans distal to ilv5 on chromosome XII.

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XIV. Laboratory of Microbiology, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal. Communicated by N. van Uden.

The yeast culture collection of the laboratory is participating since 1987 as the "Portuguese Yeast Culture Collection" in project MINE (Microbial Information Network Europe) of the Commission of the European Community. Its ca. 1400 strains belonging to ca. 300 species are being reidentified. The results are computerized using software developed by the CBS in Baarn and implemented in Oeiras by Dr. David Yarrow of its Yeast Division in Delft. At a later date the strain data will be uploaded to the MINE central computer. A printed catalogue is in preparation.

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The following papers were published recently or accepted for publication.

1. Lucas, C. and N. van Uden. Interconversion and glucose-induced inactivation of glucose transport systems in Candida shehatae. J. Basic Microbiol. 28:639-643.

During starvation (derepression) glucose-grown cells of Candida shehatae IGC 3607 displayed total interconversion of facilitated diffusion of glucose into a glucose-proton symport, dependent on de novo protein synthesis (proteosynthetic interconversion). The reverse process, inactivation of the proton symport induced by glucose or 2-deoxyglucose, was not accompanied by reemergence of the facilitated diffusion function. The inactivation process had a rapid initial and a slow second phase. The rapid inactivation depended on the external sugar concentration and was reversible while the subsequent slow inactivation was irreversible and independent of the external concentration of the signalling sugar. Interaction of the latter with a surface receptor was indicated by the range of sugar concentrations that affected rapid inactivation.

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2. Loureiro-Dias, M.C. and H. Santos. 1989. Effects of 2-deoxyglucose on Saccharomyces cerevisiae as observed by in vivo ^{31}P-NMR. FEMS Microbiol. Lett. 57:25-28.

Saccharomyces cerevisiae cells were treated with 2-deoxyglucose (1 mM) and the effects induced in the levels of phosphorus compounds and in the internal pH were monitored using ^{31}P-NMR. Upon incubation with 2-deoxyglucose a strong decrease in the polyphosphate level was observed and the cytoplasmic pH decreased by about 0.4 units. This shows that 2-deoxyglucose strongly interferes with the cell conditions and consequently, the results of experiments in which 2-deoxyglucose was used to obtain deenergized cells should be carefully reanalysed.

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3. Peinado, J.M., P.J. Cameira-dos-Santos and M.C. Loureiro-Dias. 1989. Regulation of glucose transport in Candida utilis. J. Gen. Microbiol. 135:195-201.

The transport systems for glucose present in Candida utilis cells, growing in batch and continuous cultures on several carbon sources, have been studied. Two different systems were found: a proton symport and a facilitated diffusion system. The high-affinity symport (K_m for glucose about 15 μM) transported one proton per mole of glucose and was partially constitutive, appearing in cells grown on gluconeogenic substrates such as lactate, ethanol and glycerol. It was also induced by glucose concentrations up to 0.7 mM and repressed by higher ones. The level of repression depended on the external glucose concentration at which cells had grown in a way similar to that shown by the maltose-uptake, so both systems seem to be under a common glucose control. Initial uptake by facilitated diffusion, the only transport system present in cells growing at glucose concentrations higher than 10 mM, showed a complex kinetic dependence on the extracellular glucose concentration. This could be explained either by the presence of at least two different systems simultaneously active, one with a K_m of about 1 M, or by the allosteric or hysteretic behaviour of a single carrier whose apparent K_m would oscillate between 2 and 70 mM.

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4. Sampaio, J.P. and I. Spencer-Martins. 1989. Adaptive growth at high temperatures of the lactose-fermenting yeast Kluyveromyces marxianus var. marxianus. J. Basic Microbiol. 29:61-64.

The temperature profile of growth and death rates of a strain of Kluyveromyces marxianus var. marxianus was found to be essentially dissociative, with an optimum temperature for growth at around 38°C and sustained exponential growth up to 43-44°C. At temperatures near the maximum temperature for growth a second growth period was observed. This did not correspond to the second branch in the Arrhenius plots characteristic of associative profiles, since growth was not balanced and after a while the growth rate increased to values similar to those of the first period. The biomass yield on lactose was not dependent on the growth temperature, a behaviour typical of yeast strains with dissociative temperature profiles.

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5. Giménez-Jurado, G. and N. van Uden. 1989. Leucosporidium fellii sp. nov. a basidiomycetous yeast that degrades L(+)-tartaric acid. Antonie van Leeuwenhoek 55:133-141.

A new species of basidiomycetous yeast Leucosporidium fellii was isolated from soil in Portugal on a selective L(+)-tartaric acid medium. This yeast is self-sporulating but forms dikaryotic hyphae with clamp connections and is presumably homothallic. It differs from the type strain of Leucosporidium scottii in its life cycle, assimilation pattern and guanine-cytosine content and from the other described Leucosporidium species by additional characteristics. DNA-DNA reassociation between the type strains of L. scottii and L. fellii was less than 20%.

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6. Pinto, I., H. Cardoso, C. Leao and N. van Uden. 1989. High enthalpy and low enthalpy death in Saccharomyces cerevisiae induced by acetic acid. Biotechnol. Bioeng. 33:000 (in the press).

Acetic acid at concentrations as may occur during vinification and other alcoholic yeast fermentations induced death of glucose-grown cell populations of Saccharomyces cerevisiae IGC 4072 at temperatures at which thermal death was not detectable. The Arrhenius plots of specific death rates with various concentrations of acetic acid (0-2%, w/v) pH 3.3 were linear and could be decomposed into two distinct families of parallel straight lines, indicating that acetic acid induced two types of death: 1) High enthalpy death (HED) predominated at low acetic acid concentrations (<0.5%, w/v) and higher temperatures; its enthalpy of activation ($\Delta H^{\ddagger}$) approached that of thermal death (12.4 x 10^4 cal/mol); 2) Low enthalpy death (LED) predominated at higher acetic acid concentrations and lower temperatures with H of 3.9 x 10^4 cal/mol. While the $\Delta H^{\ddagger}$ values for HED induced by acetic acid were similar with those reported earlier for HED induced by other fermentation endproducts, the values for the entropy coefficients were different: 127-168 entropy units $mol^{-1}L$ for acetic acid as compared with 3.6-5.1 entropy units $mol^{-1}L$ for ethanol, which agreed with experimental results indicating that acetic acid is over 30-times more toxic than ethanol with respect to yeast cell viability at high process temperatures.

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7. Corte-Real, M., C. Leao and N. van Uden. Transport of L(-) malic acid and other dicarboxylic acids in the yeast Candida sphaerica. Appl. Microbiol. Biotechnol. (accepted)

DL-malic acid grown cells of Candida sphaerica (anamorph of Kluyveromyces marxianus) formed a saturable transport system that mediated accumulative transport of L(-) malic acid with the following kinetic parameters of pH 5.0: V_{max} 0.44 nmoles L(-)malate.s^{-1}.mg^{-1} dry weight; K_s 0.1 mM L(-)malate. Initial uptake of the acid was accompanied by disappearance of extracellular protons, the rates of which followed Michaelis-Menten kinetics as a function of the acid concentration. Variation with extracellular pH of the K_s values, calculated either as the concentrations of anions or of undissociated acid, pointed to anions as the transported form. Furthermore, accumulated free acid suffered rapid efflux after the addition of the protonophore CCCP. These results suggested that the transport system was a dicarboxylate-proton symporter. The system was inducible and was subject to glucose repression. Succinic, fumaric, α-ketoglutaric, oxalacetic and D-malic acid, but not maleic, malonic, oxalic nor L(+)-tartaric acid, apparently used the same transport system since they acted as competitive inhibitors of L(-)malic acid transport and induced proton movements that followed Michaelis-Menten kinetics. Experiments with glucose-repressed cells showed that undissociated dicarboxylic acid (measured with labelled succinic acid) entered the cells slowly by simple diffusion. The permeability of the cells for undissociated acid increased exponentially with pH, the diffusion constant increasing 100-fold between pH 3.5 and 6.0.

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XV. Department of Morphology, Institute of Medical Science, The Jikei University School of Medicine, Nishi-shinbashi, Minato-ku, Tokyo 105, Japan. Communicated by M. Yamaguchi.

The following papers have been published.

1. Yamaguchi, M., T. Hirano, K., Sugahara, M. Mizokami, M. Araki and K. Matsubara. 1988. Electron microscopy of hepatitis B virus core antigen expressing yeast cells by freeze-substitution fixation. Eur. J. Cell Biol. 47:138-143.

We have used the freeze-substitution fixation technique for electron microscopy of yeast cells that express the hepatitis B virus core antigen (HBcAg) following transformation with the clones gene. Abundant spherical particles were found within the transformed cells. These particles had a uniform size and shape, measured about 21 nm in diameter, had electron-lucent centers, and consisted of many subunits. They were localized in both the cytoplasm and the nucleus. None of these particles was found in the cells of the parent strain. Comparison of the HBcAg particles isolated from the yeast cells and the particles within the yeast cells demonstrated that the 21 nm particles were in fact ultrastructurally superimposable on HBcAg. Thus, the HBcAg particles within the yeast cells were similar to the HBcAg particles in human liver tissues infected with hepatitis B virus, not only in their size and appearance, but also in their intracellular localization. These results suggest that the yeast cell has the same machinery for synthesis and intracellular translocation of the HBcAg polypeptides as the human cell.

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2. Yamaguchi, M., T. Hirano, H. Hirokawa, K. Sugahara, H. Mizokami, and K. Matsubara. 1988. Cryo-electron microscopy of hepatitis B virus core particles produced by transformed yeast: comparison with negative staining and ultrathin sectioning. J. Electron Microsc. 37:337-341.

Hepatitis B virus core particles (core particles) produced by the transformed yeast cell were examined by cryo-electron microscopy, and the cryo-micrographs were compared with negative staining and ultrathin sectioning after freeze-substitution. The core particles by cryo-electron microscopy appeared spherical either an angular periphery suggestive of pentagonal and hexagonal profiles, and had electron-lucent centers. Their mean diameter was 28.0 nm with standard deviation of 1.9 nm. The mean diameter by negative staining was 31.3 nm, suggesting that the core particles were flattened by negative staining. By ultrathin sectioning the apparent diameter was 21.1 nm, suggesting that about 25% shrinkage occurred even in the freeze-substitution method was employed.

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XVI. Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Communicated by J.D. Cunningham.

The following is an abstract from a recent publication.

1. Bicho. P.A., Cunningham, J.D. and H. Lee. 1989. Differential fructose effect in Pachysolen tannophilus and Pichia stipitis. FEMS Microbiology Letters 57:323-328.

The yeasts Pachysolen tannophilus and Pichia stipitis differed in their ability to utilize D-xylose in the presence of D-fructose. When P. tannophilus was grown aerobically in a fructose-xylose mixture, the ketohexose was utilized preferentially over the pentose. However, in P. stipitis cultures, the converse was observed. The effect was associated with the ability of D-fructose to repress the induction of xylose reductase and xylitol dehydrogenase activities in P. tannophilus but not in P. stipitis. Both yeasts grew on D-fructose and fermented it to ethanol when it was supplied as the sole carbon source. The results suggest that there may exist some fundamental difference in the regulation of D-fructose metabolism between P. tannophilus and P. stipitis.

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XVII. Département de Biologie Végétale, Université Lyon 1, Bât. 405, 2e étage, 43, boulevard du 11 novembre 1918, F-69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

a. S. Poncet and R. Montrocher.

A detailed biochemical and physiological study of 12 strains of yeasts available commercially for winemaking has allowed the selection of 14 tests required to identify those yeasts within 48 hours. These tests have been applied to 16 randomly selected wild strains of Saccharomyces cerevisiae. Every one but two of the 28 strains studied had a unique numerical profile. The genetic or immunological techniques currently utilized are usually expensive or tedious, and consequently this set of simple tests is advantageous for the rapid characterization of such strains. (G. Martins, Diplôme d'Assistant de Recherche)

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b. Late in 1988, G. Billon-Grand has defended the doctoral thesis: "Contribution à la taxonomie et à la phylogénie des levures. Apport du critère coenzyme Q." M.Th. Smith served as external examiner.

Biosystematic data have been investigated and supplemented for many anascosporogenous and ascosporogenous yeast species. These included intracellular oxidases, vitamin requirements, GC contents, and DNA/DNA hybridization. The taxonomy of the very heterogeneous genus Pichia in particular was investigated. Furthermore, the generic value of nitrate and nitrite assimilation criteria, according to Wicherham, has been examined. A colorimetric method was devised to determine the presence and the enzymatic activity of nitrate and nitrite oxidases. Minor and major coenzymes Q have been analysed by mean of HPLC in 226 species belonging to 28 genera (quantitative and qualitative determination of coenzyme Q systems). The taxonomic and phylogenetic importance of minor coenzymes Q has been clarified.

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c. Publications

1. Billon-Grand, G. 1989. A new ascosporogenous yeast genus: Yamadazyma gen. nov. Mycotaxon 35:201-204.

The 16 species of the genus Yamadazyma are characterized with a major coenzyme Q9. It is the reason why they have been removed from the genus Pichia.

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2. Fiol, J.B. and M.L. Claisse. Spectrophotometric analysis of yeasts: cytochrome spectra of some Q7 Pichia. In preparation.

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3. Fiol, J.B., M.L. Claisse and G. Billon-Grand. Spectrophotometric analysis of yeasts: cytochrome spectra of some Yamadazyma (ex Q9 Pichia). In preparation.

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4. Fiol, J.B., M.L. Claisse and G. Billon-Grand. Spectrophotometric analysis of yeast: cytochrome spectra of some Hansenula. In preparation.

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5. Fiol, J.B., M.L. Claisse and G. Billon-Grand. Spectrophotometric analysis of yeasts: cytochrome spectra of some Williopsis. In preparation.

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6. Billon-Grand, G. Influence on minor peaks of coenzyme Q of the glucose concentration in the culture medium, of the stage of the growth cycle and of the duration of the coenzyme Q extraction; required conditions for the minor coenzyme Q determination. Submitted to J. Gen. Appl. Microbiol.

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XVIII. 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.

New appointment: Dr. J. P. van der Walt, as Honorary Professor and Research Associate. This appointment will significantly strengthen the yeast taxonomy group of the department.

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The following papers have recently appeared or are in press.

Physiology and fermentation

1. Du Preez, J.C., B. van Driessel and B.A. Prior. 1988. The relation between redox potential and D-xylose fermentation by Candida shehatae and Pichia stipitis. Biotechnol. Lett. 10:901-906.

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2. Du Preez, J.C., B. van Driessel and B.A. Prior. 1989. D-xylose fermentation by Candida shehatae and Pichia stipitis at low dissolved oxygen levels in fed-batch cultures. Biotechnol. Lett. 11:131-136.

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3. Du Preez, J.C., B. van Driessel and B.A. Prior. 1989. Ethanol tolerance of Pichia stipitis and Candida shehatae strains in fed-batch cultures at controlled low dissolved oxygen levels. Appl. Microbiol. Biotechnol. 30:53-58.

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4. Holder, N.H.M., S.G. Kilian and J.C. du Preez. Yeast biomass from bagasse hydrolysates. Biol. Wastes (in press).

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5. Prior, B.A., S.G. Kilian and J.C. du Preez. 1989. Fermentation of D-xylose by the yeasts Candida shehatae and Pichia stipitis. Prospects and problems. Process Biochem. 24(1):21-32.

Candida shehatae and Pichia stipitis ferment D-xylose to ethanol more efficiently than other yeasts previously described. The efficiency of fermentation is related to nutritional factors, temperature, pH, concentrations of substrate and product, presence of other sugars, oxygen supply, and toxic factors present in hemicellulose hydrolysates. Ethanol yields approaching the theoretical maximum can be attained by the ethanol concentrations and rates of ethanol production are considerably lower than those observed in commercial ethanol fermentations using glucose-based substrates. The physiological mechanisms underlying these characteristics are also discussed.

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6. du Preez, J.C., B. van Driessel and B.A. Prior. Effect of aerobiosis on fermentation and key enzyme levels during growth of Pichia stipitis, Candida shehatae and Candida tenuis on D-xylose. Arch. Microbiol. (in press)

The relationship between the degree of aerobiosis, xylitol production and the initial two key enzymes of D-xylose metabolism were investigated in the yeasts Pichia stipitis, Candida shehatae and C. tenuis. Anoxic conditions severely curtailed growth and retarded ethanol productivity. This, together with the inverse relationship between xylitol accumulation and aeration level, suggested a degree of redox imbalance. The ratios of NADH- to NADPH-linked xylose reductase were similar in all three yeasts and essentially independent of the degree of aerobiosis, and thus did not correlate with their differing capacities for ethanol production, xylitol accumulation or growth under the different conditions of aerobiosis. Under anoxic conditions the enzyme activity of Pichia stipitis decreased significantly, which possibly contributed to its weaker anoxic fermentation of xylose compared to C. shehatae.

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7. van Zyl, C., B.A. Prior and J.C. du Preez. Acetic acid inhibition of D-xylose fermentation by Pichia stipitis. Enzyme Microb. Technol. (accepted)

Fermentation of D-xylose by Pichia stipitis was inhibited by acetic acid and the degree of inhibition depended on the acetic acid concentration, the availability of oxygen and the pH. A 50% inhibition of the volumetric rate of ethanol production occurred at acetic acid concentrations of 0.8 and 13.8 gl^{-1} at pH 5.1 and 6.5, respectively, under anaerobic conditions. No acetic acid was utilised in the absence of oxygen. Under oxygen-limited conditions at pH 6.5 an acid hydrolysate of sugar can bagasse containing (gl^{-1}) D-xylose (40.9), D-glucose (3.1), L-arabinose (4.5) and acetic acid (9.0) was fermented to ethanol at a rate of 0.15 $g(l.h)^{-1}$ and an ethanol yield of 0.27 $g.g^{-1}$ was obtained. When the hydrolysate was treated with an anion exchange resin, 84% of the acetic acid was removed and the subsequent fermentation resulted in a rate of ethanol production (0.56 $g(l.h)^{-1}$) and an ethanol yield (0.37 $g.g^{-1}$ sugar) similar to that obtained in a xylose-arabinose-glucose medium lacking acetic acid.

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Taxonomy

8. Miller, M., J.L.F. Kock and P.J. Botes. The significance of long-chain fatty acid compositions and other phenotypic characteristics in the yeast Pichia Hansen emend. Kurtzman, Syst. Appl. Microbiol. (accepted)

The cellular long-chain fatty acid compositions of eighty three species representing the genus Pichia Hansen emend. Kurtzman were analysed by gas-liquid chromatography. These results were compared with other criteria (viz. carbon source utilization patterns, coenzyme Q systems and G+C contents) in order to determine relationships between these species. It was found that yeasts characterized by similar cellular long-chain fatty acid compositions, do not necessarily have similar coenzyme Q systems, carbon source utilization patterns and G+C values. In the case of varieties of a species and species considered to be conspecific, similar phenotypic results were obtained. In some instances, differences in cellular long-chain fatty acid compositions were in agreement with DNA/DNA reassociation studies viz. P. opuntiae and P. thermotolerans. The results obtained were also compared with the phylogenetic scheme proposed by WICKERHAM (1970). The yeast species which occurred on any one phylogenetic line did not contain identical long-chain fatty acid compositions. A progressive change in long-chain fatty acid compositions in some lines were evident. The species considered to be primitive did not produce linolenic acid (C18:3).

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9. Viljoen, B.C., J.L.F. Kock, M. Miller and D.J. Coetzee. The value of orthogonal/field alternation gel electrophoresis and other criteria in the delimitation of anamorphic/teleomorphic relations. Syst. Appl. Microbiol. (accepted)

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10. Miller, M., J.L.F. Kock, D.J. Coetzee and G.H.J. Pretorius. The value of orthogonal-field-alternation gel electrophoresis and other criteria in the taxonomy of the genus Pichia Hansen emend. Kurtzman. Syst. Appl. Microbiol. (accepted)

The relationships among one hundred and twenty-one Pichia strains were determined by orthogonal-field-alternation gel electrophoresis (OFAGE). In order to evaluate this genomic character, we compared the results obtained with other criteria, such as carbon source utilization patterns, coenzyme Q types and G+C contents. We found that similar DNA banding patterns do not necessarily coincide with similar physiological appearances, G+C values or coenzyme Q types. Strains within a species produced the same number of chromosomal bands, except for P. membranaefaciens. In the case of varieties of a species and species considered to be conspecific, similar OFAGE patterns were observed. The results obtained were also compared with Wickerham's (1970) phylogenetic scheme for nitrate positive Pichia species. it is interesting to note that the more "primitive" ancestors, P. finlandica, P. holstii and P. capsulata produced only two DNA bands, while species placed some distance from the origin of Wickerham's (1970) phylogenetic tree are mainly characterized by more than two chromosomal bands.

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11. Viljoen, B.C., J.L.F. Kock and K. Thoupou. The significance of cellular long-chain fatty acid compositions and other criteria in the study of the relationship between sporogenous ascomycete species and asporogenous Candida species. Syst. Appl. Microbiol. (accepted)

The relationship 34 Candida species and 51 ascosporogenous yeasts were studied on the basis of longchain fatty acid composition, coenzyme Q systems, carbon-source utilization patterns and other phenotypic characteristics. Close relationships were found between nine teleomorph and anamorph yeasts i.e. Candida norvegensis and Pichia norvegensis, Candida parapsilosis and Lodderomyces elongisporus, Candida krusei and Issatchenkia orientalis, Candida utilis and Pichia jadinii, Canadida lusitaniae and Clavispora lusitaniae, Candida pelliculosa and Pichia anomala, Candida cifferri and Stephanoascus famata and Debaryomyces hansenii and Candida pelliculosa and Pichia anomala, Candida cifferri and Stephanoascus famata and Debaryomyces hansenii and Candida lipolytica and Yarrowia lipolytica.

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12. Viljoen, B.C. and J.L.F. Kock. The value of FAME and OFAGE in the taxonomy of the genus Candida. Syst. Appl. Microbiol. (accepted)

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13. Viljoen, B.C. and J.L.F. Kock. A taxonomic study of the yeast genus Candida Berkhout. Syst. Appl. Microbiol. (accepted)

The cellular long-chain fatty acid composition and electrophoretic karyotypes of 117 strains of Candida were determined and used as a basis for a taxonomic study of this genus. Three major groups subdivided into 15 entities were proposed which are differentiated by the absence or presence of linoleic (C18:2) and linolenic acid (C18:3) and different electrophoretic karyotypes. Interesting correlations between fatty acid composition, electrophoretic karyotypes, coenzyme Q system, G+C contents, serological data and proton magnetic resonance spectra were observed. The coordinate use of these different phenotypic characters, provides a convenient method to establish close relationships between the different stains and species. Cellular long-chain fatty acid composition and electrophoretic karyotypes both proved to be valuable criteria in the differentiation of species of the genus Candida.

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14. Viljoen, B.C. and J.L.F. Kock. The genus Candida Berkhout nom. conserv. - A historical account of its delimitation. (accepted)

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15. Smit, E.J., J.L.F. Kock, J.J. van der Westhuizen and T.J. Britz. Taxonomic relationships of Cryptococcus and Tremella based on fatty acid composition and other phenotypic characters. J. Gen. Microbiol. (accepted)

The cellular long-chain fatty acids present in 33 strains, representing 15 species of Cryptococcus, and 4 species of Tremella, were determined by gas chromatography. According to the relative amounts of fatty acid methyl esters, the Cryptococcus species studied were divided into four main groups. Possible relationships between species representing the two genera are presented in a new model, where cellular long-chain fatty acid compositions and other phenotypic characteristics are included.

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16. Augustyn, O.P.H. and J.L.F. Kock. Differentiation of yeast species and strains within a species by cellular fatty acid analysis. I. Application of an adapted cellular fatty acid analysis technique to differentiate between strains of Saccharomyces cerevisiae. J. Microbiol. Methods. (accepted)

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XIX. Institut für Mikrobiologie, Technische Hochschule Darmstadt, Schnittspahnstr. 10, D-6100 Darmstadt, Fed. Rep. Germany. Communicated by F.K. Zimmermann.

The following are abstracts of papers in preparation, submitted, or recently published.

1. Schaaff, I., S. Hohmann and F.K. Zimmermann. Genetic analysis of the pentosephosphate pathway in Saccharomyces cerevisiae. (in preparation)

The nonoxidative part of the pentosephosphate pathway (PPP) interconverts sugar phosphates. Following textbook descriptions this pathway results in the net production of hexose- and triose-phosphates starting from pentose-phosphates and provides metabolites for the synthesis of some amino acids and related compounds and for the synthesis of nucleic acids. In E. coli the PPP can substitute for the upper part of glycolysis for the breakdown of glucose. Yeast mutants deleted for the phosphoglucose isomerase gene do not grow with glucose as carbon source indicating that the capacity of the PPP is not sufficient for energy production or that the yeast PPP follows a different route. Interestingly, mutants deleted for one of the two structural genes for phosphofructokinase ferment glucose and they accumulate sedoheptulose-7-phosphate, an intermediate of the nonoxidative part of the PPP. A mutant defective in the postulated bypass of the phosphofructokinase reaction does not show a deficiency for any of the four enzymes of the nonoxidative part of the PPP. To clarify the actual importance of this pathway we initiated a genetic analysis. We isolated the structural gene for transaldolase by means of antibody probes from a lambda gt11 library. Transformants carrying the gene TAL1 on a multicopy vector overproduce transaldolase. Mutants deleted for TAL1 have no detectable enzyme activity and both isoenzymes are missing from yeast crude extracts. These deletion mutants do not show an obvious phenotype on complex medium but they require one or several factors for growth on synthetic complete medium which can be provided by yeast extract. The TAL1 gene has been sequenced.

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2. Hohmann, S. and D. Gozalbo. Comparison of the nucleotide sequences of a yeast gene family. 1. Distribution and spectrum of spontaneous base substitutions. (submitted for publication)

The nucleotide sequences of closely related members of a gene family can be used to investigate spontaneous mutations. Here we analyse the sequences of different yeast invertase genes which are more than 93% identical in the coding region and share some very similar, but not identical sequences in the noncoding flanking regions. Since all except of one of the invertase genes are active, most of the base substitutions are silent. Within the coding region the base substitutions are unevenly distributed, indicating that parts of the genes were homogenized, probably via gene conversion. Transitions occurred more frequently than transversions in both coding and noncoding region. In the coding region pyrimidine transitions were the most abundant event due to silent changes mainly in the third codon position. In the noncoding region pyrimidine and purine transitions were found at equal frequencies. Transversions inverting base pairs (A-T and G-C) outnumber transversions changing base pairs (A-C and G-T). While the spectrum of mutations in the coding region is influenced by selective pressure to maintain the amino acid sequence, the spectrum in the noncoding region may be much less affected by selective pressure.

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3. Gozalbo, D. and S. Hohmann. Comparison of the nucleotide sequences of a yeast gene family. 2. Analysis of spontaneous deletions and insertions. (submitted for publication)

We compared the nucleotide sequences of three yeast invertase genes in regions where the homology is better than 90%. In the noncoding region 40 gaps of 1 to 61 bases were found. This is about half as much as the nucleotide substitutions in the same sequences. We grouped the gaps into five categories by their length and characteristics of their sequences. Group 1 gaps are about 20 nucleotides long and flanked by a six bases repeated sequence which may trigger the deletion of one of the repeats and the sequence between the repeats. Group II gaps are characterized by a small repeated sequence which is missing in one of the invertase genes. Gaps which occur in sequences exclusively made up of one of the four bases were summarized in group III. The four gaps in group IV do not show any of these sequence characteristics and they are all just one base long. A 61 nucleotide sequence only found in one of the invertase genes seems to be of complex origin. We conclude that small repeated sequences or monotonous sequences are prone to deletion or insertion mutations.

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4. Schaaff, I., J.B.A. Green, D. Gozalbo and S. Hohmann. 1989. A deletion of the PCD1 gene for pyruvate decarboxylase of yeast causes a different phenotype than previously isolated point mutations. Curr. Genet. 15:75-81.

The mutant pdc1-8 has not detectable pyruvate decarboxylase activity and does not grow with glucose as carbon source. Mutants deleted for PDC1 ferment glucose and have about 50% residual enzyme activity in vitro. These mutant strains still have mRNA homologous to PDC1 suggesting that a sequence homologous to PDC1 encodes a second pyruvate decarboxylase structural gene.

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5. Hohmann, S. and H. Cederberg. Comparison of the two pyruvate decarboxylase structural genes from Saccharomyces cerevisiae. (in preparation)

We have isolated the second pyruvate decarboxylase structural gene using the homology of the two genes. Sequence analysis revealed that both genes are 87% identical on the DNA and on the protein level. The gene product of this second gene (PDC3) is 14 amino acids longer because of the insertion of one histidine codon and because of a frame shift close to the end of the coding region. The two genes use different stop codons. There is no significant homology in the noncoding flanking regions.

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XX. Alko Research Laboratories, The Finnish Sate Alcohol Company, POB 350, SF-00101 Helsinki, Finland. Communicated by M. Korhola.

The following is the summary of an academic dissertation at the Helsinki University, Department of Genetics. It is based on seven papers: I. FEMS Microbiol. Lett. 34:179-185. II. Nucl. Acids Res. 13:7257-7268. III. Nucl. Acids Res. 15:2213-2220. IV. J. Inst. Brew. 92:588-590. V. Appl. Environ. Microbiol. 54:245-249. VI. In: M. Korhola & H. Nevalainen (eds.) Industrial Yeast Genetics. Found. Biotech. Ind. Ferment. Res. 5:127-136. VII. Manuscript.

1. Suominen, P.L. 1988 Characterization and applications of the yeast MEL1 gene.

In this study a genomic library of S. cerevisiae var. uvarum was constructed and the MEL1 gene coding of secreted α-galactosidase was isolated from this library. The complete nucleotide sequence of the MEL1 gene was determined and it was found to code for a 52 kDa polypeptide containing a signal sequence for secretion. Regulatory sequences in the 5' region was identified by comparison of the MEL1 upstream sequence to that of other genes in the same regulon. The complete nucleotide sequence of the E. coli melA gene encoding a cytoplasmic α-galactosidase was determined. The primary structure of the secreted α-galactosidase coded by the MEL1 gene was compared with the primary structures of a prokaryotic (E. coli), a human (secreted) and a plant (secreted) α-galactosidase. The yeast α-galactosidase was more similar to the secreted, eukaryotic enzymes than to the prokaryotic (cytoplasmic) one, indicating a closer relationship to these enzymes. A short region common to all four enzymes was found, which therefore probably has some crucial functional importance.

Molasses is widely used as a substrate for commercial yeast production. Raffinose, present in beet molasses is not completely hydrolysed by commercially available baker's yeast strains (mel^0) because they lack α-galactosidase. The MEL1 gene was used to construct α-galactosidase-producing derivatives of a commercial baker's yeast. To facilitate analysis of the stability of the Mel$^+$ phenotype in the transformed strains, a colony-colour method differentiating α-galactosidase positive and negative strains was developed. Stable α-galactosidase positive baker's strains were constructed both by integration of the MEL1 gene into the genome and by transforming with autonomous plasmids. Increase in yields of cell mass due to complete hydrolysis of raffinose was demonstrated using these strains. Both of them would be technically suitable as commercial baker's yeast strains. One contains only yeast DNA and should thus be acceptable for use in the food industry.

Components of the isolated MEL1 gene were also used to construct sets of expression vectors for production of foreign proteins in yeast. The first set includes promoter vectors, which carry the MEL1 promoter and terminator only. The other set includes, in addition, the signal sequence of MEL1 followed by a suitable cloning site. All vectors are available as integrating and autonomously replicating plasmids. To test the function of these vectors, the eg11 cDNA of T. reesei was expressed in yeast using these vectors.

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The following communication was presented recently.

2. Aho, S., M. Paloheimo and M. Korhola. 1988. Cellulolytic yeast and monoclonal antibodies used as tools to study the enzymatic properties of Trichoderma cellulases. Kemia-Kemi 15(10 B):1068. (Kemian Paivat - Finnish Chemical Congress Symposium on Biotechnology, Helsinki, Finland)

Some strains of bacteria and filamentous fungi secrete β-glucanases, which degrade β-1,4-glycosidic bonds in cellulose. Three types of cellulases are involved in the hydrolysis of native cellulose. These are 1) endo-1,4-β-glucanases (EC 3.2.1.4). 2) cellobiohydrolases or exoglucanases (EC 3.2.1.91) and 3) β-glucosidase (EC 3.2.1.21). Genomic and cDNA clones for two cellobiohydrolases (1,2) and two endoglucanases (3,4) have been isolated from Trichoderma reesei strain VTT-D-80133. We have expressed Trichoderma cellulase cDNAs under the control of the yeast ADC1 promoter in a laboratory yeast strain. Monoclonal antibodies (Mab) against CBHI, CBHII and EGI proteins were made by Dr. D. Bamford, Department of Genetics, University of Helsinki. Proteins for immunizations were kindly provided by Dr. H.-L. Niku-Paavola, Technical Research Centre of Finland. Each monoclonal antibody against CBHII reacted on the Western Blot only against samples from the yeast strain containing CBHII cDNA in the expression vector, but did not show any reaction with yeast containing any other cellulose cDNA in the expression vector. To characterize the binding site for each monoclonal antibody, a series of 3'-deletions were made by exonuclease and the deleted cDNAs were expressed in yeast under the control of the ADC1 promoter. Only the intact CBHII protein showed enzymatic activity on the β-glucan plate. A yeast strain with a deletion of about 30 carboxyterminal amino acids of CBHII protein did not show any detectable enzyme activity. When grown on nitrocellulose-covered agar-plates, each strain containing a deleted cDNA of either CBHI or CBHII produced a protein, detectable by a polyclonal antiserum. Against CBHII protein, three clones detected only the full length protein. Two clones still detected protein with about a 60 amino acid deletion but did not bind if around 200 amino acids were deleted. Similar studies on EGI protein, and deletions thereof, with monoclonal antibodies are under progress.

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XXI. Czechoslovak Academy of Sciences, Institute of Microbiology, 142 20 Praha 4 - KRC, Videnska 1083, Czechoslovakia. Communicated by J. Vorísek.

The following is a summary of an article accepted for publication in Histochemistry (Springer).

1. Vorísek, J. Ultracytochemical localization of the vacuolar marker enzymes alkaline phosphatase, adenosine triphosphatase, carboxypeptidase Y and amino-peptidase reveal new concept of vacuole biogenesis in Saccharomyces cerevisiae.

Logarithmic cultures of Saccharomyces cerevisiae strains LBG H 1022, FL-100, X 2180 1A and 1B were studied together with the mutants pep4-3, sec18-1 and sec7-1. The necessary ultrastructural observations showed that, as a rule, juvenile vacuoles were formed de novo from perinuclear endoplasmic reticulum cisternae (ER) packed and inflated with electron-dense (polyanionic) matrix material. This process was disturbed solely in the sec18-1 mutant under non-permissive conditions. The vacuolar marker enzymes adenosine triphosphatase (ATPase) and alkaline phosphohydrolase

(ALPase) were assayed by the ultracytochemical cerium precipitation technique. The neutral ATPase was active in vacuolar membranes and in the previously shown (coated) microglobules nearby. ALPase activity was detected in microglobules inside juvenile vacuoles, inside nucleus and in the cytoplasm as well as in the membrane vesicles and in the periplasm. The sites of vacuolar protease carboxypeptidase Y (CPY; yscC) activity were assayed using N-CBZ-L-tyrosine-4-methoxy-2-naphthylamide (CBZ-Tyr-MNA) as substrate and sites of the aminopeptidase yscI activity Leu-MNA as substrate. Hexazotized p-rosaniline served as a coupler for the primary reaction product of both the above proteases (MNA) and the resulting azo-dye was osmicated during postfixation. The CPY reaction product was found in both polar layers of vacuolar membranes (homologous to ER) and in ER membranes enclosing condensed lipoprotein bodies which were taken up by the vacuoles of late logarithmic yeast. Both before and after the uptake into the vacuoles the bodies contained the CPY reaction product in concentric layers or in cavities. Microglobules with CPY activity were also observed. Amino-peptidase was localized in microglobules inside the juvenile vacuoles. These findings combined with the previous cytochemical localizations of polyphosphates and X-prolyl-dipeptidyl (amino)peptidase in S. cerevisiae suggest the following cytologic mechanism for the biosynthetic protein transport: coated microglobules convey metabolites and enzymes either to the cell surface for secretion or enter the vacuoles in all phases of the cell cycle. The membrane vesicles represent an alternative secretory mechanism present in yeast cells only during budding. The homology of the ER with the vacuolar membranes and with the surface membranes of the lipoprotein condensates (bodies) indicates a contranslational entry of the CPY into these membranes. The secondary transfer of a portion of CPY into vacuoles is probably mediated by the lipoprotein uptake process.

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XXII. Mikrobiologisches Institut, ETH-Zentrum/LFV, CH-8092 Zürich, Switzerland. Communicated by G. Braus.

The following articles have been published recently.

1. Paravicini, G., H.U. Mösch, T. Schmidheini, and G. Braus. 1989. The general control activator protein GCN4 is essential for a basal level of ARO3 gene expression in yeast. Mol. Cell. Biol. 9:144-151.

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2. Braus, G., H.U. Mösch, K. Vogel, A. Hinnen, and R. Hütter. 1989. Interpathway regulation of the TRP4 gene of yeast. The EMBO J. 8:939-945.

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3. Schmidheini, T., P. Sperisen, G. Paravicini, R. Hütter and G. Braus 1989. A single point mutation results in a constitutively activated and feedback-resistent chorismate mutase of Saccharomyces cerevisiae. J. Bacteriol. 171:1245-1253.

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XXIII. Department of Genetics and Development, Columbia University College of Physicians and Surgeons, 701 West 168th Street, New York, N.Y. 10032. Communicated by R. Rothstein.

The following are abstracts from two recently published papers.

1. Thomas, B. J. and R. Rothstein. 1989. Elevated recombination rates in transcriptionally active DNA. Cell 56:619-630.

We have examined the effect of RNA polymerase II-dependent transcription on recombination between directly repeated sequences of the GAL10 gene is S. cerevisiae. Direct repeat recombination leading either to plasmid loss or conversion was examined in isogenic strains containing null mutations in the positive activator, GAL4, or the repressor, GAL80. A 15-fold increase in the rate of plasmid loss is observed in cells constitutively expressing the construct compared with cells that are not. Conversion events that retain the integrated plasmid are not stimulated by expression of the repeats. Northern analysis of strains containing plasmid inserts with various promoter mutations suggests that the stimulation in recombination is mediated by events initiating within the integrated plasmid sequences.

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2. Wallis, J. W.,[1] G. Chrebet, G. Brodsky,[1] M. Rolfe, and R. Rothstein. 1989. A hyper-recombination mutation in Saccharomyces cerevisiae identifies a novel eukaryotic topoisomerase. Cell, in press. [1]Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis Mo 63104.

A hyper-recombination mutation was isolated that causes an increase in recombination between short repeated δ sequences surrounding the SUP4-o gene in Saccharomyces cerevisiae. The wild type copy of this gene was cloned by complementation of one of its pleiotropic phenotypes, slow growth. The mutation maps on chromosome XII, 3 cM centromere-proximal to CDC42. DNA sequence of the clone revealed a 656 amino acid open reading frame capable of encoding a protein homologous to the bacterial type I topoisomerase. No homology ws detected with previously identified eukaryotic topoisomerases. Construction of double mutants with either of the two known yeast topoisomerase genes revealed synergistic effects on growth suggesting overlapping functions. Expression of bacterial topoisomerase I in yeast [Giaever and Wang, Cell 55:849-856 (1988)] can fully complement the slow growth defect of a null mutation. We have named this locus TOP3 and suggest that it defines a novel eukaryotic topoisomerase gene.

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XXIV. Department of Microbiology and Enzymology, Faculty of Chemical Engineering and Materials Science, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands. Communicated by W.A. Scheffers.

The following papers have recently appeared:

1. Giuseppin, M.L.F., H.M.J. van Eijk, A. Bos, C. Verduyn and J.P. van Dijken. 1988. Utilization of methanol by a catalase-negative mutant of Hansenula polymorpha. Appl. Microbiol. Biotechnol. 28:286-292.

In methanol-utilizing yeasts, catalase is an essential enzyme for the destruction of hydrogen peroxide generated by methanol oxidase (E.C. 1.1.3.13). It was found however that a catalase-negative mutant of Hansenula polymorpha is able to consume methanol in the presence of glucose in continuous cultures. At a dilution rate of 0.1 h^{-1}, stable continuous cultures could be obtained during growth on methanol/glucose mixtures with a molar ratio of methanol/glucose between 0 to 2.4. In these cultures methanol oxidase was induced up to a level of 40% of that obtained in the wild-type strain. The hydrogen peroxide-decomposition activity of the mutant ws studied in more detail by pulsing methanol to samples of steady-state cultures. Only after the addition of excess methanol the hydrogen peroxidase-decomposing system became saturated, and the cells excreted hydrogen peroxidase. This was accompanied by excretion of formaldehyde and a rapid loss of viability. The presence of extracellular catalase during a methanol pulse prevented the loss of viability. The nature of the alternative hydrogen peroxide-decomposing enzyme system remains to be elucidated. Its capacity strongly depended on the cultivation conditions and pretreatment of the cells. Cells grown on formaldehyde/glucose mixtures showed a lower methanol tolerance than those grown on the methanol/glucose mixtures. Freeze-drying of cells drastically enhanced the excretion of hydrogen peroxide, probably as a result of an inactivation of the decomposing system.

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2. Van Urk, H., P.R. Mak, W.A. Scheffers and J.P. van Dijken. 1988. Metabolic responses of Saccharomyces cerevisiae CBS 8066 and Candida utilis CBS 621 upon transition from glucose limitation to glucose excess. Yeast 4:283-291.

When chemostat cultures of Saccharomyces cerevisiae CBS 8066 and Candida utilis CBS 621, grown under glucose limitation, were pulsed with excess glucose, both organisms initially exhibited similar rates of glucose and oxygen consumption. However, striking differences were apparent between the two yeasts with respect to the production of cell mass in the culture and metabolite excretion. Upon transition from glucose limitation to glucose excess. S. cerevisiae produced much ethanol but the growth rate remained close to that under glucose limitation. C. utilis, on the other hand, produced little ethanol and immediately started to accumulate cell mass at a high rate. This high production rate of cell mass was probably due to synthesis of reserve material and not caused by a high rate of protein synthesis. Upon a glucose pulse both yeasts excreted pyruvate. In contrast to C. utilis, S. cerevisiae also excreted various tricarboxylic acid cycle intermediates, both under steady-state conditions and after exposure to glucose excess. These results and those of theoretical calculations on ATP flows support the hypothesis that the ethanol production as a consequence of pyruvate accumulation in S. cerevisiae, occurring upon transition from glucose limitation to glucose excess, is caused by a limited capacity of assimilatory pathways.

3. Postma, E., C. Verduyn, W.A. Scheffers and J.P. van Dijken. 1989. Enzymic analysis of the Crabtree effect in glucose-limited chemostat cultures of Saccharomyces cerevisiae. Appl. Environ. Microbiol. 55:468-477.

The physiology of Saccharomyces cerevisiae CBS 8066 was studied in glucose-limited chemostat cultures. Below a dilution rate of 0.30 h^{-1} glucose was completely respired, and biomass and CO_2 were the only products formed. Above this dilution rate acetate and pyruvate appeared in the culture fluid, accompanied by disproportional increases in the rates of oxygen consumption and carbon dioxide production. This enhanced respiratory activity was accompanied by a drop in cell yield from 0.50 to 0.47 g (dry weight) g of glucose^{-1}. At a dilution rate of 0.38 h^{-1} the culture reached its maximal oxidation capacity of 12 mmol of O_2 g (dry weight)$^{-1}$$h^{-1}$. A further increase in the dilution rate resulted in aerobic alcoholic fermentation in addition to respiration, accompanied by an additional decrease in cell yield from 0.47 to 0.16 g (dry weight) g of glucose^{-1}. Since the high respiratory activity of the yeast at intermediary dilution rates would allow for full respiratory metabolism of glucose up to dilution rates close to μ_{max}, we conclude that the occurrence of alcoholic fermentation is not primarily due to a limited respiratory capacity. Rather, organic acids produced by the organism may have an uncoupling effect on its respiration. As a result the respiratory activity is enhanced and reaches it maximum at a dilution rate of 0.38 h^{-1}. An attempt was made to interpret the dilution rate-dependent formation of ethanol and acetate in glucose-limited chemostat cultures of S. cerevisiae CBS 8066 as an effect of overflow metabolism at the pyruvate level. Therefore, the activities of pyruvate decarboxylase. NAD^+- and $NADP^+$-dependent acetaldehyde dehydrogenases, acetyl coenzyme A (acetyl-CoA) synthetase, and alcohol dehydrogenase were determined in extracts of cells grown at various dilution rates. From the enzyme profiles, substrate affinities, and calculated intracellular pyruvate concentrations, the following conclusions were drawn with respect to product formation of cells growing under glucose limitation. (i) Pyruvate decarboxylase, the key enzyme of alcoholic fermentation, probably already is operative under conditions in which alcoholic fermentation is absent. The acetaldehyde produced by the enzyme is then oxidized via acetaldehyde dehydrogenases and acetyl-CoA synthetase. The acetyl-CoA thus formed is further oxidized in the mitochondria. (ii) Acetate formation results from insufficient activity of acetyl-CoA synthetase, required for the complete oxidation of acetate. Ethanol formation results from insufficient activity of acetaldehyde dehydrogenases. The observed pattern of metabolite production in chemostat cultures is in agreement with the conditions under which these insufficiencies can be calculated to occur. The upcoming effect of weak acids on respiration and the associated triggering of alcoholic fermentation were confirmed in chemostat experiments in which the yeast was grown in the presence of propionate. In this case, the maximal respiratory activity was attained at a dilution rate of 0.30 h^{-1}, above which alcoholic fermentation occurred.

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XXV. Departamento de Bioquimica, Universidade Federal do Rio de Janeiro, 21941 Rio de Janeiro - Brazil. Communicated by A.D. Panek.

The following are abstracts from papers submitted for publication.

1. Paschoalin, V.M.F., J.T. Silva and A.D. Panek. Identification of an ADPG-dependent trehalose synthase in Saccharomyces.

Uridine diphosphoglucose is not the only donor for trehalose synthesis in yeast cells. Mutant strains with undetectable UDPG-dependent trehalose-6-P synthase activity have allowed the identification of an ADPG-dependent trehalose synthase. Genetic and chromatographic approaches indicate that the two activities correspond to different proteins. The apparent Km for the nucleotide is similar for both enzymes and Mg^{2+} is also required for both activities, however, a striking difference was observed with respect to ATP.Mg activation. This new enzymatic activity in Saccharomyces clarifies our previous contradictory results with mutant strains which are able to accumulate trehalose during growth yet UDPG-dependent trehalose synthase activity is undetectable in vitro.

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2. Silva, J.T., A.C. Panek and A.D. Panek. Regulation of the trehalose-6-phosphate synthase complex in Saccharomyces.

Active trehalose-6-phosphate synthase from Saccharomyces cerevisiae was purified 90-fold, by chromatography on phosphocellulose and precipitation by acetone. This preparation was specific for UDPG as the glucosyl donor with a Km of 0.71 mM and for G-6-P. Phosphocellulose fractionation led to the identification of an activating factor of this enzymatic activity. Evidence is presented for the protein nature of the activator. Further investigation is needed to determine the site of action of this new protein.

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

The following are recent publications from our department.

1. Vaughan-Martini, A. and A. Martini. 1988. Killer sensitivity patterns as a tool for the fingerprinting of strains within the yeast species Kluyveromyces lactis and K. marxianus. Biotech. Letters. 2:293-296.

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2. Vaughan-Martini, A. and G. Rosini. 1989. Killer relationships within the yeast genus Kluyveromyces. Mycologia 8(2).

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3. Vaughan-Martini, A. 1989. A newly separated species of the Saccharomyces sensu stricto complex based upon nDNA/nDNA homologies. Syst. Appl. Microbiol. (in press).

Saccharomyces paradoxus (Batschinskaya), a yeast species isolated exclusively from natural sources such as tree exudates, insects and soil was compared by the nDNA/nDNA optical reassociation technique to the species of the Saccharomyces sensu stricto complex S. cerevisiae, S. bayanus and S. pastorianus (sensu Vaughan Martini and Martini, 1987). Low homology values indicate that significant evolutionary divergence has taken place between S. paradoxus and the other three species of the group.

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4. Vaughan-Martini, A. and A. Martini. 1989. A proposal for correct nomenclature of the domesticated species of the genus Saccharomyces. In: Biotechnology Applications in Beverage Production. G. Lanzarini and C. Cantarelli (eds.), Elsevier Science Publishers, Amsterdam.

A brief history of the domesticated species of the Saccharomyces sensu stricto complex which are most frequently utilized for alcoholic fermentation, with mention of habitat, isolation studies, and variations over the years in the taxonomic positions of the various strains.

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5. Rosini, G., M. Ciani and V. Palpacelli. 1989. Use of a probe pO_2 for control of wine-making starter vitality. Biotech. Letters. (in press)

A simple technique for rapid determination of fermentation starters vitality which eliminates the need for determination of viable cell counts is described. The mathematical relationship between cell number and oxygen consumption of either strains of Saccharomyces cerevisiae was studied.

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6. Rosini, G. 1989. Killer yeasts: notes on properties and technical use of the character. In: Biotechnology Applications in Beverage Production. G. Lanzarini and C. Cantarelli (eds.), Elsevier Science Publishers, Amsterdam (in press)

* * *

XXVII. Department of Biotechnology, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland. Communicated by A. Fiechter.

The following paper has been recently published.

1. Sanglard, D. and J.C. Loper.[1] 1989. Characterization of the alkane-inducible cytochrome P450 (P450alk) gene from the yeast Candida tropicalis: identification of a new P450 gene family. Gene 76:121-136. [1]Department of Microbiology and Molecular Genetics, University of Cincinnati Medical Center, Cincinnati, Oh 45267-0524 USA.

The P450alk gene, which is inducible by the assimilation of alkane in Candida tropicalis, was sequenced and characterized. Structural features described in promoter and terminator regions of Saccharomyces yeast genes are present in the P450alk gene and some particular structures are discussed for their possible role in the inducibility of this gene. Expression of the P450alk gene was achieved in Saccharomyces cerevisiae using the yeast alcohol dehydrogenase (ADH1) expression system after removal of the P450alk gene flanking regions. The resultant expressed protein had a molecular mass slightly greater than P450alk from C. tropicalis. This alteration did not prevent the function and the localization of P450alk expressed in S. cerevisiae, as this organism showed an acquired microsomal-bound activity for the terminal hydroxylation of lauric acid. The deduced P450alk amino acid sequence was compared with members of the nine known P450 gene families. These comparisons indicated that P450alk had a low relationship with these members and therefore was the first member (A1) of a new P450 gene family (LII).

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The following papers have been recently submitted.

2. Sanglard, D., I. Beretta, M. Wagner and A. Fiechter. Functional expression of the alkane-inducible monooxygenase system of the yeast Candida tropicalis in Saccharomyces cerevisiae.

The genes for the alkane-inducible monooxygenase system of the yeast Candida tropicalis, namely a cytochrome P450alk (P450alk) and a NADPH cytochrome P450 oxidoreductase (NCPR) gene, were transferred in Saccharomyces cerevisiae. The P450alk gene was expressed in this host with the help of the yeast alcohol dehydrogenase I (ADHI) promoter and terminator, whereas the NCPR gene could be expressed with its own structural elements. The presence of P450alk in S. cerevisiae microsomal fractions resulted in a new acquired lauric acid terminal hydroxylation activity. Moreover, the same activity, coupled with the appearance of 12-hydroxylauric acid derivates, could be obtained by the addition of lauric acid to intact cells expressing P450alk. The coordinate expression of the P450alk and NCPR genes in S. cerevisiae elevated the turnover rate of the P450alk monooxygenase activity about 2-fold.

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3. Sanglard, D. and A. Fiechter. Isolation of a cytochrome P450 gene (P450alk2) related to the alkane-inducible cytochrome P450 gene (P450alk) from the yeast Candida tropicalis.

Reexamination of a genomic lambda-gt11 Candida tropicalis expression library for the presence of P450alk-related genes resulted in the isolation of a DNA fragment having similarity with a portion of a previously reported P450alk gene from the same yeast. This new P450 gene was also induced by tetradecane when C. tropicalis was grown on this carbon source and was named therefore P450alk2, P450alk1 corresponding to the first isolated P450 gene. Furthermore, the presence of multiple P450alk-related genes in the genome of C. tropicalis is suggested by the hybridization pattern of P450alk1 and P450alk2 gene probes with the yeast genomic DNA.

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4. Glumoff, V., O. Käppeli,[1] A. Fiechter, and J. Reiser. Genetic transformation of the filamentous yeast Trichosporon cutaneum using dominant selection markers. [1]BIDECO AG, CH-5401 Baden, Switzerland.

An efficient transformation system for the filamentous yeast Trichosporon cutaneum has been developed. Transformation was obtained with plasmids carrying either the E. coli hygromycin B (Hmb) phosphotransferase gene (hph) or the Streptoalloteichus hindustanus phleomycin (Phleo) resistance gene (ble) as dominant selection markers. Expression of both resistance genes was controlled by the gpd promoter and the trpC terminator from Aspergillus nidulans. The transformation frequency was up to 500 colonies per µg of transforming DNA using the ble gene and up to 100 colonies per µg of transforming DNA using the hph gene. Co-transformation frequencies using unselected DNA varied between 50 and 65%. The transforming DNA was found to exist as multiple tandem plasmid copies of high molecular weight. This polymeric structure, in non-selective media, was mitotically unstable, indicating that it possibly existed in an episomal state.

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XXVIII. Caribbean Industrial Research Institute (CARIRI), University of the West Indies Campus, St-Augustine, Trinidad. Communicated by D.A. Ali.

The following is a summary of a paper which was recently presented at the Second Cuban and International Seminar on Biotechnology which was held in Cuba, 17-22 April 1989. Part of this work is also being published in the MIRCEN Journal of Applied Microbiology and Biotechnology.

1. Nagassar-Mohit, G.[1] and D.A. Ali. 1989. Studies on yeasts in the sugar cane industry in Trinidad and Tobago. [1]University of the West Indies, St-Augustine, Trinidad.

Identification and classification studies are being carried out on 250 yeast isolates from the sugar cane industry. These isolates have come from various areas of the sugar mills, the sugar refinery and the rum distilleries. Identification was based on morphological, cultural, physiological and biochemical studies using methods described by van der Walt and Yarrow (1984). Based on initial studies of glucose, sucrose, maltose, galactose and lactose fermentations,

150 of the isolates were found to fall into 16 clusters. Fifty representative strains from the clusters were selected for full identification. The test results were taken at intervals over three (3) weeks and were assessed:

a. using taxonomic keys formulated by Barnett, Payne and Yarrow (1983) and Kreger-van Rij (1984)

b. using a probabilistic identification computer programme formulated by Barnett, Payne and Yarrow (1985) and used at the National Collection of Yeast Cultures, Norwich, England (NCYC 1986). Major species identified are as follows:

Cryptococcus laurentii	13.15%
Pichia membranaefaciens	13.15%
Rhodotorula glutinis	7.89%
Zygosaccharomyces bailii	7.89%
Rhodotorula rubra	5.2%
Hansenula uvarum	5.2%
Candida krusei	5.2%
Saccharomyces cerevisiae	2.6%

The species isolated in Trinidad and Tobago appear to be similar to those found in similar environments elsewhere. Additional studies are now being undertaken. These include surface topography and internal morphology using scanning electron microscopy (SEM) and transmission electron micrography (TEM). Whole cell protein patterns using semi-automated Phast Gel (Pharmacia, Sweden) techniques are also being used.

* * *

XXIX. Department of Microbiology, M.A.C.S., Research Institute, Law College Road, Pune 411 004, India. Communicated by P.P. Kanekar.

The following is the abstract of a paper presented in the poster session of 28th Annual Conference (Golden Jubilee Celebrations) of Association of Microbiologists of India organized in January 1988. The paper was selected for the "Best Poster Award".

1. Neeta, J., S.H. Godbole and Kanekar, P.P. 1988. Role of Hansenula silvicola in 'Dhokla' fermentation.

'Dhokla' is an indigenous Indian fermented food, popular as a snack dish all over the country and liked for its spongy texture. 'Dhokla' is prepared by fermenting batter of bengal gram flour and curds (2:3) for 18 hours and steaming as a pancake. The leavened texture of the product is due to gas produced during fermentation by the microorganisms. Microbiological analysis of five samples each of the ingredients and 28 samples of fermented batters revealed the presence of Hansenula silvicola along with the two lactic acid bacteria namely Lactobacillus fermentum and Leuconostoc mesenteroides. Experiments performed to study growth rate of this yeast during 18 hours of fermentation demonstrated rise in its population from 0.7×10^7 to 2.7×10^7 c.f.u./g. To find out role of this yeast in 'Dhokla' fermentation, sterile bengal gram flour was inoculated with the three microbial species singly and in combinations. The data collected on the extent of fermentation (indicating change in the volume of the batter) and the organoleptic qualities of the product showed highest spongy texture but no sour taste of the product fermented with the yeast alone. The product with two lactic cultures was good in taste but poor in sponginess while the product prepared with the three species exhibited the best organoleptic qualities viz. good sour taste and highly spongy texture. The studies indicated that the spongy texture of the 'Dhokla' could be attributed to the yeast Hansenula silvicola.

* * *

XXX. Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie, 91198 Gif-Sur-Yvette Cedex, France. Communicated by J. Schwencke.

The following is an abstract of a published paper.

1. Martinez, A.F. and J. Schwencke. 1988. Chitin synthetase activity is bound to chitosomes and to the plasma membrane in protoplasts of Saccharomyces cerevisiae. Biochim. Biophys. Acta 946:328-336.

The sub-cellular distribution of chitin synthetase was studied in homogenates of Saccharomyces cerevisiae protoplasts. Use of a mild disruption method minimized rupture of vacuoles and ensuing contamination of subcellular fractions by vacuolar proteinases. After fractionation of whole or partially purified homogenates through an isopycnic sucrose gradient chitin synthetase activity was found to be distributed between two distinct particulate fractions with different buoyant density and particle diameter. When whole homogenates were used, about 52% of the chitin synthetase loaded was localized in a microvesicular population identified as chitosomes (diameter 40-110 nm; buoyant density (d) = 1.146 g/cm^3). Another vesicular population containing 26% of the activity was identified as plasma membrane vesicles because of its large mean diameter (260 nm), its high buoyant density (d = 1.203 g/cm^3) and by the presence of the vanadate-sensitive ATPase activity. Moreover, after surface labeling of protoplasts with ^{3}H-concanavalin A, the label cosedimented with the presumed plasma membrane vesicles. There was a negligible cross-contamination of chitosome fraction by yeast plasma membrane markers. In both the plasma membrane and the chitosome reactions, the chitin synthetase was stable and essentially zymogenic. Activation of the chitosome fraction produces microfibrils 100-250 nm in length. Our results support the idea that chitosomes do not originate by plasma membrane vesiculation but are defined sub-cellular organelles containing most of the chitin synthetase in protoplasts of Saccharomyces cerevisiae.

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XXXI. Center of Biological Research, Division of Experimental Biology, P.O. Box 128, La Paz B.C.S., México. Communicated by R.V. Juárez.

The following is a summary of the results of research on the isolation of marine yeasts from the west coast of Baja California Sur, Mexico, by R.V. Juárez, D. Hernandez S., and J.L. Ochoa.

In the west coast of Baja California Sur occurs the border of two oceanic masses: one is temperate and the other is tropical, creating a zone of transition with ecological implications that affect the fishery activities of the region. Numerous studies have been carried out on the distribution and abundance of planctonic communities in this zone, but there is a clear shortage of information concerning the presence and activities of microorganisms, specially yeasts. To fill in this gap, we have studied the area comprised between 27°10'10"N, 115°18'45"W; 27°26'38"N, 114°45'0"W; 23°53'20"N, 110°11'15"W; and 23°8'40"N, 112°30'0"W. The stations are distributed according to the CalCoFi network where samples were taken at 0, 50 and 100 m. From 96 samples, 227 strains of yeasts were isolated by enrichment. Since we lost of a number of strains after some subculturing, we believe that some strains are unable to survive in laboratory conditions and/or that some marine yeasts require special conditions for maintenance other than refrigeration and subculturing.

The identification so far has shown that the anasporogenous yeast are the predominant group, mainly in the localities far off to the coast suggesting that Ascomycetes are less able to adapt to the marine environment than the members of Basidiomycetes and Deuteromycetes. The prevalent genera were the basidiomycetes Rhodosporidium, Leucosporidium, Sporidiobolus and the Deuteromycetes Candida, Cryptococcus, Rhodotorula as well as the "black yeast". Aureobasidium pullulans. These yeasts had been previously isolated by Dr. Jack W. Fell from the Antarctic Ocean were the temperatures of the water masses are lower, suggesting that this factor is not essential for the presence of those organisms in marine environments.

* * *

XXXII. Department of Genetics and Microbiology, Charles University, Prague 2, Vinicná 5, Czechoslovakia. Communicated by V. Vondrejs and O. Bendová.

A. The following papers will be presented on the 4th Trilateral Conference of Yeasts, Sáraspatch, Hungary, July 24-28, 1989.

1. Janderová, B., F. Cvrcková, M. Fabianová, and O. Bendová. Properties of starch-degrading industrial hybrid strains.

Starch degrading industrial strains were constructed by induced protoplast fusion of Saccharomyces diastaticus (pof7) and a polyploid brewing strain of S. uvarum. Hybrid strains producing glucoamylase were obtained and characterized. Several clones derived from hybrids behaved comparably with parental brewing strain under tube fermentation test conditions. They produced beer without phenolic off-flavor (pof^-). Similarly, hybrids of industrial distillery strains producing glucoamylase were constructed and characterized. These strains will serve as recipients of alpha-amylase gene in the next step of manipulation.

* * *

2. Puta, F. and R. Wambutt. Plasmid pPW 362 - an appropriate tool for cloning of yeast DNA.

A new shuttle plasmid vector (E. coli-S. cerevisiae) was constructed. Possibility of positive selection of E. coli-clones transformed by recombinant plasmids, relatively small size of vector (7,8 bp), and a set of cloning sites (Eco RI, Hind III, and Bcl I) are the main advantages of this shuttle plasmid vector. A fragment containing tet^r under p^R lambda-promoter regulated by cI lambda-gene (originating from pUN 121: Nilsson B. et al. N.A.R. 11:8019, 1983) is used as a positive selection system. The yeast part of pPW362 is homologous to YEp24 (Bostian D. et al. Gene 8:17, 1979).

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B. The following papers have been completed for publication.

3. Hahnová, Z., I. Pavlícek, and V. Vondrejs. Production and reversion of local protoplasts in Schizosaccharomyces pombe cells (running title).

Different conditions for preparation of local protoplasts in S. pombe cells were tested in order to obtain the largest yield of cells with local protoplasts at one pole of cylindric cell-body (PLP). It was shown that the frequency of reversion depends on the osmolarity of the medium.

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4. Pavlícek, I., M. Kothera, V. Vondrejs, and Z. Palková. Electrofusion of local protoplasts in Schizosaccharomyces pombe (submitted for publication in Progress in Biotechnology).

A method for orientation of cells with PLP under nonuniform alternating electric fields was described. Electrofusion of oriented local protoplasts was demonstrated.

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5. Palková, Z., V. Vondrejs, and S. Zadrazil. Secretion of calf-chymosin from hybrids of Saccharomyces cerevisiae x Kluyveromyces lactis obtained by induced protoplast fusion (running title).

Hybrids of S. cerevisiae containing recombinant plasmid coding secretion of calf chymosin and K. lactis were obtained. The hybrid clones produced chymosin and were able to grow in media containing lactose as a sole carbon source, however, they were very unstable.

* * *

XXXIII. Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7. Communicated by M.A. Lachance.

The following paper was published recently.

1. Butler, M.J., G. Lazarovits, V.J. Higgins[1], and M.A. Lachance. 1988. Partial purification and characterization of a dehydratase associated with the pentaketide melanogenesis pathway of Phaeococcomyces sp. and other fungi. Exptl. Mycol. 12: 367-376. [1]Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

Polyacrylamide gel electrophoresis was used to identify an enzyme that catalyzed the dehydration of scytalone to 1,3,8-trihydroxynaphthalene, the next sequential component of the pentaketide melanin pathway. The dehydratase, extracted from the pentaketide melanin-producing black yeast Phaeococcomyces sp., was partially purified by gel filtration and preparative electrophoresis. Enzyme activity was indicated by the formation of a single orange bank on polyacrylamide gels after scytalone was added as substrate. The enzyme has a molecular mass of ca. 60,000-65,000 and an isoelectric point of 5.2. The pH optimum for activity with scytalone under aerobic conditions was 7.5. Anaerobic reactions resulted in the accumulation of 1,3,8-trihydroxynaphthalene, and aerobic reactions produced 2-hydroxyjuglone, which is an orange-colored autooxidation product of 1,3,8-trihydroxynaphthalene. Reduced nucleotides were not required for the dehydratase reaction. When the pentaketide pathway intermediate vermelone was used as a substrate it was converted to 1,8-dihydroxynaphthalene and an unknown hydroxynaphthalene. Extracts of the plant pathogenic pentaketide melanin-producing fungi Verticillium dahliae. Verticillium albo-atrum, Alternaria solani, and Cladosporium cucumerinum were also shown to have the dehydratase activity on polyacrylamide electrophoresis gels. The dehydratase activity did not occur with extracts of yeasts that do not form pentaketide melanins or with extracts from V. dahliae and V. albo-atrum that had not yet formed melanin.

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XXXIV. Department of Food Science and Technology, University of California, Davis California 95616 USA. Communicated by H.J. Phaff.

The following article, whose abstract appeared in the last issue of the YNL, has now been published.

1. Hagler, A.N.[1], L.C. Mendonça-Hagler[1], and H.J. Phaff. 1989. Candida populi, a new species of yeast occurring in exudates of Populus and Betula species. Int. J. Syst. Bacteriol. 39:97-99. [1]Instituto de Microbiologia da UFRJ, Cidade Universitaria CCS bloco I, Rio de Janeiro, R.J. CEP 21.944, Brazil.

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MEETINGS

Meetings held recently

1. XIIth International Specialized Symposium on Yeast, Genetics of Non-conventional Yeasts, September 13-19, 1987, Weimar, German Democratic Republic.

This yeast meeting considered preferably the genetics and molecular biology of yeasts that are of interest for basic research, that are being used in industrial processes or show potential applications in future biotechnology. Recent results of research on more than 50 species of Candida, Hansenula, Yarrowia, Kluyveromyces, Pichia, Rhodosporidum, Saccharomyces, Schwanniomyces, Zygosaccharomyces and other genera have been published in separate numbers (3,4,5) of volume 28 of the Journal of Basic Microbiology.

Topics of the symposium were:

- Life cycles, Sexuality, Parasexuality
- Genome Structure, Recombination
- Gene Transfer and Cloning
- Regulation of Gene Expression
- Application in Biotechnology

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Beutenbergstraße 11
Jena 6900
German Democratic Republic

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2. XIXth Annual Conference of the Czechoslovak Commission for Yeasts held in Smolenice Castle, 8th to 10th February 1989. Communicated by A. Kocková-Kratochvílová.

Section 1: Cell surfaces and membranes

- M. Opekarová: Vesicles from plasma membranes as a tool for the studies of transport processes.
- J. Voríšek: Classical and alternative imaginations of yeast secretor pathways.
- M. Kopecká: New approaches and knowledges about the cell wall structure of yeasts.
- A. Pichová, I. Rupes and E. Streiblová: The expression of protooncogenes in the relation to the yeast cell wall structure.
- M. Havelková, I. Pokorná, J. Hasek and E. Streiblová: The influence of antitubular toxin nocodazole on the growing yeast protoplasts.

Section 2: Useful yeast enzymes

- D. Smogrovicová and L. Polivka: Starch utilizing yeasts.
- J. Gasperík and E. Hostinová: Structural and functional analysis of the amylolytic complex in Saccharomycopsis fibuligera
- O. Markovic: Pectolytic enzymes in yeasts
- P. Biely: Xylanolytic enzyme systems in yeasts
- O. Volfová, J. Dvoracková and E. Kyslíková: Enzymes of methylotrophic yeasts and their application
- E. Sturdík and R. Kolár: Autolytic enzymes in yeasts
- M. Janderová: Amylolytic yeasts and their construction by gene manipulations
- J. Subík and M. Obernauerová: Biochemistry and genetics of the invertase formation

Posters:

- Briestianská, J. and Svoboda, A.: Microtubuli in secreting mutants of Saccharomyces cerevisiae
- Kotyk, A. and Knotková, A.: The transport of glucosamine into the cells of Saccharomyces cerevisiae
- Sigler, K. and Höfer, M.: H^+ and K^+ flows induced by hydrogen peroxide in yeasts
- Fabianová, M., Janderová, B., Lopatníková, J. and Bendová, O.: Distillery yeasts with the ability to produce glucamylase
- Cvrcková, F., Janderová, B. and Bendová, O.: Construction of brewing yeast strain DEX^+ pof^-
- Janderová, B., Smardová, I. and Cvrcková, F.: The preparation of a strain S. diastaticus without POF gene
- Palková, Z. and Vondrejs, V.: Characterization of hybrids Kluyveromyces lactis x Saccharomyces cerevisiae
- Stepánek, V. and Janderová, B.: Induced fusion of protoplasts of Schwanniomyces alluvius with brewing yeast
- Puta, F. and Wambutt, R.: Construction of double plasmids S. cerevisiae - E. coli prepared for positive selection of recombinants
- Ruttkay-Nedecky, B. and Subík, J.: Study of a common interaction of the mutant pdr3-1 and mutant ogd1 and cyh^R in yeast S. cerevisiae
- Sulo, P., and Michalcáková, S.: Construction of vine yeasts with the killer feature.
- Zigová, M., Lichnerová, Z., Kaclíková, E. and Subík, J.: Partial supression ogd1 mutation by cloned fragment of DNA of standard strain S. cerevisiae
- Pasková, J., Behalová, B. and Spacek, S.: Production of ergosterol and yeast autolysate after the cell plasmolysis of S. cerevisiae
- Demová, E.: Trigonopsis variabilis - the possibility of its utilization in enzymic preparation of the precursor of cephalosporine antibiotics
- Kossaczká, Z., Machová, M. and Vojtková-Lepsíková, A.: Xylose reductase and xylitol dehydrogenase in yeasts.
- Omelková, J.: The influence of saponins on the β-glucosidase activity in yeast cell free extract
- Breierová, E., Sajbidor, J. and Kocková-Kratochvílová, A.: Resistance against freezing and the composition of fatty acids in yeasts
- Michalcaková, S., Sulo, I. and Sláviková, E.: Killer yeasts in the genera Hansenula and Kluyveromyces
- Pecková, M. and Másová, I.: Yeast-like organisms in Czech peloids

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Forthcoming meetings

1. The Genetics and Cellular Biology of Basidiomycetes. The University of Toronto, Centre for Plant Biotechnology. August 3-6, 1989, Erindale Campus.

For further information contact:

Paul A. Horgen, Director
The Centre for Plant Biotechnology
University of Toronto
Erindale Campus
Mississauga, Ontario
Canada L5L 1C6

Telephone (416) 828-5424
Fax: (416) 828-5328

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2. Yeast Cell Biology, August 15-20, 1989, Cold Spring Harbor, N.Y.

Contact:

Meetings Office,
Cold Spring Harbor Laboratory,
Bungtown Rd.,
Cold Spring Harbor, NY 11724 U.S.A.

Telephone: (516) 367-8346.

* * *

3. 13th International Specialized Symposium on Yeast: Production of ethanol and fermented beverages. 18-22 September 1989, Leuven, Belgium.

Contact:

Prof. H. Verachtert,
Laboratory of Industrial Microbiology,
Kardinaal Mercierlaan 92,
3030 Heverlee, Belgium

Tel 016 22 09 31.

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4. Cryptococcus and Cryptococcosis Meeting, 12-16 November, 1989, Jerusalem, Israel.

Contact:

Melia Teum,
P.O. Box 8388,
Jerusalem 91082, Israel

Tel 02-667-402, 02-637-572

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5. 6th International Symposium on Genetics of Industrial Microorganisms (GIM 90), Strasbourg, France, 12-18 August 1990. Communicated by H. Heslot.

The 6th International Symposium of Genetics of Industrial Microorganisms (GIM 90) will be held at the Palais des Congrès, Strasbourg (France), 12-18 August, 1990 under the auspices of the GIM-International Committee.

The GIM 90 is organized by the SOCIETE FRANCAISE DE MICROBIOLOGIE (SFM) with the support of the French Ministries of Agriculture, Education, Industry, Research, the Institut National de la Recherche Agronomique, the Centre National de la Recherche Scientifique.

ORGANIZING COMMITTEE:
Chairman: H. Heslot; Co-chairman, J. Davies; Members: L. Bobichon, J. Davies, P. Dupuy, G. Durand, J. Florent, H. Heslot, L. Penasse, A. Rambach, M. Wegbescher.

SCIENTIFIC PROGRAMME:
The scientific programme, of international scope, will include lectures by renowned scientists and industrialists. Poster sessions will play a key role in the scientific programme. Lectures and posters will be arranged in sessions covering the following themes: protein engineering, genetic instability heterologous expression, secretion, extrachromosomal elements - transposons, metabolic pathways - manipulation, corynebacteria -production of aminoacids, streptomycetes - antibiotics - resistance mechanisms, lactic and bacteria, industrial yeasts, filamentous fungi, methylotrophs - methanogens, genetics of bacillus, clostridia, plant-bacterial interactions, alcoholic and malolactic fementations - aroma, biomass degradation, toxins of microorganisms, bioconversions.

English will be the official language of the Symposium.

In order to help us ascertain your interest in the Symposium and to ensure that you will receive the next circular, please write to:

Symposium Secretariat
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28, rue du Docteur Roux
75724 Paris Cedex 15 (France)

Phone: (1) 45.68.81.79
Telecopy: (1) 45.67.46.98 - Telex 214 403 F

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6. Fourth International Mycological Congress, Regensburg, Fed. Rep. of Germany, August 28 - September 3 1990).

Topics of general mycological interest will be covered. A symposium on the systematics ans evolution of yeasts will be convened by Dr. J.P. van der Walt.

Deadline for abstracts of contributions (including posters) will be June 15 1990. To receive the final Circular (not later than March 1st, 1990), contact:

Prof. Dr. Andreas Bresinsky,
Botanisches Institut der Universitat,
D-8400 Regensburg,
Federal Republic of Germany

Tel: 941 9433108

* * *

7. 14th International Specialized Symposium on Yeasts, Yeast Taxonomy, Theoretical and Practical Aspects, September 3-7 1990, Smolenice, Czechoslovakia.

Organized by the Commission for Yeasts of the Czechoslovak Microbiological Society; Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, Bratislava, in accordance with resolution of the meeting of the International Yeast Commission held in Perugia in 1988, the 14th International Specialized Symposium on Yeasts, YEAST TAXONOMY, THEORETICAL AND PRACTICAL ASPECTS will be held in the Smolenice Castle near Bratislava on September 3-7, 1990. The Symposium will cover the following topics:

1. Traditional and modern approaches in yeast taxonomy
2. New properties of yeasts
3. Evolution of yeasts
4. Taxonomy in relation to biotechnology
5. Environment-induced changes in yeasts
6. Morphological properties and surface structures
7. Preservation of yeasts

Those who are interested in attending the meeting are kindly requested to write before September 30, 1989 to:

Secretariat of the 14th ISSY
Institute of Chemistry
Dubravska cesta 9
842 38 Bratislava
Czechoslovakia

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8. XIth Congress of the International Society for Human and Animal Mycology, Montréal, Québec, Canada, June 24-28, 1991.

To receive the second announcement contrat:

XIth Congress of the International Society for Human and Animal Mycology
c/o JPdl Multi-Management Inc.
1410 Stanley, Suite 609
Montréal, Québec, Canada
H3A 1P8

Tel: (514) 287-1070

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BRIEF NEWS ITEMS

1. A search for strains of Hansenula polymorpha (Pichia angusta?).

We are attempting to assemble a comprehensive collection of Hansenula polymorpha strains (including natural or induced mutants) from various institutes and culture collections.

Should your collection contain strains of this species, we would be extremely grateful if you could send us as many of these (and of related species) as possible, or inform us how cultures may be obtained. Any information on Hansenula polymorpha would be most welcome, together with any papers by yourselves or associates on the same.

All the cultures will be stored in this Institute's Collection for research purposes and made available for the scientific community; also, a current catalogue of wild strains and mutants will be published, and sources and authors mentioned.

Thank you for your time and any assistance you are able to render.

Please contact:

Dr. E. Berardi,
Istituto di Microbiologia A. Capriotti,
Via E. De Nicola
I 07100 Sassari, Italia

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2. Faculty position in Food Microbiology. Communicated by H. J. Phaff.

The Department of Food Science and Technology seeks a microbiologist for a tenure-track position as Assistant Professor and Assistant Microbiologist in the Agricultural Experiment Station. The candidate will be expected to use molecular or classical approaches in developing a research program that investigates microorganisms or microbiological problems of importance to foods or food processing. Applicants will be selected for interviews on the basis of research accomplishments, potential for developing a strong research program, and ability to train, teach, and advise students in food science and related basic disciplines. Graduate training at Davis uses the Graduate Group system, and the appointee would direct Ph.D. and M.S. students from the Microbiology, Food Science, Biochemistry, and/or Genetics Graduate Groups, depending on research interests. Undergraduate teaching responsibilities will be coordinated with the four microbiologists currently in the Department and will be within the appointee's area of specialization. A Ph.D. in Microbiology or related field is required. Send curriculum vitae, publication list, statement of research interests, and the names and addresses of four references to: Dr. C. W. Price, Department of Food Science and Technology, University of California, Davis, 95616. To assure consideration, please submit application by May 1, 1989. U.C. Davis is an Equal Opportunity/Affirmative Action Employer.

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3. International Commission on Yeasts and Yeast-like Organisms - ICY News. Communicated by A. Martini.

Proceedings of 7th ISY

The Proceedings of the 7th ISY which was held in Perugia, Italy in 1988 have just come out as a special supplement of the journal Yeast (April 1989, volume 5). For those who have made prepayments, the volumes were sent off by surface mail in early May, 1989. Additional copies (surface mail) may be obtained from A. Martini, at the price of Italian Lit. 50,000, payable to:

The 7th International Symposium on Yeasts
Account #051546/01
Cassa di Risparmio di Perugia
Piazza Universita
06100 Perugia, Italy

Anyone interested in purchasing the volume should write directly to A. Martini at the address below.

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Participation in ISY

As a consequence of the growing interest in yeasts as protagonists of genetics and biotechnology, in recent years many new yeast-centered communities of scientists, such as the Yeast Molecular Biologists and Geneticists, have been formed, while already existing scientific societies, such as the International Society for Human and Animal Mycology, have known a renewed renaissance.

These groups in turn organize periodic meetings, always attended by many people. For example, in 1988, in the space of less than 40 days, four major meetings were held: the ISHAM meeting in Barcelona, Spain, the Biotechnology Symposium in Paris, France, the 7th International Symposium on Yeasts in Perugia, Italy and the Yeast Molecular Biology and Genetics meeting in Helsinki, Finland.

These circumstances could explain why our general symposia have experienced a progressive decline in attendance, from 400 delegates in Den Hague (1969), Vienna (1974), and London, Ontario (1980), to less than 300 in Montpellier (1984) and 225 in Perugia (1988). Fortunately the same trend cannot be observed in relation to the attendance of Specialized Symposia sponsored by ICY.

As members of the oldest community of persons interested in yeasts, it could be useful to undertake some initiatives in order to stop this progressive loss of identity and reverse the present trend.

As the recently elected Chairman of the International Commission on Yeasts and Yeast-like Organisms, I would appreciate any suggestions, advice and criticism from national representatives of the Commission representatives or write directly to me at the address below.

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Directory of persons associated with ICY

During the last meeting of the International Commission on Yeasts and Yeast-like Organisms (ICY), held during the 1988 General Symposium in Perugia, it was suggested that the Chairman should explore the possibility of publishing a Directory of all persons presently and in the past associated with general and specialized symposia of ICY. Specific requests for name lists will be addressed in due time to all national members of ICY. Suggestions and criticism on this initiative are welcome.

Prof. A. Martini,
Dipartimento di Biologia Vegetale,
Borgo 20 Giugno 74,
I-06100 Perugia, Italy.

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TITLES OF INTEREST

The following reviews, which may be of general interest to our readers, have been published in Microbiological Reviews recently (see editorial).

1. Herskowitz, I.[1] 1988. Life Cycle of the Budding Yeast Saccharomyces cerevisiae. Microbiol. Rev. 52:536-553. [1]Department of Biochemistry & Biophysics, University of California, San Francisco, San Francisco, California 94143 USA.

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2. Newlon, C.S.[1] 1988. Yeast Chromosome Replication and Segregation. Microbiol. Rev. 52:568-601. [1]Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, New Jersey 07103 USA.

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3. Fincham, J.R.S.[1] 1988. Transformation in Fungi. Microbiol. Rev. 52:148-170. [1]Department of Genetics, University of Cambridge, Cambridge CB2 3EH, United Kingdom.

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4. Warner, J.R.[1] 1989. Synthesis of ribosomes in Saccharomyces cerevisiae. Microbiol. Rev. 53:256-271. [1]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461 USA.

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