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Y E A S T

A Newsletter for Persons Interested in Yeast

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

Editorials

Dorothy Spencer 1933-1995

I and the entire community of researchers interested in yeasts were shocked by the news of Dorothy Spencer's passing on July 24, as a result of liver cancer. I have a special debt to Dorothy, as she acted as a guide, coordinator, navigator, and host during my collection trip in northern Argentina with Herman Phaff, in 1990.

Frank informed me that several people have enquired about Dorothy's favourite charity, intending to make donations in her memory. Dorothy was indeed particularly impressed with the work of an organization that takes care of homeless children, in Tucuman. Anyone interested in contributing should forward donations to Señora Martine Siñeriz, c/o F. Siñeriz, Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Avenida Belgrano y Pasaje Caseros, 4000 S.M. de Tucuman, Argentina.

Possible delays in publication

I am currently on sabbatical leave until June 30 1996. As a result, some delays can be expected in the publication of the June 1996 issue. I therefore ask our readers for their patience in the circumstances.

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

M. A. Lachance
Editor

I. Celebrating 50 years of scientific excellence: Herman J. Phaff

The recent publication of a special issue of the Journal of Industrial Microbiology [**14**(6), June 1995] marked the 50th anniversary of Herman J. Phaff's career in yeast research. I quote Sally A. Meyer's dedicatory paragraph: "This Special Issue of the Journal of Industrial Microbiology is dedicated to Herman Jan Phaff by his students, post-doctoral associates and close colleagues. Herman has been a world leader in the ecology, physiology and taxonomy of yeasts. The purpose of the Special Issue is to celebrate Herman's fifty years of active research which is still continuing. Many of us owe the success of our careers to his scholarship, teaching, professionalism and, above all, his deep friendship." Sally's eloquent words summarize the feelings of many Yeast Newsletter readers, myself included. Not the least of Herman's contributions was his 34 year term as Editor of the Yeast Newsletter. On behalf of all readers, I wish to express my most heartfelt congratulations to Herman, as well as my gratitude for all he has done for us, his students and colleagues. The table of contents of the Special Issue, reprinted here, attests to the diversity of interests generated by Herman's teachings.

Dedication: Herman Jan Phaff.
Sally A. Meyer.

Life with yeasts during retirement.
Herman J. Phaff.

Carbon source nutrition of rapamycin biosynthesis in *Streptomyces hygroscopicus*.
I. Kojima, Y.R. Cheng, V., Mohan and A.L. Demain.

Chemical and cytological changes during the autolysis of yeasts.
Tatang Hernawan and Graham Fleet.

Increased resistance to antifungal antibiotics of *Candida* spp. adhered to silicone.
A.V. Kalya and D.G. Ahearn.

The phylogenetic relationships of *Eeniella nana* Smith, Batenburg-van der Vegte et Scheffers based on the partial sequences of 18S and 26S ribosomal RNAs (Candidaceae).
Yuzo Yamada, Minako Matsuda and Kozaburo Mikata.

Flocculation of industrial and laboratory strains of *Saccharomyces cerevisiae*.
Carmen Sieiro, Natalia M. Reboredo and Tomas G. Villa.

Industrial production of soy sauce.
B.S. Luh.

Yeasts associated with algarrobo trees (*Prosopis* spp.) in northwest Argentina: a preliminary report.
Dorothy M. Spencer, J.F.T. Spencer, E. Fengler and L.I. de Figueroa.

rDNA targeted oligonucleotide primers for the identification of pathogenic yeasts in a polymerase chain reaction.
Jack W. Fell.

Glucanases and chitinases of *Bacillus circulans* WL-12.
Hirosato Tanaka and Takeshi Watanabe.

Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: a comparison with western communities.
Marc-André Lachance, Donald G. Gilbert and William T. Starmer.

Continuous production of non-alcohol beer by immobilized yeast at low temperature.
M.F.M. van Iersel, E. Meersman, W. Swinkels, T. Abee and F.M. Rombouts.

Carotenoids protect *Phaffia rhodozyma* against singlet oxygen damage.
William A. Schroeder and Eric A. Johnson.

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

Facts, myths and legends on the prime industrial microorganism.
Ann Vaughan-Martini and Alessandro Martini.

Relationships among the genera *Ashbya, Eremothecium, Holleya* and *Nematospora* determined from rDNA sequence divergence.
Cletus P. Kurtzman.

Candida amapae, a new amino acid-requiring yeast from the Amazonian fruit *Parahancornia amapa*.
Paula B. Morais, Carlos A. Rosa, Sally A. Meyer, Leda C. Mendonça-Hagler and Allen N. Hagler.

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

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

NAME	ATCC# DEPOSITOR/STRAIN	SIGNIFICANCE/REFERENCE
Arthroascus fermentans	96488 F.L. Lee 80D2303	Type culture (Int. J. Syst. Bacteriol. **44**:303-307, 1994)
Candida utilis	96621 P-L. Yu Y15	Fermentation of apple pomace to produce animal feed (World J. Microbiol. & Biotechnol. **11**:168-170, 1995)
Kloeckera apiculata	96620 P-L. Yu	Fermentation of apple pomace to produce animal feed (World J. Microbiol. & Biotechnol. **11**: 168-170, 1995)
Kluyveromyces lactis	96395 G.D. Clark-Walker CK56-7C	Genotype: *MATα uraA1 ade1 ade2 sir2-1.* Hypersensitive to ethidium bromide (Mol. Cell. Biol. **14**:4501-4508, 1994)
Kluyveromyces lactis	96396 G.D. Clark-Walker CK53/1	Genotype: *MATa uraA1 ade2 sir2::URA3.* Hypersensitive to ethidium bromide (Mol. Cell. Biol. **14**:4501-4508, 1994)
Phaffia rhodozyma	96594 C. Vagvolgyi	Produces astaxanthin (FEMS Microbiol. Lett. **123**:315-318, 1994; Biotechniques **18**:64-65, 1995)
Pichia angusta	96694 M.D. Ter-Avanesyan 1B	Genotype: *ade2 leu2.* Transformation host (Yeast **11**:343-353, 1995)
Pichia angusta	96695 M.D. Ter-Avanesyan 8V	Genotype: *leu2.* Transformation host (Yeast **11**:343-353, 1995)
Rhodosporidium babjevae	90942 W.I. Golubev VKM Y-2275	Type, mating type A_1 (Syst. Appl. Microbiol. **16**:445, 1993)
Rhodosporidium babjevae	90943 W.I. Golubev VKM Y-2276	Type, mating type A_2 (Syst. Appl. Microbiol. **16**:445, 1993)
Saccharomyces cerevisiae	20750 Collaborative Res. CGY1285	Genotype: *MATα ura3-52 pep4-3 ssc1-1.* Transformation host (U.S. Pat. 5,057,416)
Saccharomyces cerevisiae	20751 Collaborative Res. CGY1083	Genotype: *MATα ura3-52 his4-27 pep4-3 ssc3-1.* Transformation host (U.S. Pat. 5,057,416)
Saccharomyces cerevisiae	20752 Collaborative Res. CGY1291	Genotype: *MATα ura3-52 his4-27 pep4-3 ssc2-1.* Transformation host (U.S. Pat. 5,057,416)
Saccharomyces cerevisiae	20753 Collaborative Res. CGY998	Genotype: *MATα ura3-52 his4-27 pep4-3.* Transformation host (U.S. Pat. 5,057,416)
Saccharomyces cerevisiae	76193 A. Tzagoloff M7-40/A1	
Saccharomyces cerevisiae	76194 A. Tzagoloff aM6-200-2C	
Saccharomyces cerevisiae	76195 A. Tzagoloff M6-200/A1	
Saccharomyces cerevisiae	76196 A. Tzagoloff M9-94/A7	
Saccharomyces cerevisiae	76197 A. Tzagoloff M9-3/A3	
Saccharomyces cerevisiae	76198 A. Tzagoloff DS31	
Saccharomyces cerevisiae	76199 A. Tzagoloff X14-25	
Saccharomyces cerevisiae	96361 N.A. Woychik WY-35	Genotype: *MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3/leu2-3 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2 rpb6/rpb6Δ2::HIS3.* Transformation host (Mol. Cell. Biol. **14**:4155-4159, 1994)
Saccharomyces cerevisiae	96362 M. Gobbetti 141	Maltose-positive leavening agent in sourdough (World J. Microbiol. Biotechnol. **10**:275-279, 1994).
Saccharomyces cerevisiae	96364 S. Hohmann YSH 3.131.-6D	Genotype: *MATα leu2-3 leu2-112 ura3-52 trp1-92 byp1-3.* Transformation host (Curr. Genet. **26**:295-301, 1994)
Saccharomyces cerevisiae	96397 S.J. Elledge Y166	Genotype: *MATa gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3 leu2-112 rnr3::GAL1--->URA3 [GAL1-lacZ].* Two-hybrid system.

Saccharomyces cerevisiae	96398	S.J. Elledge Y153	Genotype: *MATa leu2-3 leu2-112 ura3-52 trp1-901 his3-Δ200 ade2-101 gal4Δgal80Δ URA3::GAL-lacZ LYS2::GAL-HIS3.* Transformation host in two-hybrid system (Genes & Dev. **7**:555-569, 1993)
Saccharomyces cerevisiae	96399	S.J. Elledge Y187	Genotype: *MATα gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3 leu2-112 URA3 GAL1--->lacZ met-.* Two-hybrid system (Cell **75**:805-816, 1993)
Saccharomyces cerevisiae	96400	S.J. Elledge Y190	Genotype: *MATa leu2-3 leu2-112 ura3-52 trp1-901 his3-Δ200 ade2-101 gal4Δgal80ΔURA3 GAL-lacZ LYS GAL-HIS3 cyh(r).* Transformation host in two-hybrid system (Cell **75**:805-816, 1993).
Saccharomyces cerevisiae	96408	S. Pestka AB1380	Carrying a 540 kb YAC with genes for human GART and IFN-gamma accessory factor (Genet. Anal. Tech. Appl. **10**:109-112, 1993; Proc. Natl. Acad. Sci. USA **90**:8737-8741, 1993; J. Biol. Chem. **269**:7013-7018, 1994)
Saccharomyces cerevisiae	96469	S.C. Jong HB-1	Edme active dry brewers' yeast for beer, Essex, England
Saccharomyces cerevisiae	96470	S.C. Jong HB-2	Coopers active dry brewers' yeast, Australia
Saccharomyces cerevisiae	96471	S.C. Jong HB-3	Muntons active dry brewers' yeast for ale, United Kingdom
Saccharomyces cerevisiae	96472	S.C. Jong HB-4	Nottingham active dry brewers' yeast for English ale, Canada
Saccharomyces cerevisiae	96473	S.C. Jong HB-5	Windsor active dry brewers' yeast for English ale, Canada
Saccharomyces cerevisiae	96474	S.C. Jong HB-6	Kent active dry brewers' yeast for Amsterdam lager, Ann Arbor, MI
Saccharomyces cerevisiae	96475	S.C. Jong HB-7	Yeast liquid brewers' yeast for American ale, Mt. Hood, OR
Saccharomyces cerevisiae	96476	S.C. Jong HB-8	Kent dry brewers' yeast for European lager, Ann Arbor, MI
Saccharomyces cerevisiae	96482	J.M. Birmingham HB-9	Kent dry brewers' yeast for Whitbread ale, Ann Arbor, MI
Saccharomyces cerevisiae	96483	J.M. Birmingham HB-10	Kent dry brewers' yeast for European lager, Ann Arbor, MI
Saccharomyces cerevisiae	96484	J.M. Birmingham HB-11	Brewers' yeast
Saccharomyces cerevisiae	96485	N. Gunge YTR-R1	Genotype: *MATa RAD4 leu2 his1 his4 [rho$^+$].* UV-resistant transformation host (Curr. Genet. **26**:369-373, 1994)
Saccharomyces cerevisiae	96486	N. Gunge YTR-r1	Genotype: *MATa rad4 leu2 his1 his4 [rho$^+$].* UV-hypersensitive transformation host (Curr. Genet. **26**:369-373, 1994)
Saccharomyces cerevisiae	96487	N. Gunge YAT547 Japan	Transformation host (Curr. Genet. **26**:369-373, 1994)
Saccharomyces cerevisiae	96494	NRRL Y-17592	Genotype: *ID41-6/161/AIO MATα ade$^-$ his$^-$ [rho^o].* Killer toxin-sensitive (Yeast **10**:403-414, 1994)
Saccharomyces cerevisiae	96495	J.M. Birmingham HB-12	Willliam's liquid brewers' yeast for Czechoslovakian pilsner, San Leandro, CA
Saccharomyces cerevisiae	96496	J.M. Birmingham HB-13	William's liquid brewers' yeast for American lager, San Leandro, CA
Saccharomyces cerevisiae	96497	J.M. Birmingham HB-14	William's brewers' yeast for English ale, San Leandro, CA
Saccharomyces cerevisiae	96498	J.M. Birmingham HB-15	William's brewers' yeast for German alt, San Leandro, CA
Saccharomyces cerevisiae	96499	J.M. Birmingham HB-17	William's brewers' yeast for Burton ale, San Leandro, CA
Saccharomyces cerevisiae	96500	J.M. Birmingham HB-18	William's liquid brewers' yeast for Bavarian lager, San Leandro, CA
Saccharomyces cerevisiae	96501	J.M. Birmingham HB-19	William's brewers' yeast for Abbey ale, San Leandro, CA
Saccharomyces cerevisiae	96502	J.M. Birmingham HB-20	William's liquid brewers' yeast for California ale, San Leandro, CA
Saccharomyces cerevisiae	96503	J.M. Birmingham HB-21	William's liquid brewers' yeast for Bay Area lager, San Leandro, CA

Saccharomyces cerevisiae	96504 J.M. Birmingham HB-22	William's brewers' yeast for Munich lager, San Leandro, CA
Saccharomyces cerevisiae	96505 J.M. Birmingham HB-23	William's liquid brewers' yeast for Delbruckii wheat, San Leandro, CA
Saccharomyces cerevisiae	96506 J.M. Birmingham HB-24	William's liquid brewers' yeast for German Kolsch, San Leandro, CA
Saccharomyces cerevisiae	96507 J.M. Birmingham HB-27	William's brewers' yeast for cask ale, San Leandro, CA
Saccharomyces cerevisiae	96508 J.M. Birmingham HB-28	William's brewers' yeast for Scottish ale, San Leandro, CA
Saccharomyces cerevisiae	96515 D. Jenness DJ211-5-3	Genotype: *MATa cry1 bar1-1 ade2-1 his4-580 leu2 lys2 trp1 tyr1 ura3 SUP4-3.* Temperature- and α-factor-sensitive transformation host (Mol. Cell. Biol. **14**:7245-7255, 1994)
Saccharomyces cerevisiae	96517 F. Messenguy 10R34d-II	Genotype: *ura3 argRII::CAR1.* Transformation host (Yeast **10**:923-933, 1994)
Saccharomyces cerevisiae	96518 F. Messenguy 12T7C1	Genotype: *ΔCAR1 ura3*$^{-}$
Saccharomyces cerevisiae	96519 P. Heiter YPH250	Genotype: *MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1*. Transformation host (Genetics **122**:19-27, 1989; Gene (Amst.) **148**:179-185, 1994). Phytosphingosine-sensitive (Proc. Natl. Acad. Sci. USA **91**:7899-7902, 1994). Revised: August 1995
Saccharomyces cerevisiae	96542 M. Johnson YM3293	Genotype: *MATα tyr1 ura3-52 his3Δ200 ade2-101 lys2-801::[UAS Leu2-URS Gal1-lacZ] (pBM1926) can1*. Transformation host (Mol. Cell. Biol. **14**:3834-3841, 1994)
Saccharomyces cerevisiae	96543 M. Johnson YM3296	Genotype: *MATα tyr1 ura3-52 hisΔ200 ade2-101 lys2-801: :[UAS GAL1-lacZ] (pBM1927)*. Transformation host (Mol. Cell. Biol. **14**:3834-3841, 1994)
Saccharomyces cerevisiae	96581 H. Jeppsson 3	Produces high ethanol, heavily flocculating (Appl. Environ. Microbiol. **58**:1661-1669, 1992)
Saccharomyces cerevisiae	96604 C. Fairhead FY1679	Genotype: *MATa MATα ura3-52/ura3-52 trp1-Δ63/TRP1 his3-Δ200/HIS3 leu2-Δ1/LEU2*. (Yeast **10**:1403-1413, 1994; ibid., **11**:53-55, 1995)
Saccharomyces cerevisiae	96606 C.W. Lawrence CL1265-2	Genotype: *MATα rev7-1 arg4-17 ura3-52 leu2-3 leu2-112 his3-Δ1 trp1*. Transformation host (Yeast **10**:1503-1509, 1994)
Saccharomyces cerevisiae	96607 C.W. Lawrence AH8-3D	Genotype: *MATa rev7-2 arg4-17 ura3-52 leu2-3 leu2-112 lys2-1*. Transformation host (Yeast **10**:1503-1509, 1994)
Saccharomyces cerevisiae	96609 S. Hahn SHY93	Genotype: *MATα leu2 ura3 his4 ΔTOA1::HIS4/pSH325 (ARS CEN URA3 TOA1)*. Complementation analysis (Mol. Cell. Biol. **15**:1234-1243, 1995)
Saccharomyces cerevisiae	96610 S. Hahn SHY94	Genotype: *MATα ade*$^{-}$ *ura3 his4 leu2 ΔTOA2::HIS4/pSH342 (ARS CEN URA3 TOA2)*. Complementation analysis (Mol. Cell. Biol. **15**:1234-1243, 1995)
Saccharomyces cerevisiae	96665 A.G. Hinnebusch H1816	Genotype: *MATa ura3-52 leu2-3 leu2-112 trp1-Δ63 sui2Δ gcn2Δ p1108 [GCN4-lacZ TRP1] at trp1-Δ63, p1097 [SUI2 LEU2]*. Transformation host (Mol. Cell. Biol.**15**:365-378, 1995)
Saccharomyces cerevisiae	96666 A.G. Hinnebusch H1894	Genotype: *MATa ura3-52 leu2-3 leu2-112 gcn2Δ trp1-Δ63.* Transformation host (Mol. Cell. Biol. **15**:365-378, 1995)
Saccharomyces cerevisiae	96667 A.G. Hinnebusch H2384	Genotype: *MATa ura3-52 gcn4-103 leu2-3 leu2-112 trp1-63 ino1 [HIS4-lacZ LEU2]*. Transformation host (Mol. Cell. Biol. **15**:1220-1233, 1995)

Saccharomyces cerevisiae	96668	A.G. Hinnebusch H2032	Genotype: *MATα ura3-52 his1-29 leu2-3 leu2-112 trp1-63 gcn4::LEU2*. Transformation host (Mol. Cell. Biol. **15**:1220-1233, 1995
Saccharomyces cerevisiae	96669	A.G. Hinnebusch H2036	Genotype: *MATa ura3-52 gcn4-103 leu2-3 leu2-112 trp1-63 ino1*. Transformation host (Mol. Cell. Biol. **15**:1220-1233, 1995)
Saccharomyces cerevisiae	96670	A.G. Hinnebusch H2359	Genotype: *MATa ura3-52 gcn4-103 leu2-3 leu2-112 trp1-63 ino1 [HIS4-lacZ LEU2]*. Transformation host (Mol. Cell. Biol. **15**:1220-1233, 1995)
Saccharomyces cerevisiae	96673	T.D. Petes AS4	Genotype: *MATa trp1 arg4-17 tyr7 ade6 ura3*. Wild-type transformation host (Mol. Cell. Biol. **15**:1679-1688, 1995)
			Saccharomyces cerevisiae 96674 T.D. Petes AS13Genotype: *MATα leu2 ade6 ura3*. Wild-type transformation host (Mol. Cell. Biol. **15**:1679-1688, 1995)
Saccharomyces cerevisiae	96686	D.J. Jamieson S150-2B	Genotype: *MATa leu2-3 leu2-112 ura3-52 trp1-289 his3-Δ1 Gal*$^+$. Transformation host (Microbiology (Reading) **140**: 3277-3283, 1994)
Saccharomyces cerevisiae	96687	D.J. Jamieson DJY118	Genotype: *MATa leu2-3 leu2-112 ura3-52 trp1-289 his3-Δ1 sod1::URA3 GAL*$^+$. 96686 transformed with *Eco*Rl/*Sal*I cut pDJ51 (Microbiology (Reading) **140**:3277-3283, 1994)
Saccharomyces cerevisiae	96688	D.J. Jamieson DJY119	Genotype: *MATa leu2-3 leu2-112 ura3-52 trp1-289 his3-Δ1 sod2::TRP1 GAL*$^+$. 96686 transformed with *Eco*Rl/*Hin*dIII-cut pDJ50 (Microbiology (Reading) **140**:3277-3283, 1994)
Saccharomyces exiguus	96363	M. Gobbetti M14	Maltose-negative leavening agent in sourdough (World J. Microbiol. Biotechnol. **10**:275-279, 1994)
Saccharomyces kluyveri	76530	CBS 5828	Type culture of *Saccharomyces silvestris* (K. Vet. Landbohojsk. Arsskr. **1967**:179-194, 1967)
Schizosaccharomyces pombe	96529	K. Wolf NG5/6	Genotype: *h*$^+$ *ade7 [cox1I1*$^-$ *cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]*. Respiratory-deficient nuclear petite mutant (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96530	K. Wolf 46/1	Genotype: *h*$^+$ *ade7 [cox1I1Δ cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]*. Respiratory-deficient, deletion in mitochondrial *cox1*$^-$ intron (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96531	K. Wolf P4E(R)	Genotype: *h*$^-$ *ade7 [eryr cox1I1*$^-$ *cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]*. Erythromycin-resistant, no mitochondrial introns (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96532	K. Wolf P4D(R)	Genotype: *h*$^-$ *ade7 [diur cox1I1*$^-$ *cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]* Diuron-resistant, no mitochondrial introns (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96533	K. Wolf X39E(R)	Genotype: *h*$^+$ *ade7 [eryr cox1I1*$^+$ *cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]* Erythromycin-resistant, only one mitochondrial intron in cox1 (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96534	K. Wolf X39D(R)	Genotype: *h*$^+$ *ade7 [diur cox1I1*$^+$ *cox1I2a*$^-$ *cox1I2b*$^-$ *cox1I3*$^-$ *cobI*$^-$*]* Diuron-resistant, only one mitochondrial intron in *cox1* (Curr. Genet. **25**:336-341, 1994)
Schizosaccharomyces pombe	96611	C.E. Ballou LBP6-6B	Mating type h$^{(-S)}$, genotype: *gmn1*. (Proc. Natl. Acad. Sci. USA **91**:9327-9331, 1994; ibid., **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96612	C.E. Ballou LBP6-6A	Mating type h$^{(+N)}$, genotype: *gmn1*. (Proc. Natl. Acad. Sci. USA **91**:9327-9331, 1994; ibid., **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96613	C.E. Ballou LBP12-5B	Mating type h$^{(+N)}$, genotype: *gmn2*. (C.E. Ballou, personal communication)

Schizosaccharomyces pombe	96614	C.E. Ballou LBP17-6A	Mating type $h^{(-S)}$, genotype: *gmn2*. (C.E. Ballou, personal communication)
Schizosaccharomyces pombe	96615	C.E. Ballou LBP17-5A	Mating type $h^{(+N)}$, genotype: *gmn3*. (C.E. Ballou, personal communication)
Schizosaccharomyces pombe	96616	C.E. Ballou LBP14-3B	Mating type $h^{(-S)}$, genotype: *gmn1 gmn2*. (Proc. Natl. Acad. Sci. USA **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96617	C.E. Ballou LBP14-2A	Mating type $h^{(+N)}$, genotype: *gmn1 gmn2*. (Proc. Natl. Acad. Sci. USA **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96618	C.E. Ballou LBP17-2B	Mating type $h^{(-S)}$, genotype: *gmn1 gmn3*. (Proc. Natl. Acad. Sci. USA **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96619	C.E. Ballou LBP17-5B	Mating type $h^{(+N)}$, genotype: *gmn1 gmn3*. (Proc. Natl. Acad. Sci. USA **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96622	C.E. Ballou LBP16-2A	Mating type $h^{(-S)}$, genotype: *gmn1*. (Proc. Natl. Acad. Sci. USA **92**:2790-2794, 1995)
Schizosaccharomyces pombe	96696	O. Fleck LH110	Genotype: h^{90} *swi4::ura4+ ura4-D18.* Segregates heterothallic h^{+} due to duplication in the mating type region (Nucl. Ac. Res. **20**:2271-2278, 1992; ibid., **22**:5289-5295, 1994)
Stephanoascus smithiae	96582	G. Gimenez-Jurado IGC 4646.	Type culture (Syst. Appl. Microbiol. **17**:240, 1994)
Stephanoascus smithiae	96583	G. Gimenez-Jurado IGC 5052	Taxonomy (Syst. Appl. Microbiol. **17**: 237-246, 1994)
Yamadazyma acaciae	96491	NRRL Y-17589	Contains linear plasmid pPacl-1, lacks pPacl-2 (Yeast **10**:403-414, 1994)
Yamadazyma acaciae	96492	NRRL Y-17590	Contains linear plasmids pPacl-1 and pPacl-2Δ1 (Yeast **10**:403-414, 1994)
Yamadazyma acaciae	96493	NRRL Y-17591	Cured of linear plasmids pPacl-1 and pPacl-2 (Yeast **10**:403-414, 1994)
Yamadazyma farinosa	90009	H. Ohta IAM 4682	

Workshops offered by ATCC in 1996.

February 15-16
Anaerobic Bacteriology
April 1-5
Recombinant DNA: Techniques & Applications
April 9-12 & November 19-22
Polymerase Chain Reaction (PCR) Applications/Cycle DNA Sequencing
April 16-19
Basic Techniques in Molecular Mycobacteriology
September 18-20
Downstream Processing, Recovery and Purification of Proteins
September 24-27
Fermentation Microbiology
October 2-4
Microscopy/Photomicrography
October 15-18
Freezing & Freeze-Drying of Microorganisms
November 11 -15
Advanced Recombinant DNA Techniques & Applications

For information on ATCC Workshops contact:
ATCC, Workshop Coordinator, 12301 Parklawn Drive, Rockville Md U.S.A. 20852.
Telephone: (301) 231 -5566
FAX: (301) 816-4364
ATCC Internet Address:
http://www.atcc.org/workshops/workshop.html

III. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC Delft, The Netherlands. Communicated by M.Th. Smith <cbs@dutsf29.stm.tudelft.nl>.

A. New acquisitions:

Bensingtonia musae M. Takashima *et al.*
7965[T] dead leaf of *Musa paradisiaca*, Thailand, T. Nakase

Candida amapae P.B. Morais *et al.*
7872[T] fruit of *Parhancornia amapa,* Brazil, A.N. Hagler

Candida boleticola Nakase
7844 rotten leaf, China
7847 soil, China, Bai Feng-yan

Candida caseinolytica Phaff *et al.*
7881 *Opuntia linderheimeri* (cactus), USA
7882 *Opuntia phaeacantha* (cactus), USA, H.J. Phaff

Candida cellulolytica Nakase *et al.*
7920^{T} exudate of broad-leaved tree, Japan, JCM

Candida dublinensis D.J. Sullivan *et al.*
7987^{T} oral cavity of HIV-infected patient, Ireland
7988 oral cavity of HIV-infected patient, Australia, D.C. Coleman

Candida ernobii (Lodder & Kreger-van Rij) S.A. Meyer & Yarrow
7891 black gall on *Populus tremuloides*, Canada
7892 under bark of *Populus tremuloides*, Canada, L.J. Hutchison (Hutchison & Hiratsuka, Mycologia **86**:386-391, 1994)

Candida freyschussii H.R. Buckley & van Uden
7845 fermented vegetables, China, Bai Feng-yan

Candida fukuyamaensis Nakase *et al.*
7921^{T} water of pond, Japan, JCM

Candida kruisii (Kocková-Kratochvílová & Ondrusová) S.A. Meyer & Yarrow
7846 leaf, China, Bai Feng-yan

Candida rugosa (H.W. Anderson) Diddens & Lodder
7883 throat of 63-year-old woman, Finland
7884 faeces of 53-year-old woman, Finland
7885 throat of 83-year-old woman, Finland, J. Issakainen

Candida sophiae-reginae C. Ramírez & A. González
7954 bitter-lemon drink, Netherlands, C.J.M. Geurts

Candida sorbophila (Nakase) S.A. Meyer & Yarrow
7922 ear of baby, Germany, R. Kappe

Candida tenuis Diddens & Lodder
7880 liquid sweetener, Netherlands, E.S. Hoekstra (gives atypical growth results on methyl-α-glucoside, L-arabinitol, 50% glucose)

Cryptococcus skinneri Phaff & do Carmo-Sousa
7890 slightly blue-stained wood of *Populus tremuloides*, Canada, L.J. Hutchison (Hutchison & Hiratsuka, Mycologia **86**:386-391, 1994)

Cryptococcus terreus di Menna
7943 (IGC 4697) decaying toadstool, Portugal
7944 (IGC 4698) soil, Portugal
7945 (IGC 4700) shelf-fungus, Portugal, IGC

Debaryomyces polymorphus (Klöcker) C.W. Price & Phaff var. *africanus* van der Walt *et al.*
7902 uncultivated soil, South Africa, J.P. van der Walt

Filobasidiella neoformans Kwon-Chung var. *neoformans*
7926 Cap59^{-} mutant of NIH B-3501
7927 Cap55^{-} mutant of NIH B-3501
7928 Cap60^{-} mutant of NIH B-3501
7929 Cap64^{-} mutant of NIH B-3501
7930 Cap66^{-} mutant of NIH B-3501
7931 Cap67^{-} mutant of NIH B-3501
7932 Cap81^{-} mutant of NIH B-3501
7933 Cap^{+} mutant of NIH B-3501
7934 Cap55^{-} mutant of NIH B-3501
7935 Cap60^{-} mutant of NIH B-3501
7936 Cap67^{-} mutant of NIH B-3501
7937 Cap331^{-} mutant of NIH B-3501
7938 Cap172^{-} mutant of NIH B-3501, E.S. Jacobson (Jacobson & Tingler, J. Med. Vet. Mycol. **32**:401-404, 1994)
7952 woman, aged 41 years, HIV positive (atypical strain), Sweden, C. Jarstrand

Kluyveromyces marxianus (E.C. Hansen) van der Walt
7858 M. Ingram -> J.A. Barnett -> 1954, NCYC (NCYC 426) -> 1994, J.I. Castrillo

Lipomyces tetrasporus Krasil'nikov *et al.* ex Nieuwdorp *et al.*
7939 soil, South Africa
7940 soil, South Africa
7941 soil, Canada
7942 soil, South Africa, J.P. van der Walt

Malassezia furfur (Robin) Baillon
7982 skin of ear, France
7983 systemic infection, France
7984 healthy skin of ear of elephant, France
7985 wing of *Struthio camelus* (ostrich), France, E. Guého

Malassezia pachydermatis (Weidman) C.W. Dodge
7925 axilla of healthy beagle dog, UK, R. Bond, requires lipids for growth (Bond & Antony, J. Appl. Bacteriol. **78**:537-542, 1995)

Malassezia sympodialis Simmons & E. Guého
7977 skin of patient with pityriasis versicolor, France
7978 skin of patient with pityriasis versicolor, UK
7979 healthy skin, UK
7980 healthy skin, UK
7981 skin of female patient with pityriasis versicolor, France, E. Guého

Myxozyma neotropica Spaaij *et al.*
7953 soil, Italy, E. Berardi

Pichia farinosa (Lindner) E.C. Hansen
7911 (T of *Pichia petrophila* Mou) soy sauce, China, NRRL (Mou *et al.*, Acta Microbiol. Sin. **19**:259-264, 1979)

Pichia euphorbiiphila (van der Walt) Kurtzman
7912 (NRRL Y-12743) MT a
7913 (NRRL Y-12744) MT α

Pichia kluyveri Bedford ex Kudryavtsev var. *kluyveri*
7907 (NRRL Y-17734) MT h^+
7908 (NRRL Y-17752) MT h^-, NRRL

Pichia kluyveri Bedford ex Kudryavtsev var. *cephalocereana* Phaff *et al.*
7909 (NRRL Y-17718) MT h^+
7910 (NRRL Y-17738) MT h^-, NRRL

Pichia kluyveri Bedford ex Kudryavtsev var. *eremophila* Phaff *et al.*
7905 (NRRL Y-17751) MT h^-, *Opuntia* sp., Mexico
7906 (NRRL Y-17753) MT h^+, NRRL

Pichia veronae K. Kodama
7924 log of oak infested with ambrosia beetles, Slovenia, B. Gorald

Rhodotorula bacarum (Buhagiar) Rodrigues de Miranda & Weijman
7946 (IGC 4666) leaf of *Platanus* sp., Portugal (differs from type by growing on inositol)

Rhodotorula bogoriensis (Deinema) von Arx & Weijman
7947 (IGC 4918) leaf litter, Portugal, IGC

Rhodotorula ferulica J.P. Sampaio & van Uden
7948 (IGC 4825) stagnant water, IGC

Rhodotorula fujisanensis (Soneda) Johnson & Phaff
7949 (IGC 4692) dry leaf, Portugal
7950 (IGC 4693) wood of Quercus sp., Portugal
7951 (IGC 4695) root of tree, Portugal, IGC

Saccharomyces cerevisiae Meyen ex E.C. Hansen
7957 factory producing cassava flour, Brazil
7958 factory producing cassava flour, Brazil (Laluce *et al.*, Appl. Environ. Microbiol. **54**:2447-2451, 1988)
7959 factory producing ethanol from cane-sugar syrup, Brazil
7960 factory producing ethanol from cane-sugar syrup, Brazil (Laluce *et al.*, Biotechnol. Bioeng. **37**:528-536, 1991; Ernandes *et al.*, Biotechnol. Lett. **12**:463-468, 1990)
7961 fermenting concentrated syrup from sugar cane, Brazil (Ernandes *et al.*, Biotechnol. Lett. **12**:463-468, 1990)
7962 fermenting concentrated syrup from sugar cane, Brazil (Laluce *et al.*, Biotechnol. Bioeng. **37**:528-536, 1991; Bertolini *et al.*, Biotechnol. Lett. **13**:197-202, 1991)
7963 fermenting concentrated syrup from sugar cane, Brazil
7964 fermenting concentrated syrup from sugar cane, Brazil, C. Laluce (Laluce *et al.*, Biotechnol. Bioeng. **37**:528-536, 1991)

Saccharomyces exiguus Reess
7901 sour dough, Italy, M. Gobbetti (Gobbetti *et al.*, Appl. Microbiol. Biotechnol. **41**:456-460, 1994)

Sporobolomyces coprosmae Hamamoto & Nakase
7899 leaf of *Coprosma tenuifolia*, New Zealand, T. Nakase

Sporobolomyces coprosmicola Hamamoto & Nakase
7897 leaf of *Coprosma tenuifolia*, New Zealand, T. Nakase

Sporobolomyces dimmenae Hamamoto & Nakase
7896 leaf of *Pseudowintera colonata*, New Zealand
7915 leaf of *Nothofagus menziesii*, New Zealand
7916 leaf of *Gaulthera antipoda*, T. Nakase

Sporobolomyces dracophyllus Hamamoto & Nakase
7900 dead leaf of *Dracophyllum filifolium*, T. Nakase

Sporobolomyces linderae Nakase *et al.*
7893 dead leaf of *Linderae obtusilobae*, Japan, T. Nakase

Sporobolomyces novazealandicus Hamamoto & Nakase
7895 leaf of *Pseudowintera colonata*, New Zealand
7917 leaf of *Hebe stricta* var. *stricta*, New Zealand, T. Nakase

Sporobolomyces taupoensis Hamamoto & Nakase
7898 leaf of *Nothofagus fusca*, New Zealand, T. Nakase

Xanthophyllomyces dendrorhous Golubev
7918 *Betula verrucosa*, Russia
7919 *Betula tauschii*, Japan, V.I. Golubev

The CBS databases can be consulted on Internet at *http://www.cbs.knaw.nl/www/cbshome.html*. **Please note, our fax numbers are now, international: 31 15 2782355 and national: 015 2782355.**

The following book will soon appear. It can be ordered from CBS, P.O. box 273, 3740 AG Baarn, the Netherlands. Fax 31-35-5416142. E-mail <sales@cbs.knaw.nl>.

1. T. Boekhout & R.A. Samson, eds. In press. Proceedings of the contributed symposium on Heterobasidiomycetes. Studies in Mycology **38**.

This volume, entitled Heterobasidiomycetes: Systematics and Applied Aspects, contains the following contributions.

T. Boekhout & J.W. Fell. Heterobasidiomycetes: systematics and applications.
R.J. Bandoni. Dimorphic heterobasidiomycetes: taxonomy and parasitism.
J.P. Sampaio & A. Fonseca. Physiological aspects in the systematics of heterobasidiomycetous yeasts.
W.I. Golubev & T. Boekhout. Sensitivity to killer toxins as a taxonomic tool among heterobasidiomycetous yeasts.

D.H. Howard & K.J. Kwon-Chung. Zoopathogenic heterobasidiomycetous yeasts.

K.J. Kwon-Chung, Y.C. Chang, R. Bauer, E.C. Swann, J.W. Taylor & R. Goel. The characteristics that differentiate *Filobasidiella depauperata* from *Filobasidiella neoformans*.

E.A. Johnson & W. Schroeder. Astaxanthin from the yeast *Phaffia rhodozyma*.

D.J. McLaughlin, E.M. Frieders & H. Lü. A microscopist's view of heterobasidiomycete phylogeny.

W.H. Müller, A.C. van Aelst, T. van der Krift & T. Boekhout. Novel approaches to visualize the septal pore cap.

P. Blanz & H. Döring. Taxonomic relationships in the genus *Exobasidium* (Basidiomycetes) based on ribosomal DNA analysis.

J.W. Fell, T. Boekhout & D.W. Freshwater. The role of nucleotide analysis in the systematics of the yeast genera *Cryptococcus* & *Rhodotorula*.

E.C. Swann & J.W. Taylor. Toward a phylogenetic systematics of the Basidiomycota: integrating yeasts and filamentous basidiomycetes using 18S rRNA gene sequences.

T. Nakase, S.-O. Suh & M. Hamamoto. Molecular systematics of ballistoconidium-forming yeasts.

T. Boekhout, J.W. Fell & K. O'Donell. Molecular systematics of some yeast-like anamorphs belonging to the *Ustilaginales* and *Tilletiales*.

C. The following articles have appeared, are accepted or have been submitted.

2. T. Boekhout. *Pseudozyma* Bandoni emend. Boekhout, a genus for yeast-like anamorphs of Ustilaginales. J. gen. appl. Microbiol. (accepted)

The genus *Pseudozyma* Bandoni is emended in order to accommodate anamorphic yeast-like fungi phylogenetically related to the Ustilaginales, such as *Candida fusiformata, C. tsukubaënsis, Pseudozyma prolifica, Sporobolomyces antarcticus, Stephanoascus flocculosus, S. rugulosus, Sterigmatomyces aphidis,* and *Trichosporon oryzae*. Morphologically, the genus is characterized by the presence of septate hyphae without clamp connections, with the cytoplasm retracted in cells separated by lysed cells and retraction septa. Fusiform blastoconidia originate on denticles, sterigma-like structures or attenuating hyphae. Aerial mycelium consists of acropetal, branched chains of fusiform to ellipsoidal ramoconidia. Xylose is absent in whole-cell hydrolyzates, CoQ = 10, urease and DBB reactions are positive, *myo*-inositol and D-glucuronate are assimilated, and extracellular starch-like compounds are not produced.

3. T. Boekhout, H. Roeijmans & F. Spaaj. 1995. A new pleomorphic ascomycete, *Calyptrozyma arxii* gen. et sp. nov., isolated from the human lower oesophagus. Mycol. Res. (accepted).

A new pleomorphic ascomycete, *Calyptrozyma arxii*, isolated from the human lower oesophagus, is described. Initial growth results in yeast cells, followed by formation of hyphae. Asexual reproduction occurs by blasto-, aleurio- and arthroconidia. In addition, unicellular to multicellular conidia-like cells occur, which initially are somewhat thick-walled. Finally, the outer wall layers of these cells become loose. Sexual reproduction is by eight-spored asci formed on aggregations of generative hyphae. Although no ascomata are formed the fungus belongs to the Euascomycetes, as was deduced from septal ultrastructure and partial ribosomal RNA sequences.

4. T. Boekhout & J.W. Fell. 1995. Heterobasidiomycetes: Systematics and applications. Studies in Mycology **38**.

5. T. Boekhout, J.W. Fell & K. O'Donnell. 1995. Molecular systematics of some yeast-like anamorphs belonging to the *Ustilaginales* and *Tilletiales*. Studies in Mycology **38**.

Partial nuclear large subunit ribosomal DNA sequences were analysed from species of *Entyloma, Itersonilia, Melanotaenium, Microbotryum, Tilletiopsis, Tilletiaria, Tilletia, Ustilago* and a number of ustilaginaceous anamorphs in order to assess phylogenetic relationships. The results indicate that *Tilletiopsis* is polyphyletic, comprising four independently derived groups of species. The teleomorphic species *Tilletiaria anomala* forms a monophyletic group with *Tilletiopsis fulvescens*, *T. flava* and *T. minor*. *Cryptococcus tsukubaënsis, Trichosporon oryzae, Sporobolomyces antarcticus, Candida fusiformata, Sterigmatomyces aphidis, Pseudozyma prolifica, Stephanoascus flocculosus* and *S. rugulosus* form a monophyletic group with *Ustilago maydis*. All these species, and probably *T. pallescens* as well, belong to the Ustilaginomycetes. However, phylogenetic relationships of *Microbotryum violaceum, Itersonilia perplexans* and *Tilletiopsis pallescens* are unresolved by the present data. The position of *M. violaceum* and *I. perplexans* is not in conflict with their placement in the Urediniomycetes (sensu Swann) and Hymenomycetes, respectively.

6. J.W. Fell, T. Boekhout & D.W. Freshwater. 1995. The role of nucleotide sequence analysis in the systematics of the yeast genera *Cryptococcus* and *Rhodotorula*. Studies in Mycology **38**.

Phylogenetic relationships of basidiomycetous yeasts and yeast-like organisms were studied using partial large-subunit ribosomal DNA and RNA sequences. Three main clusters could be distinguished, which correspond with the orders *Sporidiales, Tremellales* and Ustilaginales, and correlate with the biochemical composition of the cell wall and septal ultrastructure. Among the

red yeasts (*Rhodotorula, Rhodosporidium, Sporobolomyces, Sporidiobolus*) several clades are apparent, which also contain some species of *Erythrobasidium, Bensingtonia, Leucosporidium* and *Microbotryum*. This indicates that the red yeasts are polyphyletic. Additionally, the results demonstrate that phenetic characters are not always reliable indicators for a phylogenetic classification. A PCR technique for species identification is discussed.

7. W.I. Golubev & T. Boekhout. 1995. Sensitivity to killer toxins as a taxonomic tool among heterobasidiomycetous yeasts. Studies in Mycology **38**.

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

8. W.H. Müller, A.C. van Aelst, T. van der Krift & T. Boekhout. 1995. Novel approaches to visualize the septal pore cap. Studies in Mycology **38**.

This paper describes some non-conventional techniques for electron microscopy applied to the study of septal pore caps occurring in Basidiomycetes. The septal pore caps of *Schizophyllum commune* and *Trichosporon sporotrichoides* have been studied by the use of freeze-substitution, preferential staining of the endoplasmic reticulum with zinc iodide-osmium tetroxide and intracellular scanning electron microscopy, and for *S. commune* freeze fracturing and replication as well. The septal pore cap of *S. commune* differs from the endoplasmic reticulum in reactivity with zinc iodide-osmium tetroxide, whereas in *T. sporotrichoides* both showed the same reactivity. The septal pore cap of *T. sporotrichoides* was found to be made up of little differentiated tubular and vesicular endoplasmic reticulum.

9. W.H. Müller, A.C. van Aelst, T.P. van der Krift & T. Boekhout. 1995. Cellular organization of the hyphal tip and the dolipore septum of the heterobasidiomycetous fungus *Trichosporon sporotrichoides*. Submitted to Microbiology.

The heterobasidiomycetous fungus *Trichosporon sporotrichoides* was subjected to cryofixation and freeze-substitution to study the hyphal tip as well as the formation and the ultrastructure of the septal pore cap (SPC). The hyphal tip contained apical vesicles, tubular and vesicular endomembranous elements. In the formation of the SPC the endoplasmic reticulum was found to be differentiated into tubular structures. This resulted in a mature SPC consisting of a complex of smooth tubular elements. The 3D-ultrastructure of the SPC was visualized by two preparation methods for transmission electron microscopy: freeze-substitution and preferentially staining with zinc-iodide tetroxide (ZIP), and by a preparation method for scanning electron microscopy. The ZIO staining resulted into a positively stained tubular SPC connected with stained tubular ER. The stereo-views of ZIO pretreated hyphae showed that a membraneous structure with tubular elements covered the dolipore. Scanning electron microscopy of median fractured faces of the SPC showed that small tubular elements were found between larger tubular elements and the dolipore. Lateral views of the SPC revealed that it comprised a complex of tubular, globular and sausage-like membraneous structures. The results of the hyphal tip presented are in agreement with previous hyphal tip cell studies, but add as a new finding a system of smooth endomembranes located between presumed Golgi and apical vesicles. The SPC of *T. sporotrichoides* shows a different morphology as compared with earlier studies.

10. M. Th. Smith, J.P. van der Walt & W.H. Batenburg-van der Vegte. 1994. *Babjevia* gen. nov., a new genus of the Lipomycetaceae. Antonie van Leeuwenhoek **67**:177-179.

11. M. Th. Smith, A.W.A.M. de Cock, G.A. Poot & H.Y. Steensma. 1995. Genome comparisons in the yeastlike fungal genus *Galactomyces* Redhead et Malloch. Int. J. System. Bacteriol. **45**:826-831.

The G+C contents of the DNAs of 41 strains belonging to the genus *Galactomyces* Redhead et Malloch were determined by the thermal denaturation method. Melting profiles revealed that the DNAs of these strains are heterogenous. Four groups were recognized on the basis of this heterogeneity. However, DNA similarity values, which were calculated by using DNA-DNA reassociation kinetics, revealed that the strains could be divided into six subgroups. Strains belonging to the same subgroup exhibited high levels of DNA similarity (84 to 100%). The members of two subgroups, corresponding to *Galactomyces citri-aurantii* and *Galactomyces reessii*, exhibited low levels of DNA similarity with the members of the other subgroups (20 to 27%). The members of the four remaining subgroups, which contained only strains previously identified as *Galactomyces geotrichum*, exhibited intermediate levels of reassociation (41 to 59%). Some combinations of phenotypic characteristics correlated with the subgroups; a key based on phenotypic characteristics that can be used to distinguish the subgroups is presented.

12. M. Th. Smith, G.A. Poot, W.H. Batenburg-van der Vegte & J.P. van der Walt. 1995. Species delimitation in the genus *Lipomyces* by nuclear genome comparison. Antonie van Leeuwenhoek **68**: 75-87.

Species delimitation in *Lipomyces* was attempted by nuclear genome comparison in conjunction with the re-evaluation of 48 physiological characters of 65 strains. High intraspecific (>75%) and low interspecific (<28%) similarity values established that *L. japonicus, L. lipofer* and *L. tetrasporus* are genetically isolated, and also distinct from *L. kononenkoae* and *L. starkeyi*. Ambiguous similarity values were obtained with *L. kononenkoae* and *L. starkeyi*. Strains previously assigned to *L. kononenkoae* constitute two related clusters. While similarity values within each cluster range from 76-99%, representatives of the two clusters reassociate for only 47%. Since these clusters are differentiated by their ecologically relevant maximum growth temperature, *L. kononenkoae* is subdivided. Strains previously assigned to *L. starkeyi* resolve into four closely related clusters. While similarity values within each cluster range from 78-100%, representatives of the four clusters reassociate for only 59-69%. Since these four clusters are poorly differentiated, the subdivision of *L. starkeyi* does not appear possible without recourse to other criteria. Four unassigned strains constitute a further two clusters. reassociation within these clusters is of the order of 91-100%, while reassociation between them occurs only at 59%. Reassociation of representatives of these clusters with those of the *L. kononenkoae* and *L. starkeyi* complexes is around 40% and 31%, respectively. These two clusters consequently appear to be intermediate between *L. kononenkoae* and *L. starkeyi*, and will, as such, have to be considered in any delimitation of these two species. A key to the taxa of *Lipomyces* and related genera of the Lipomycetaceae is given.

Search for strains

In view of my revision of the *Galactomyces/Geotrichum/Dipodascus* complex, I am looking for strains of these genera. Therefore, I would be obliged if people who have such strains in their collection, would let me have some of their isolates. Cultures can be sent to: Maudy Smith, CBS Yeast Division, Julianalaan 67, 2628 BC DELFT, The Netherlands. E-mail: <m.t.smith@stm.tudelft.nl>

IV. Biochemisches Institut der Universität Freiburg, Hermann-Herder-Straβe 7, D-79104 Freiburg im Breisgau, Germany. Communicated by H. Holzer.

Recent publication.

1. S. Nwaka, B. Mechler, M. Destruelle & H. Holzer. 1995. Phenotypic features of trehalase mutants in *Saccharomyces cerevisiae*. FEBS Lett. **360**:286-290.

In the yeast *Saccharomyces cerevisiae*, some studies have shown that trehalose and its hydrolysis may play an important physiological role during the life cycle of the cell. Recently, other studies demonstrated a close correlation between trehalose levels and tolerance to heat stress, suggesting that trehalose may be the protectant which contributes to thermotolerance. We had reported lack of correlation between trehalose accumulation and increase in thermotolerance under certain conditions (Nwaka, S., *et al.* (1994) FEBS Lett. **344**:225-228). Using mutants of the trehalase genes, *NTH1* and *YBR0106*, we have demonstrated the necessity of these genes in thermotolerance (Nwaka, S., Kopp, M. and Holzer, H. (1995) J. Biol. Chem. **270**:10193-10198). In the present paper, we have analyzed the expression of the trehalase genes under heat stress conditions and present genetic evidence for the 'poor-heat-shock-recovery' phenotype associated with *NTH1* and *YBR0106* mutants. Furthermore, we show a growth defect of neutral and acid trehalase deficient mutants during transition from glucose to glycerol, which is probably related to the 'poor-heat-shock-recovery' phenomenon.

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

The following paper was submitted to Patterns in Fungal Development.

1. B.F. Johnson, G.B. Calleja[1] & B.Y. Yoo.[2] A new model for hyphal tip extension; its application to differential fungal morphogenesis.

[1]Diliman Institute, P-9 Dalan Roces, Area 14, UP Campus, Diliman, Lunsod Quezon, The Philippines.
[2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada

I. Historical Introduction
II. Analysis of Models
  A. The endolytic model
  B. The steady-state model
  C. The actin knob model
III. Testing the Endolytic and the Steady-State Models
IV. A New Hybrid Model for Extension
V. Adjuncts to the Model
  A. The role of turgor
  B. The role of actin
  C. The role of the spitzenkorper
VI. Function of the New Hybrid Model
  A. Monaxial, in-line extension
  B. Extension at septa
  C. Conjugation
  D. Protoplasting revisited
VII. Perturbations
  A. Saltatory extension rates
  B. Malforming morphogenesis
  C. Bent hyphae
  D. Central controls
VIII. The Future

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

Recent publications.

1. Besançon X., Ratomahenina R. & Galzy P. 1995. Isolation and partial characterization of an esterase (E.C.3.1.1.1.) from a *Debaryomyces hansenii* strain. Netherlands Milk and Dairy Journal **49**:97-110.
2. Boutur O., Dubreucq E & Galzy P. 1995. Factors influencing ester synthesis catalysed in aqueous media by the lipase from *Candida deformans* (Zach) Langeron and Guerra. J. Biotechnol. **42**:23-33.
3. Perrier V., Dubreucq E. & Galzy P. 1995. Fatty acid and carotenoid composition of *Rhodotorula* strains. Arch. Microbiol. **164**:173-179.
4. Briand D., Dubreucq E., Grimaud J. & Galzy P. 1995. Substrate specificity of the lipase from *Candida parapsilosis* (Ashford) Langeron and Talice. Lipids **30**:747-754.
5. Vasserot Y., Drider D., Arnaud A. & Galzy P. 1995. Monoterpenol glycosides in plants and their biotechnological transformation. Acta Biotechnol. **15**:77-95.
6. Janbon G., Derancourt J., Chemardin P., Arnaud A. & Galzy P. 1995. A very stable ß-glucosidase from a *Candida molischiana* mutant strain: enzymatic properties, sequencing and homology with other yeast ß-glucosidases. Biosci. Biotechnol. Biochem. **59**:1320-1322.
7. Janbon G., Magnet R., Bigey F., Arnaud A. and Galzy P. 1995. Karyotype studies on different strains of *Candida molischiana* by pulsed field gel electrophoresis. Curr. Genet. **28**:150-154.

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

The following papers were accepted recently or are submitted.

1. Linardi, V.R., Amancio, M.C. & Gomes, N.C.M. 1995. Maintenance of *Rhodotorula rubra* isolated from liquid samples of gold mine effluents. Folia Microbiologica, in press.

2. Morais, P.B., Martins,[1] M.B., Klazcko,[2] L.B., Mendonça-Hagler,[3] L.C. & Hagler,[3] A.N. 1995. Yeast succession in the Amazon fruit *Parahancornia amapa* as resource partitioning among *Drosophila.* Appl. Environ. Microbiol. **61**(12), in press.

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[2]Departamento de Genetica, UNICAMP, Campinas-SP.
[3]Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ.

The succession of yeasts colonizing the fallen ripe amapa fruit, from *Parahancornia amapa*, was examined. The occupation of the substrate depended on both the competitive interactions of yeast species, such as the production of killer toxins, and the selective dispersion by the drosophilid guild of the amapa fruit. The yeast community associated with this Amazon fruit differed from those isolated from other fruits in the same forest. The physiological profile of these yeasts was mostly restricted to the assimilation of a few simple carbon sources, mainly L-sorbose, D-glycerol, DL-lactate, cellobiose, and salicin. Common fruit-associated yeasts of the genera *Kloeckera* and *Hanseniaspora, Candida guilliermondii*, and *Candida krusei* colonized fruits during the first three days after the fruit fell. These yeasts were dispersed and served as food for the invader *Drosophila malerkotliana*. The resident flies of the *Drosophila willistoni* group fed selectively on patches of yeasts colonizing fruits 3 to 10 days after the fruit fell. The killer toxin-producing yeasts *Pichia kluyveri* var. *kluyveri* and *Candida fructus* were probably involved in the exclusion of some species during the intermediate stages of fruit deterioration. An increase in pH, inhibiting toxin activity and the depletion of simple sugars, may have promoted an increase in yeast diversity in the later stages of decomposition. The succession provided a patchy environment for the drosophilids sharing this ephemeral substrate.

3. Morais, P.B., Rosa, C.A., Abranches, J., Mendonça-Hagler, L.C. & Hagler, A.N. 1995. Yeast communities associated with *Drosophila quadrum* (calloptera group) in Atlantic Rain Forest. Submitted.

The following M.Sc. Dissertation was presented recently, under the supervision of Dr. J.R. Nicoli.

4. A.C.P. Rodrigues. 1995. Effects of *Saccharomyces boulardii* against the enteropathogens *Salmonella enteridis* var. *typhimurium*, *Shigella flexneri*, and enteroinvasive *Escherichia coli* in conventional and NMRI gnotobiotic mice.

This work was carried out to demonstrate, *in vivo*, the protective effect of *Saccharomyces boulardii* against infection due to enteropathogens, suggested by *in vitro* experiments, and to study two possible mechanisms (ecological and immunological) to explain this protection, using gnotobiotic (GN) and conventional (CV) mice. The main conclusions were that: 1) the yeast reached populational levels potentially functional in the gastrointestinal portions where the enteropathogens act; 2) the mortality was lower in CV animals treated with the yeast and infected with *Salmonella enteridis* var. *typhimurium* and in GN animals monoassociated with *S. boulardii* and infected with *Shigella flexneri* than in the respective control groups; 3) the anatomopathological examination showed that lesions due to *S. enteridis* var. *typhimurium* and *S. flexneri* infections were less severe in the animals treated with the yeast when compared with the control mice; 4) the *S. enteridis* var. *typhimurium* DL50 was higher in CV animals treated with the yeast than in control mice; 5) there was no significant inhibition of *S. enteridis* var. *typhimurium* translocation to mesenteric lymphonods, spleen and liver from CV mice treated with *S. boulardii*, but an initial delay and a more efficient posterior resolution of this translocation were observed in these animals; 6) there was no antagonism between *S. boulardii* and the enteropathogens *S. enteridis* var. *typhimurium*, *S. flexneri* and an enteroinvasive *Escherichia coli* in the digestive tract of GN mice; 7) there was no difference in the levels of total or S. *enteridis* var. *typhimurium*-specific type A secreted immunoglobulins and serical IgG and IgM from CV animals treated with the yeast when compared with control mice.

The following communications were presented at the 7th International Symposium on Microbial Ecology, Santos-SP, Brazil in September 1995.

5. Morais, P.B., Rosa, C.A., Linardi, V.R. & Maia, A.B.R.A. Characterization of yeasts isolated from spontaneous fermentations for the sugar-cane "aguardente" production.

6. Santos, A., Morais, P.B., Rosa, C.A., Linardi, V.R. & Carazza, F. Screening for fuel alcohol production of *Saccharomyces* strains isolated from tropical habitats.

The following short course on yeasts was given in our Department by Dr. M.A. Lachance as visiting professor.

7. Lachance, M.-A. Molecular taxonomy of yeasts: present and perspectives.

Topics:

I. Conventional (pre-molecular) yeast systematics.
II. Molecular yeast systematics:
   a. Molecular definition of species;
   b. Sequence characterization methods;
   c. Application to species identification;
   d. Application to strain characterization (finger-printing);
   e. Application to phylogeny and genus definition.
III. Perspectives on yeast taxonomy:
   a. Limitations of the current methods;
   b. Characteristics of a perfect approach.

We would like to thank Dr. Lachance for his kindness in accepting our invitation to spend two months (August and September) as visiting professor in our Department. It was a very pleasant and useful time for us, on account of Dr. Lachance's capacities to communicate his knowledge of yeast taxonomy to all the participants, and the congenial relationship that he established with all our colleagues in the Department. We hope that our field work will reap the result of continued collaboration in yeast ecology, and that this visit will not be his last. We would like to thank CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnologico) for the visiting professor fellowship that was granted to Dr. Lachance, and Dr. J.R. Nicoli for his submission of the proposal for the fellowship.

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

As part of my sabbatical leave, I spent two months in the Department of Microbiology, Universidade Federal de Minas Gerais, Belo Horizonte MG, Brasil, where I collaborated with Prof. C.A. Rosa and colleagues on research of mutual interest in yeast ecology and taxonomy. I thank Prof. Rosa for his hospitality and his various colleagues who have made my visit most enjoyable. Warm thanks to Prof. J.R. Nicoli, who was particularly kind. The generosity of the Brazilian funding agency CNPq is greatly appreciated. During the time spent in Brazil, I have presented various seminars on the evolution and ecology of cactophilic yeasts, at the Universidade Federal de Rio de Janeiro (Department of Microbiology), the Universidade Federal de Minas Gerais (Department of Microbiology and Department of Ecology, and the Universidade Federal de Ouro Preto (Biochemistry Laboratory).

I later joined Profs. J.S.F. Barker, J.M. Bowles, W.T. Starmer, and L.L. Wolf on a 6-week investigation of the yeast-insect-*Hibiscus* ecosystem in Queensland and New South Wales, Australia. Special thanks to Prof. Barker for his hospitality and his superb coordination efforts in this endeavour.

The following articles whose abstracts appeared in the June issue are now in print.

1. Lachance, M.A., D.G. Gilbert & W.T. Starmer. 1995. Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: a comparison with western communities. J. Industr. Microbiol. **14**:484-494.

2. Lachance, M.A. 1995. Yeast communities in a natural tequila fermentation. Antonie van Leeuwenhoek **68**:151-160.

IX. Department of General and Marine Microbiology, University of Göteborg, Medicinariegatan 9C, S-413 90 Göteborg, Sweden. Communicated by R. Vázquez Juárez <ricardo.vazquez@gmm.gu.se>.

The following is the abstract of a Ph.D. thesis completed at the University of Göteborg, Lundberg Institute, under the supervision of Dr. Lena Gustafsson.

1. T. Andlid. Ecological Physiology of Yeasts Colonizing the Intestine of Fish.

The present thesis principally deals with two scientific issues. First, the ability of certain yeast strains to colonize the gastrointestinal tract of fish and second, the intra-cellular lipid-accumulation in yeasts. Both these issues are discussed in the applied terms of yeasts as probionts and yeasts as feed. The primarily target has been aquaculture, but in the longer perspective we also consider probiotic effects of yeasts for other animals and man. Yeasts were isolated from the intestine of farmed rainbow trout, turbot and from free living flat-fish. The dominant species were *Debaryomyces hansenii, Saccharomyces cerevisiae, Rhodotorula rubra* and *Rh. glutinis.* Colonization experiments were performed by inoculating rainbow trout and turbot with fish-isolated yeast strains and by examining the microbial intestinal colonization at intervals. Inoculation of cultures of *Rh. glutinis, D. hansenii* HF1 and *S. cerevisiae* CBS7764 to experimental fish yielded colonization levels up to 3.8×10^4, 3.1×10^6 and 2.3 x 109 viable yeast cells/g intestine or faeces in three separate colonization experiments, respectively. The high level of colonizing yeasts persisted for several weeks. The concentrations of yeasts in the tank water never exceeded 10^3 viable cells/ml. For periods of the experiments, the concentration of aerobic bacteria in the fish intestine was much lower than the intestinal yeast concentration. Scanning electron microscopy studies demonstrated a close association of the yeasts with the mucus gel of the intestine. No bacteria were detected on the micrographs, indicating that their affinity for the intestinal mucosa was less than that of yeasts. The adhesion of fish-isolated yeast strains to intestinal mucus, lipids and proteins was analysed and is discussed as a putative colonization factor. The strains tested were generally adhesive to mucus, polystyrene and to BSA at approximately equal levels, indicating that non-specific forces are involved. The adhesion was, however, stronger to phosphatidylserine (PS) than to all other substrata tested. The much stronger adhesion to PS compared with the adhesion to phosphatidylcholine and phosphatidylethanolamine indicates participation of specific or electrostatic forces. The adhesion was also strong to the extracellular matrix proteins collagen type I, fibronectin, laminin, whereas the binding to collagen IV was in all cases sparse. In addition, growing yeast strains isolated from fish were all largely hydrophobic. The hydrophobicity was found to be dependent on the physiological state of these cells as demonstrated by a high expression of cell surface hydrophobicity (CSH) during exponential phase of growth whereas stationary cells expressed a low CSH. The level of adhesion to immobilised mucus coincided with the degree of CSH, indicating hydrophobic interactions to be important in the mucosal association. In addition to adhesion, growth is necessary for colonization. The yeasts were shown to be able to utilize fish mucus as the sole source of energy and nutrients for growth. The isolated fish-mucus promoted rapid growth comparable to or faster than the rate achieved in defined yeast medium. In further studies, *S. cerevisiae,* CBS7764, isolated from the intestine of rainbow trout, was compared in more detail with the laboratory reference *S. cerevisiae* SKQ with respect to growth parameters and protein expression. The data achieved showed a large degree of resemblance between the two strains, however, also clear differences were detected. For example, the heat shock proteins were generally expressed at a higher rate of synthesis in CBS7764 than in SKQ. Energy content and fatty acid composition of the lipid accumulating yeast *Rh. glutinis* was studied. Microcalorimetry was demonstrated to be a suitable on-line method for monitoring the lipid production phase of *Rh. glutinis.* The choice of lipid extraction method for the oil accumulated by oleaginous yeasts was shown to be highly important both regarding the accuracy in quantifying the lipid level and determination of the fatty acid composition. The energy content of *Rhodotorula glutinis* increased from 23.0 to 30.6 kJ/g dry biomass during the lipid accumulating phase and was directly correlated to the analyzed level of lipids. Consequently, bomb-calorimetric measurements of the energy content was shown to be an indirect quantification method of the lipid content in oleaginous yeasts. A novel method of quantification of accumulated lipids, based on microcalorimetric data, is also suggested. The fatty acid composition remained rather constant during the batch growth of *Rh. glutinis* with approximately 70% unsaturated C18 fatty acids. These data make this yeast a better candidate for use as aquaculture feed compared with the commonly used *Saccharomyces cerevisiae.*

Göteborg 1995 ISBN 91-86022-96-2.

This thesis is based on the following papers.

1. T. Andlid, R. Vázquez-Juárez & L. Gustafsson. 1995. Yeast colonizing the intestine of rainbow trout *(Salmo gairdneri)* and turbot *(Scophtalmus maximus)*. Microb. Ecol., in press.
2. R. Vázquez-Juárez, F. Ascencio, T. Andlid, L. Gustafsson & T. Wadström. 1993. The expression of potential colonization factors of yeasts isolated from fish during different growth conditions. Can. J. Microbiol. **39**:1135-1141.
3. R. Vázquez-Juárez, T. Andlid & L. Gustafsson. 1993. Cell surface hydrophobicity and its relation to adhesion of yeasts isolated from fish gut. Colloids and Surfaces B: Biointerfaces **2**:199-208.
4. T. Andlid, C. Larsson, C. Liljenberg, I. Marison & L. Gustafsson. 1995. Enthalpy content as a function of lipid accumulation in *Rhodotorula glutinis*. Appl.Microbiol. Biotechnol. **42**:818-825.
5. T. Andlid, R. Vazquez-Juarez & L. Gustafsson. 1995. Adhesion and growth of *Debaryomyces hansenii* and *Saccharomyces cerevisiae,* isolated from the intestine of rainbow trout. Submitted to Microbial Ecology.
6. T. Andlid, L. Blomberg, L. Gustafsson & A. Blomberg. 1995. Metabolic characterization of *Saccharomyces cerevisiae* isolated from the intestine of rainbow trout. Submitted for publication.

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

Research in the Delft Yeast Physiology Group is focused on the regulation of metabolic fluxes in *Saccharomyces cerevisiae* and other industrial yeasts, and in particular on pyruvate metabolism, sugar transport and redox metabolism. Within the BSDL (Biotechnological Sciences Delft-Leiden) research school, intensive collaboration exists with the Yeast Genetics group of Dr. Yde Steensma and the Bioprocess Engineering Group of Prof. Dr. Sef Heijnen. Inside the Netherlands, the group participates in a large programme focusing on the regulation of glycolytic flux in yeasts and filamentous fungi. In the context of the EC Framework IV programme, the Delft group will coordinate a research project entitled "From gene to product in yeast - a quantitative approach". This project, which involves engineers and biologists from 10 research groups (8 university groups and 2 major biotechnological industries from 8 European countries), aims at a quantitative description of metabolic fluxes leading to the formation of high-added-value products by yeasts. The following are our publications for 1995.

1. De Jong-Gubbels P., Van Rolleghem P., Heijnen J.J., van Dijken J.P. & Pronk J.T. 1995. Regulation of carbon metabolism in chemostat cultures of *Saccharomyces cerevisiae* grown on mixtures of glucose and ethanol. Yeast **11**:407-418.
2. Hensing M.C.M., Rouwenhorst R.J., Heijnen J.J., van Dijken J.P. & Pronk J.T. 1995. Physiological and technological aspects of large-scale heterologous-protein production with yeasts. Antonie van Leeuwenhoek **67**:261-279.
3. Hensing M.C.M., Vrouwenvelder J.S., Hellinga C., van Dijken J.P & Pronk, J.T. 1995. Use of chemostat data for modelling extracellular-inulinase production by *Kluyveromyces marxianus* in a high-cell-density fed-batch process. J. Ferment. Bioeng. **79**:54-58.
4. Hensing M.C.M., Bangma K.A., Raamsdonk L.M., de Hulster E., van Dijken J.P. & Pronk, J.T. 1995. Effects of cultivation conditions on the production of heterologous β-galactosidase by *Kluyveromyces lactis.* Appl. Microbiol. Biotechnol. **43**:58-64.
5. Kaliterna J., Weusthuis R.A., Castrillo J.I., van Dijken J.P. & Pronk J.T. 1995. Transient responses of *Candida utilis* to oxygen limitation: regulation of the Kluyver effect for maltose. Yeast **11**:317-325.
6. Kaliterna J., Weusthuis R.A., Castrillo J.I., van Dijken J.P. & Pronk J.T. 1995. Coordination of sucrose uptake and respiration in the yeast *Debaryomyces yamadae.* Microbiology **141**:1567-1574.
7. Meijer J.J. & van Dijken J.P. 1995. Effects of glucose supply on myeloma growth and metabolism in chemostat culture. J. Cellular Physiol. **162**:191-198.
8. Visser W., van Spronsen E.A., Nanninga N., Pronk J.T., Kuenen J.G. & van Dijken J.P. 1995. Effects of growth conditions on mitochondrial morphology in *Saccharomyces cerevisiae.* Antonie van Leeuwenhoek **67**:243-255.

XI. Center for Process Biotechnology, Department of Biotechnology, Building 223, The Technical University of Denmark, DK-2800 Lyngby, Denmark. Communicated by L. Olsson <lo@ibt.dtu.dk> and U. Schulze <us@ibt.dtu.dk>.

The philosophy of the research activities on yeast at the Center for Process Biotechnology is to combine physiological studies with advanced analytical techniques and mathematical modelling with the objective of increasing our understanding of *Saccharomyces cerevisiae*. Presently the research on yeast is focused on four aspects:

· Minimization of the glycerol formation under anaerobic conditions. The project aims at minimizing the formation of glycerol in the production of ethanol by applying Metabolic Engineering.

· Mixed sugar utilization. The aim of the project is to study the mixed sugar utilization of *S. cerevisiae* in continuous cultures. The primary focus will be on the interaction between glucose and other sugars.

· Glucose repression, in relation to utilisation of other sugars, is studied. By means of genetic engineering, strains are constructed which are glucose derepressed, *i.e.* they can simultaneously utilise glucose together with other sugars. The physiology of "glucose-derepressed" recombinant *S. cerevisiae* strains is studied and compared to that of wild-type strains.

· Production of recombinant proteins. The production of recombinant proteins by *S. cerevisiae* is studied in continuous cultures using proteinase A as the model system.

Recent publications.

1. Christensen, L.H., Marcher, J., Schulze, U., Carlsen, M., Min, R.W., Nielsen, J. & Villadsen, J. 1995. Fast and precise monitoring of bioreaction processes. Biotechnol. Bioeng. In press.
2. Christensen, L.H., Schulze, U., Nielsen, J. & Villadsen, J. 1995. Acoustic gas analysis for fast and precise monitoring of bioreactors. Chem. Eng. Sci. **50**:2601-2610.
3. Johumsen, K.V. 1995. Production of proteinase A by *Saccharomyces cerevisiae.* Ph.D. thesis. Department of Biotechnology. Technical University of Denmark.
4. Schulze, U. 1995. Anaerobic physiology of *Saccharomyces cerevisiae.* Ph.D. thesis. Department of Biotechnology. Technical University of Denmark.
5. Schulze, U., Larsen, M.E. & Villadsen, J. 1995. Determination of intracellular trehalose and glycogen in *Saccharomyces cerevisiae.* Anal. Biochem. **228**:143-149.
6. Schulze, U., Liden, G. & Villadsen, J. 1995. Dynamics of ammonia uptake in nitrogen limited anaerobic cultures of *Saccharomyces cerevisiae.* J.Biotechnol. In press.

XII. Sezione di Microbiologia Applicata, Dipartimento di Biologia Vegetale, Università degli Studi, Perugia, 06121 Italy. Communicated by A. Vaughan-Martini.

We at DBVPG regret to announce the untimely death of our friend and colleague, Gianfranco Rosini in December of 1994. Gianfranco Rosini was a dedicated teacher of general and wine microbiology and his many studies of wine-related yeasts gave a significant contribution in a field of great economic and social importance. Those who knew him remember Gianfranco a conscientious worker and loyal friend with a keen ability for communication, in spite of his so-so command of the English language. The following papers have been published by our group in the last year.

1. Cardinali, G. & Martini, A. 1994. Electrophoretic karyotypes of authentic strains of the *sensu stricto* group of the genus *Saccharomyces.* Int. J. Syst. Bacteriol. 44:791-797.
2. Cardinali, G., Pellegrini, L. & Martini, A. 1995. Improvement of chromosomal DNA extraction from different yeast species by analysis of single preparation steps. Yeast **1**:1027-1029.
3. Vaughan-Martini, A. & A. Martini. 1994. The importance of correct nomenclature in biotechnology: a brief history of *Saccharomyces sensu stricto.* In: L. Albergina, L. Frontali and P. Sensi (Eds.) Proceedings of the 6th European Congress on Biotechnology. pp. 355-358.
4. Vaughan-Martini, A. & A. Martini. 1995. Facts, myths and legends on the prime industrial micro-organism. J. Ind. Microbiol. **14**:514-522.
5. Vaughan-Martini, A. 1995. *Saccharomyces barnetti* and *Saccharomyces spencerorum:* two new species of *Saccharomyces sensu lato* (van der Walt). Antonie van Leeuwenhoek. **68**:111-118.

6. Ciani, M. & Rosini, G. 1995. Analisi della validità dell'equazione di Genevois nella selezione di colture per vinificazione. Ann. Microbiol. (in press).

7. Ciani, M. 1995. Continuous deacidification of wine by immobilized *Schizosaccharomyces pombe* cells: evaluation of malic acid degradation rate and analytical profiles. J. Applied Bacteriol. (in press).

8. Ciani, M. & Diriye, F.U. 1995. Presence of rhizobia in soils in Somalia. World J. Microbiol. Biotechnol. (in press).

9. Ciani, M. & Picciotti, G. 1995. The growth kinetics and fermentation behaviour of some non-*Saccharomyces* yeasts associated with wine-making. Biotech. Letters. **17**:1247-1250.

The following are in press.

10. Vaughan-Martini, A. & A. Martini. 1996. Isolation, purification and analysis of nuclear DNA in yeast taxonomy. In: I. Evans (ed.) Methods in Yeast Molecular Biology. In press.

11. Vaughan-Martini, A. & Barcacaccia, S. 1996. A reconsideration of species related to *Saccharomyces dairensis* (Naganishi). Int. J. Syst. Bacteriol. In press.

A study of DNA reassociation kinetics and electrophoretic karyotypes of several strains classified as *Saccharomyces castellii, S. dairensis* and *Pachytichospora transvaalensis* showed that the group includes at least five distinct species. Two of the four strains classified as *P'spora transvaalensis* had very high DNA sequence homology with the type strain of *S. castellii,* while the type and another strain demonstrated an intermediate relationship between the type strains of the unrelated taxa *S. castellii* and S. *dairensis.* Five strains, classified as *S. dairensis,* were not related to any of the type strains of the three taxa studied. Two showed an intermediate interrelationship (65% base sequence homology), while another pair had a 98% DNA reassociation rate among themselves. The remaining strain was distinct from all others studied both for DNA base sequences and electrophoretic karyotype.

12. Vaughan-Martini, A. & Pollacci, P. 1996. Synonomy of the yeast genera *Saccharomyces* Meyen ex Hansen and *Pachytichospora* van der Walt. Int. J. Syst. Bacteriol. In press.

The type and other strains of the phenotypically similar taxa *Saccharomyces castellii* Capriotti, *S. dairensis* Naganishi and *Pachytichospora transvaalensis* van der Walt were studied for comparable ascospore morphology by ultra-thin sections for transmission electron microscopy (TEM). The results of this and another investigation studying DNA base sequence homologies have demonstrated the invalidity of the monospecific genus *Pachytichospora* van der Walt. It is proposed to reinstate the species *S. transvaalensis* van der Walt.

13. Vaughan-Martini, A., Cardinali, G. & Martini, A. 1996. Differential killer sensitivity as a tool for fingerprinting wine-yeast strains of *Saccharomyces cerevisiae.* J. Ind. Microbiol. In press.

The extreme variability of the killer phenomenon in nature, expressed differently in different strains of the same yeast species, embodies an exceptional potential for the discrimination of yeasts at the strain level. Data presented show that killer-sensitive relationships between a killer reference panel of 24 yeasts belonging to 13 species of 6 genera and different industrial wine-starters of *Saccharomyces cerevisiae* can be profitably used for a rapid and simple fingerprinting procedure.

14. Ciani, M. & Ferraro, L. 1996. Enhanced glycerol content in wines made by immobilized *Candida stellata* cells. Appl. Environ. Microbiol. (in press).

Screening tests carried out for 10 strains of *Candida stellata* confirmed high glycerol production, although low fermentation rate and reduced ethanol content were observed. To overcome the poor competition with *Saccharomyces cerevisiae,* fermentation tests with immobilized *C. stellata* cells, alone or in combination with *S. cerevisiae,* have been carried out. The immobilization of *C. stellata* cells consistently reduced the fermentation when compared with that obtained with free cells, exhibiting about 30- and 2-fold improvement in fermentation rate compared with rates for *C. stellata* and *S. cerevisiae* free cells respectively. Moreover, immobilized *C. stellata* cells produced a two fold increase in ethanol content and a strong reduction in acetaldehyde and acetoin production in comparison with levels for free cells. The evaluation of different combinations of *C. stellata* immobilized cells and *S. cerevisiae* showed interesting results with regard to analytical profiles for practical application in wine-making. In fact, analytical profiles of combination showed, apart from high glycerol content, a reduction of acetic acid and higher alcohols and a consistent increase in succinic acid content in comparison with values for the *S. cerevisiae* control strain. Sequential fermentation first with immobilized *C. stellata* cells and then after 3 days with an added inoculum of *S. cerevisiae* free cells was the best combination, producing 15.10 g of glycerol per litre, i.e., 136% more than the *S. cerevisiae* control strain produced. Fermentation with immobilized *C. stellata* cells could be an interesting process by which to enhance glycerol content in wine.

XIII. Biocenter 1 A, P.O. Box 56 (Viikinkaari 9), FIN-00014, University of Helsinki, Finland. Communicated by M. Korhola <matti.korhola@yeast.pp.fi>.

After 15+ years at the Research Laboratories of Alko Ltd., I have resigned and will move my yeast research to the University of Helsinki new Biocenter in January, 1996. Alko has gone through extensive reorganizations and has practically eliminated all research; the biomedical and sociological alcohol research will be moved out of the company to government organizations by 1.5.1996. About third of the industrial R&D was saved and almost 30 people were moved to the Rajamäki factories last summer to do product development and applications work for the industrial enzymes business unit Primalco Biotec. My address for research contacts from 1.1.1996 will be as given above. Phone: + 358 0 70851 (operator). Fax: + 358 0 708 59 262.

At the university I will be working as a Docent in Industrial Microbiology at the Department of Biological Sciences, Division of General Microbiology. I have also established a company, Alkomohr Biotech Ltd. The company concentrates on industrial yeasts and products (specialty enzymes). The office is at Alkomohr Biotech Ltd., Lehtotie 8, FIN-00630 Helsinki, Finland. Phone & fax: + 358 0 728 39 37.

The following papers have recently appeared or are in press.

1. Naumov, G.I., Naumova, E.S. & Korhola, M. 1995. Karyotypic relationships among species of *Saccharomyces sensu lato: S. castellii, S. dairensis, S. unisporus* and *S. servazzii.* System. Appl. Microbiol. **18**:103-108.
2. Naumov, G.I., Naumova, E.S., Sancho, E.D. and Korhola, M.P. 1995. Polymeric *SUC* genes in natural populations of *Saccharomyces cerevisiae.* FEMS Microbiol. Lett. (in press).

XIV. Department of Applied Microbiology, Lund Institute of Technology/Lund University, P.O. Box 124, S-221 00 Lund, Sweden. Communicated by B. Hahn-Hägerdal <barbel.hahn-hagerdal@tmb.lth.se>.

The fermentation of the pentose sugar xylose in lignocellulose hydrolysates is under investigation. The influence of inhibitors in lignocellulose hydrolysates has been studied (4, 7, 18, 19). The analysis of substrates and products as well as the production of preparative amounts of xylulose have been demonstrated (1, 3, 17). The physiology of pentose utilisation in different yeasts has been investigated and a cyanide insensitive respiration has been identified in the xylose fermenting yeast *Pichia stipitis* (5, 10-12, 21). The genes for the xylose metabolising enzymes xylose reductase and xylitol dehydrogenase from *P. stipitis* have been cloned and expressed in *Saccharomyces cerevisiae* and their influence on the metabolism of *S. cerevisiae* has been studied (2, 6, 8, 13-16). An arabinitol dehydrogenase gene from *P. stipitis* has also been cloned (9). Overexpressing the genes for the pentose phosphate pathway enzymes transketolase and transaldolase in recombinant *S. cerevisiae* harbouring genes for xylose utilising enzymes enhances the xylose utilisation (20).

Recent publications.

1. Buttler T, Gorton L, Jarskog H, Marko-Varga G, Hahn-Hägerdal B, Meinander N, Olsson. 1994. Monitoring of ethanol during fermentation of a lignocellulose hydrolysate by on-line microdialysis sampling, column liquid chromatography and an alcohol biosensor. Biotechnol Bioeng **44**:322-328.
2. Carlsen H, Hallborn J, Gorwa M-F, Hahn-Hägerdal B. 1994. Bioconversion of xylose to xylitol with in situ generation of NAD(P)H in recombinant *Saccharomyces cerevisiae.* ECB6: Proc 6th Eur Congr Biotechnol, Eds L Alberghina, L Frontali, P Sensi, pp. 313-316.
3. Dominguez E, Marko-Varga G, Hahn-Hägerdal B, Gorton L. 1994. Optimization of enzyme ratios in a co-immobilized enzyme reactor for the analysis of D-xylose and D-xylulose in a flow system. Enzyme Microb Technol **16**:216-222.
4. Hahn-Hägerdal B, Jeppsson H, Olsson L, Mohagheghi A. 1994. An interlaboratory comparison of the performance of ethanol-producing microorganisms in a xylose-rich acid hydrolysate. Appl Microbiol Biotechnol **41**:62-72.
5. Hahn-Hägerdal B, Jeppsson H, Skoog-Holmgren K, Prior BA. 1994. Biochemistry and physiology of xylose fermenting yeast. Enzyme Microb Technol **16**:933-943.

6. Hahn-Hägerdal B, Hallborn J, Jeppsson H, Meinander N, Walfridsson M, Ojamo H, Penttilä M, Zimmermann FK. Redox balances in *Saccharomyces cerevisiae.* Ann N Y Acad Sci (in press).
7. Hahn-Hägerdal B. Ethanolic fermentation of lignocellulose hydrolysates - A minireview. Appl Biochem Biotechnol (accepted for publication).
8. Hallborn J, Gorwa M-F, Meinander N, Penttilä M, Keränen S, Hahn-Hägerdal B. 1994. The influence of co-substrate and aeration on xylitol formation by a recombinant *Saccharomyces cerevisiae* expressing the *XYL1* gene. Appl Microbiol Biotechnol **42**:326-333.
9. Hallborn J, Walfridsson M, Penttilä M, Keränen S, Hahn-Hägerdal B. 1995. A short chain dehydrogenase gene from *Pichia stipitis* having D-arabitol dehydrogenase activity. Yeast **11**:839-847.
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13. Meinander N, Hallborn J, Keränen S, Ojamo H, Penttilä M, Walfridsson M, Hahn-Hägerdal B. 1994. Utilization of xylose with recombinant *Saccharomyces cerevisiae* harbouring genes for xylose metabolism from *Pichia stipitis.* ECB6: Proc 6th Eur Congr Biotechnol, Eds L Alberghina, L Frontali, P Sensi. pp. 1143-1146.
14. Meinander N, Hahn-Hägerdal B, Linko P, Linko M, Ojamo H. 1994. Fed-batch xylitol production with recombinant, XYL1-expressing *Saccharomyces cerevisiae* using ethanol as a co-substrate. Appl Microbiol Biotechnol **42**:334-339.
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16. Thestrup HN, Hahn-Hägerdal B. 1995. Xylitol formation and reduction equivalent generation during anaerobic xylose conversion with glucose as cosubstrate in recombinant *Saccharomyces cerevisiae* expressing the *xyl1* gene. Appl Environ Microbiol **61**:2043-2045.
17. Olsson L, Lindén T, Hahn-Hägerdal B. 1994. A rapid chromatographic method for the production of preparative amounts of xylulose. Enzyme Microb Technol **16**:388-394.
18. Olsson L, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb Technol (in press).
19. Palmqvist E, Hahn-Hägerdal B, Galbe M, Zacchi G. The effect of water-soluble inhibitors from steam-pretreated willow on enzymatic hydrolysis and ethanol fermentation. Enzyme Microb Technol (accepted for publication).
20. Walfridsson M, Hallborn J, Penttilä M, Keränen S, Hahn-Hägerdal B. 1995. Xylose metabolising *Saccharomyces cerevisiae* overexpressing the *TKL1* and *TAL1* genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase. Appl Environ Microbiol (in press).
21. Yu S, Jeppsson H, Hahn-Hägerdal B. Xylulose fermentation by *Saccharomyces cerevisiae* and xylose fermenting yeast strains. Appl Microbiol Biotechnol (in press).

XV. Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3168, Australia. Communicated by P. Nagley.

Since the previous communication from this laboratory in mid-1992, the following papers have been published on the molecular biology of mitochondria in *Saccharomyces cerevisiae.* Our work has focused on the formation and properties of the ATP synthase enzyme complex in mitochondria, that is responsible for cellular energy production by oxidative metabolism. Aspects we have been concerned with include the genetic and biochemical analysis of mutations in mitochondrial and nuclear genes, the regulation by nuclear genes of the expression of individual mitochondrial genes, the manipulation and expression of artificial mitochondrial genes relocated to the nucleus (aimed at systematic mutational analysis of protein structure and function), the study of mitochondrial ATP synthase *in vivo* and *in vitro* (analysed using monoclonal antibodies), and functional aspects of mitochondrial ATP synthesis.

1. R.J. Devenish, M. Galanis, T. Papakonstantinou, R.H.P. Law, D.G. Grasso, L. Helfenbaum & P. Nagley. 1992. Molecular biology and assembly of yeast mitochondrial ATP synthase. In: S. Papa, A. Azzi and J.M. Tager, eds. Adenine Nucleotides in Cellular Energy Transfer and Signal Transduction, Birkhauser Verlag, Basel pp. 1-12.
2. R.J. Devenish, T. Papakonstantinou, M. Galanis, R.H.P. Law, A.W. Linnane & P. Nagley. 1992. Structure/function analysis of yeast mitochondrial ATP synthase subunit 8. Annals New York Acad. Sci. **671**:403-414.
3. G.B. Cox, R.J. Devenish, F. Gibson, S.M. Howitt & P. Nagley. 1992. The structure and assembly of ATP synthase. In L. Ernster, ed. Molecular Mechanisms in Bioenergetics. Elsevier Scientific Publishers, Amsterdam, pp. 283-315.
4. M. Galanis, L. Wang, P. Nagley & R.J. Devenish. 1993. Duplication of secretion signal sequences is deleterious for the secretion of human interferon *a*4 from *Saccharomyces cerevisiae* and *Bacillus subtilis.* Biochem. Mol. Biol. Int. **30**:271-282.
5. T. Papakonstantinou, M. Galanis, P. Nagley & R.J. Devenish. 1993. Each of three positively charged amino acids in the C-terminal region of yeast mitochondrial ATP synthase subunit 8 is required for assembly. Biochim. Biophys. Acta, **1144**:22-32.
6. M.J. Payne, P.M. Finnegan, P.M. Smooker & H.B. Lukins. 1993. Characterisation of a second nuclear gene, *AEP1*, required for expression of the mitochondrial *OLI1* gene in *Saccharomyces cerevisiae*. Curr. Genet. **24**:126-135.
7. A.F.L. Straffon, P. Nagley & R.J. Devenish. 1994. Rescue of yeast defective in mitochondrial ATP synthase subunit 8 by a heterologous gene from *Aspergillus nidulans*. Biochem. Biophys. Res. Comm. **203**:1567-1573.
8. M. Prescott, N.S. Bush, P. Nagley & R.J Devenish. 1994. Properties of yeast cells depleted of the OSCP subunit of mitochondrial ATP synthase by regulated expression of the *ATP5* gene. Biochem. Mol. Biol. Int. **34**:789-799.
9. P.M. Smooker, I.J. Macreadie, J.L. Wright & H.B. Lukins. 1994. Suppression of a yeast mitochondrial RNA processing defect by nuclear mutations. Curr. Genet. **25**:239-244.
10. R.H.P. Law & P. Nagley. 1995. Import into isolated yeast mitochondria of radiolabelled proteins synthesised *in vitro*. In M.J. Tymms, ed., In Vitro Transcription and Translation Protocols (Methods in Molecular Biology, Volume 37), Humana Press Inc., Totowa, New Jersey, pp. 293-315.
11. T. Papakonstantinou, R.H.P. Law, S. Manon, R.J. Devenish & P. Nagley. 1995. Relationship of subunit 8 of yeast ATP synthase and the inner mitochondrial membrane: subunit 8 variants containing multiple lysine residues in the central hydrophobic domain retain function. Eur. J. Biochem. **227**:745-752.
12. M. Prescott, T. Higuti, P. Nagley & R.J. Devenish. 1995. The functional expression of a rat cDNA encoding OSCP in the yeast *Saccharomyces cerevisiae.* Biochem. Biophys. Res. Comm. **207**:943-949.

13. P.M. Finnegan, T.P. Ellis, P. Nagley & H.B. Lukins. 1995. The mature *AEP*2 gene product of *Saccharomyces cerevisiae,* required for the expression of subunit 9 of ATP synthase, is a 58 kDA mitochondrial protein. FEBS Lett. **368**:505-508.

The following articles are currently in press.

14. M. Prescott, R.J. Devenish & P. Nagley. 1995. Assembly of multisubunit complexes in mitochondria, In F.-U. Hartl, ed., Protein Import into Mitochondria, (Advances in Molecular and Cell Biology, Volume 43), JAI Press, Greenwich, Connecticut, USA (in press).
15. R.H.P. Law, S.T. Manon, R.J. Devenish & P. Nagley. 1995. ATP synthase from *Saccharomyces cerevisiae*. Methods in Enzymology, Vol. 260 (in press).
16. R.E. Gray, R.H.P. Law, R.J. Devenish & P. Nagley. 1995. Allotopic expression of mitochondrial ATP synthase genes in the nucleus of *Saccharomyces cerevisiae*. Methods in Enzymology, Vol. 264 (in press).

XVI. Departamento de Biotecnología, Universidad Autónoma Metropolitana, Iztapalapa, Apartado Postal 55-535, México D.F. 09340, Mexico. Communicated by M. García-Garibay <jmgg@xanum.uam.mx> and L. Gómez-Ruiz <lcgr@xanum.uam.mx>.

The following are recent papers published by our group.

1. M. García-Garibay. 1994. Brewing Fermentation (in Spanish). Bebidas Mexicanas **3**(1):11-18.

Fermentation is a key operation in the brewing process, which defines important characteristics of the beer. The alcoholic fermentation is performed in the hopped wort using selected strains of yeasts. In this paper the most important aspects concerning fermentation are reviewed such as fermenters design, biochemistry and physiology of fermentation, and the most important characteristics of brewing yeasts.

2. R. Caballero, P. Olguín, A. Cruz-Guerrero, F. Gallardo, M. García-Garibay & L. Gómez-Ruiz. 1995. Evaluation of *Kluyveromyces marxianus* as baker's yeast. Food Res. Internat. **28**(1):37-41.

Two strains of *Kluyveromyces marxianus* (NRRL-Y-2415 and NRRL-Y-1109) were assessed as baker's yeasts comparing them with two strains of *Saccharomyces cerevisiae* isolated respectively from compressed yeast and active dry yeast. Strains were tested for dough proofing activity in lean dough and rich doughs (prepared with sucrose, lactose or whey) and sensory evaluation of breads. In rich doughs containing lactose or whey, both strains of *K. marxianus* had superior proofing activity compared to commercial baker's yeast strains but no difference in bread flavor was seen.

3. M. Bonilla-Salinas, P. Lappe, M. Ulloa, M. García-Garibay & L. Gómez-Ruiz. 1995. Isolation and identification of killer yeasts from sugar cane molasses. Lett. Appl. Microbiol. **21**(2):115-116.

A total of 13 yeast strains were isolated from ten samples of sugar cane molasses obtained from sugar mills in Mexico; nine of them were killer strains. There was at least one killer strain in every sample. The resulting species were the following: *Torulaspora delbrueckii* (2), *Schizosaccharomyces pombe* (7), *Cryptococcus albidus* var. *albidus* (1), and *Saccharomyces cerevisiae* (3). A new killer species is reported: *Schizosaccharomyces pombe.*

4. A. Cruz-Guerrero, I. García-Peña, E. Bárzana, M. García-Garibay & L. Gómez-Ruiz. 1995. *Kluyveromyces marxianus* CDBB-L-278: a wild inulinase hyperproducing strain. J. Ferment. Bioengin. **80**(2):159-163.

Kluyveromyces marxianus CDBB-L-278 is an inulinase hyperproducing strain. It was able to grow in a medium containing inulin as the unique carbon source in the presence of 2-deoxyglucose. It produced up to 3.3 times the activity of the control strain *K. marxianus* NCYC-1429 in an inulin medium, and 3.6 times in a medium with glycerol as the sole carbon source. Although the strain CDBB-L-278 was able to produce inulinase in the presence of 2-deoxyglucose, it was demonstrated that it is not a de-repressed strain since enzyme production was reduced when the concentration of glucose or fructose was increased in the medium. Since inulinase was produced in a glycerol medium without an inducer, it can be considered that the enzyme production was partially constitutive in *Kluyveromyces marxianus* CDBB-L-278 as well as strain NCYC-1429. The inulinase from *K. marxianus* CDBB-L-278 was characterized. It had a higher affinity for inulin than for sucrose. Temperature and pH profiles were different for both of these two substrates. The enzyme was stable to high temperatures, with a half-life of 180 min at 50°C.

XVII. Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro. CT Bloco A, Lab 547, 21949-900 Rio de Janeiro, RJ, Brasil. Communicated by A. D. Panek.

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

1. E.C.A. Eleutherio, M.J.S. Ribeiro, M.D. Pereira, F.M. Maia & A.D. Panek. 1995. Effect of trehalose during stress in a heat-shock resistant mutant of *Saccharomyces cerevisiae*. Biochem. Mol. Biol. Internat. **36**:1217-1223.

Cells of a heat-shock resistant mutant were approximately 1000-times more resistant to lethal heat shock than those of the parental strain. We observed that exponentially growing cells of the mutant synthesized trehalose and showed increased osmotolerance, dehydration tolerance and ethanol tolerance, a fact not observed in wild type strains. The mutant synthesizes constitutively six proteins, among them two proteins of 56 and 63 kDa. Interestingly these molecular weights could correspond to the subunit of trehalose-6-phosphate synthase and to phosphoglucomutase II, respectively. Our results showed that glucose-growing cells of the *hsr1* mutant possessed high levels of activity of these enzymes when compared to the control strain.

2. M.B. Dutra, J.T. Silva,[1] D.C. Mattos & A.D. Panek. 1995. Regulation of UDPG-pyrophosphorylase isoforms in *Saccharomyces cerevisiae* and their roles in trehalose metabolism. Biochem. Biophys. Acta (accepted)

[1]Depto. de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense (UFF), Outeiro de S.J. Batista, s/n. Centro, Niterói-RJ, 24020-140, Brasil.

UDPG-pyrophosphorylase (E.C. 2.7.7.9) from *Saccharomyces cerevisiae* was studied and the presence of isoforms investigated. Its activity was monitored during growth of cultures in rich media containing glucose, galactose, sucrose, maltose or glycerol as carbon sources. The results suggest that UDPG-pyrophosphorylase is subject to both catabolite repression and catabolite inactivation. The inactivation process seems to be complex: in order to produce maximum inactivation, glucose and ammonium sulfate must be added together. Addition of glucose or ammonium sulfate separately produced little effect upon enzyme activity. Adsorption to and elution from a DEAE-Sephacel column of a crude protein extract prepared from yeast cells collected in stationary phase from a glucose medium, showed three activity peaks which we denominated isoform I, II and III. Isoform I is constitutive, it was the only form present during exponential growth on glucose medium, and did not suffer any alteration after glucose exhaustion, heat shock or by growing cells on maltose. On the other hand, isoforms II and III were shown to be repressed by glucose, and induced by heat shock. Furthermore, isoform II of UDPG-pyrophosphorylase was present together with isoform I when yeast cells were grown on maltose. The presence of a *MAL4C* allele rendered isoform II constitutive. Interestingly, a *gal3* mutant strain had low UDPG-pyrophosphorylase activity and isoforms I and II were not expressed. These results are discussed in relation to trehalose metabolism.

The following papers have been submitted.

3. E.C.A. Eleutherio, F.M. Maia, M.D. Pereira, R. Degré,[1] D. Cameron[1] & A.D. Panek. 1995. Effect of trehalose on desiccation tolerance in yeast cells subjected to osmotic treatment. J. Biotechnol.

[1]Lallemand Inc., Montréal (Canada).

Saccharomyces uvarum is heat sensitive and when dried by usual procedures exhibits very poor survival. Our results demonstrate that these cells are capable of accumulating trehalose when submitted to an osmotic treatment using 20% solutions of either sorbitol or dextrin endowing them with the capacity of surviving posterior dehydration. Additionally it was observed that trehalose plays an important role in protecting cells of *S. uvarum* from osmotic stress.

4. J.C. Ferreira, J.T. Silva[1] & A.D. Panek. 1995. A regulatory role for *tsl1* on trehalose synthase activity. Biochem. Mol. Biol. Intern.

[1]Depto. de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense, Valonguinho, 24020-140, Niterói, RJ, Brasil.

UDPG-dependent trehalose synthase activity was determined during growth on glucose medium in parental strains and yeast strains having deletions in components of the trehalose phosphate synthase complex. Deletion of *TPS3* produced no alteration. In contrast, strains harboring deletions *tsl1D* or *tsl1D/tps3D* showed no activation of the enzyme after glucose exhaustion. To evaluate the role played by *TPS3* and *TSL1* on trehalose synthase activity we have determined the effect of the addition of a cell free extract from a strain expressing only *TPS3* or *TSL1* to extracts of strains lacking *TSL1, TPS3* or both. No effect was observed on trehalose synthase activity from *tps3D* mutant. The addition of the same extract to trehalose synthase from *tsl1D* or *tsl1D/tps3D* strains showed a two-fold activating effect, indicating that *TPS3* and *TSL1* regulated differently the UDPG-dependent trehalose synthase activity in a different manner.

5. J.F. de Mesquita, V.M.F. Paschoalin & A.D. Panek. 1995. Modulation of trehalase activity in *Saccharomyces cerevisiae* by an intrinsic protein. Biochem. Biophys. Acta.

The regulation of cytosolic trehalase activity in yeast has been described as cycles of activation by phosphorylation by cAMP protein kinase at a single site of the polypeptide chain. In this paper, evidence is presented for another regulatory mechanism - the binding of an endogenous inhibitory protein. This negative modulator was isolated during the purification procedure of cytosolic cryptic trehalase from repressed wild type cells of *Saccharomyces cerevisiae.* However, in derepressed cells the inhibitor was not found nor was it present in *ras2* mutant cells submitted to a heat treatment. The trehalase inhibitory activity proved to be a calmodulin ligand protein and, therefore, involved in the modulation of trehalase activity by Ca^{2+} ions.

The following are manuscripts in preparation.

7. J.C. Ferreira, J.M. Thevelein,[1] S. Hohmann,[1] V.M.F. Paschoalin, L.C. Trugo & A.D. Panek. 1995. Trehalose accumulation in mutants deleted in the trehalose synthase-phosphatase complex.

[1]Laboratorium voor Moleculaire Celbiologie, Instituute voor Plantkunde, Katholieke Universiteit Leuven, Belgium.

In *Saccharomyces cerevisiae,* trehalose-6-phosphate synthase converts UDP-glucose and glucose-6-phosphate to trehalose-6-phosphate which is dephosphorylated by trehalose-6-phosphate phosphatase to trehalose. These two steps take place within a complex consisting of three proteins: trehalose-6-phosphate synthase encoded by the *GGS1/TPS1 (= FDP1 = BYP1 = CIF1)* gene, trehalose-6-phosphatase encoded by the *TPS2* gene and by a third protein probably encoded by both the *TSL1* and *TPS3* genes. Using three different methods for trehalose determination, we have observed trehalose accumulation in *ggs1D/tps1, tps2D* and *tsl1D* mutants, as well as in a double mutant *ggs1/tsl1D, tps2D* and also in *ggs1/tps1D* deleted mutants suppressed for growth on glucose. All these mutants harbor *MAL* genes. Trehalose synthesis in these mutants is probably performed by the ADPG-dependent trehalose synthase which can be detected in all strains tested. It is noteworthy that trehalose accumulation in these mutants was detected only in cells grown on weakly repressive carbon sources such as maltose and galactose or during the transition phase from fermentable to non-fermentable growth in a glucose medium and that α-glucosidase activity was always detectable in high amounts under these conditions. We also describe an ADPG-pyrophosphorylase activity in *Saccharomyces cerevisiae* which increased concomitantly with the accumulation of trehalose during the transition phase from fermentable to non-fermentable growth in *MAL2-8c* strains. The same was observed when *MAL1* strains were compared during growth on glucose and maltose. These results led us to conclude that maltose-induced trehalose accumulation is independent of the UDPG-dependent trehalose-6-phosphate synthase/phosphatase complex; that the ADPG-dependent trehalose synthase is responsible for maltose-induced trehalose accumulation probably by forming a complex with a specific trehalose-6-phosphatase activity and that ADPG synthesis is activated during trehalose accumulation under these conditions.

8. C.F.T. dos Santos, R.E. Larson[1], A.D. Panek & V.M.F. Paschoalin. 1995. Ca^{2+}/calmodulin affinity proteins in yeast. Catabolite repression and induction by carbon sources.

[1]Depto. de Bioquímica, Faculdade de Medicina de Ribeirão Preto, USP, Monte Alegre, 14049-900, Ribeirão Preto, SP, Brasil.

Soluble calmodulin-binding proteins from *Saccharomyces carlsbergensis* were analysed in cells grown on glucose, maltose and galactose as the carbon source. A large number of polypeptide chains showed affinity for calmodulin. The polypeptide chains of 115, 67 and 45 kDa were only detected in second exponential phase on glucose or on non-fermentative carbon sources suggesting that they might be submitted to catabolite repression. The 195 and 22 kDa chains were only observed when cells were grown on maltose, whereas the 88 kDa protein was just observed in galactose-grown cells. Among the calmodulin-binding proteins, eight were phosphorylated in a Ca^{2+}/calmodulin-dependent manner (220, 200, 175, 100, 62, 55, 31 and 16 kDa) and a protein of 51 kDa showed its dephosphorylation to be abolished in the presence of the Ca^{2+}/calmodulin complex.In this study, it was also observed that the Ca^{2+}/calmodulin dependent (g-^{32}P) incorporation was dramatically decreased in yeast cells submitted to a heat treatment.

The following is a recently defended Ph.D thesis.

9. J.J. Mansure. 1995. The Role of trehalose on ethanol tolerance. 123 pp.

The effect of ethanol on stability of intact yeast cells, liposomes and biological membranes has been investigated. Wine strains that are well known to be ethanol resistant produce trehalose during fermentation. Several strains with differences in trehalose metabolism were examined for their ability to survive in the presence of 10% (v/v) ethanol. A positive correlation was observed between cell viability and trehalose concentration. When leakage of electrolytes from the cells was recorded by observing changes in conductivity of the medium, we found that ethanol increases leakage, but the presence of trehalose reverses that effect. Similar studies were done with liposomes of similar composition to those seen in intact cells in log and stationary phases. In the presence of ethanol, carboxyfluorescein trapped in the liposomes leaked to the medium. When trehalose was added inside, outside or on both sides of the membrane, the ethanol-induced leakage was strongly inhibited. Yeast cells with different abilities to produce trehalose had their viability compared, after exposure to ethanol. A positive correlation was observed between trehalose content and cell viability. The tolerance obtained may not be entailed with degree of insaturation, because it was the same for all strains tested. Ethanol induced changes in fluidity and phase transition of several liposomes with different lipid composition, and the presence of trehalose did not seem to work

on these effects. Secondary structures of Ca-ATPase of sarcoplasmic reticulum from lobster were analyzed by FTIR. Addition of 10% (v/v) ethanol decreased b sheet and induced a helix conformation. However 250 mM trehalose, brought back b sheet levels and showed no effect on a helix. The Ca-ATPase activity showed a coupling ratio close to that observed in the control when trehalose was added to the samples containing ethanol. Trehalose seems to be enhancing ethanol tolerance by decreasing leakage.

XVIII. Center of Biological Research, Division of Experimental Biology, P.O. Box 128, La Paz 23000 B.C.S., México. Communicated by A. García-González <agarcia@cibnor.conacyt.mx>.

The following are summaries of work in progress in our laboratory.

1. Sánchez-Paz, A., Hernández-Saavedra, N. Y., Ramírez-Orozco, M., & Ochoa, J. L. Studies of partial digestion of SOD from two different sources with proteolytic enzymes.

Aerobic organisms generate several oxygen reactive species, including superoxide radical. Diverse biological and pathological processes involve these free radicals. In biology we can find many different defense strategies against the oxidative stress, which includes non-enzymatic systems (vitamin A, vitamin C, vitamin E) and enzymatic systems (catalase, peroxidase an superoxide dismutase). It has been show that the use of SOD can be effective to reduce the damage produced by some diseases and pathologies. However, the average life of Cu,ZnSOD in blood is extremely short (6 min), for this reason new techniques have been developed to modify the enzyme and to make the enzyme to last longer in the organism. Considering the above exposed will be very convenient to know if the SOD could be administered orally. In this way is necessary to study the resistance of bovine SOD and marine yeast SOD to some proteolytic enzymes (pepsin, trypsin, and chymotrypsin).

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

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

3. Ramírez-Orozco M. & Ochoa J.L. Enzymatic activity induction from the marine yeast *Debaryomyces hansenii.*

In order to obtain an increase in SOD activity in cultures of *D. hansenii,* three different culturing approaches were assayed: a) injection of copper sulfate into the culture medium; b) injection of 100% oxygen; and c) substitution of glucose by glycerol as carbon source. All the experiments were carried out in a 2L chemical reactor containing 1L of M1 culture medium. Samplings were done every 6 hours during 48 h, and to each sample SOD activity determined according to the xanthin-xanthin oxidase method. In a separated experiment used as a control, SOD showed that maximum activity was obtained at the lag phase (24-30 h) of the culture, therefore at this stage either copper sulfate or 100% oxygen were applied. In the case of the carbon source experiment, glycerol was used from the start. The conclusions indicate that copper sulfate and oxygen can increase up to 4 times the enzyme activity, while the glycerol also affected the rise on enzyme activity yields about 3.7 times.

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

Recent publications.

1. T. Wartmann, A. Krüger, K. Adler, M.D. Bui, I. Kunze & G. Kunze. 1995. Temperature-dependent dimorphism of the yeast *Arxula adeninivorans* Ls3. Antonie van Leeuwenhoek **68:**215-223.

Arxula adeninivorans Ls3 is described as an ascomycetous, arthroconidial, anamorphic, xerotolerant yeast, which was selected from wood hydrolysates in Siberia. By using minimal salt medium or yeast-extract-peptone-medium with glucose or maltose as carbon source it was shown that this yeast is able to grow at up to 48°C. Increasing temperatures induce changes in morphology from the yeast phase to mycelia depending on an altered programme of gene expression. This dimorphism is an environmentally conditioned (reversible) event and the mycelia can be induced at a cultivation temperature of 45°C. Depending on the morphology of strain Ls3 (yeast phase or mycelia) the secretion behaviour as well as the spectrum of polypeptides accumulated in the culture medium changed. The activities of the accumulated extracellular enzymes glucoamylase and invertase were 2 to 3 times higher in cultures grown at 45°C than in those grown at 30°C. While the level of the glucoamylase protein secreted from mycelia between 45 and 70 hours did not change, biochemical activity decreased after a cultivation time of 43 hours. It was shown that this effect depended on both the catabolic repression of the glucoamylase by glucose and the thermal inactivation of this enzyme in media without or with low concentrations of starch or maltose.

2. H. Rösel & G. Kunze. 1995. Cloning and characterization of a *TEF* gene for elongation factor 1α from the yeast *Arxula adeninivorans*. Curr. Genet. **28:**360-367.

The *TEF1* gene encoding the elongation factor-1α of the yeast *Arxula adeninivorans* Ls3 has been cloned from a genomic library and subsequently sequenced. The gene is localized on chromosome 2 from *Arxula adeninivorans*, comprises 1,380 bp and it encodes a protein containing 459 amino acids. Comparing the amino acid sequence from this elongation factor with those of other organisms it could be shown that the elongation factor-1α of the strain Ls3 has a high homology to the corresponding factors from *Candida albicans* (90.0%), *Saccharomyces cerevisiae* (89.1%), *Rhizomucor racemosus* -EF12 (88.0%), -EF13 (88.0%) and -EF11 (87.8%). In contrast to other fungi a second *TEF* gene encoding identical or near identical polypeptides could not been identified. The transcript quantities of isolated *TEF1* gene were nearly the same in all growth phases during the mycelial growth, whereas a slight decrease could be detected during the yeast growth.

3. T. Wartmann, I. Kunze, M.D. Bui, R. Manteuffel & G. Kunze. 1995. Comparative biochemical, genetical and immunological studies of glucoamylase producing *Arxula adeninivorans* yeast strains. Microbiol. Res. **150:**113-120.

Seven *Arxula adeninivorans* strains originating from The Netherlands (CSIR 1136, CSIR 1138 and CBS 8244T), South Africa (CSIR 1147, CSIR 1148 and CSIR 1149) and Siberia (Ls3) were compared concerning their secretory glucoamylase. Within the spectrum of secretory polypeptides obtained by SDS-polyacrylamide gel electrophoresis, the glucoamylase corresponding polypeptide could be identified by means of specific antibodies directed against the glucoamylase of strain Ls3. The molecular masses of the glucoamylases vary from 84 to 95 kDa. After endoglycosidase F treatment of the secretory proteins, the N-linked carbohydrate content for each glucoamylase could be quantified at about 25%. Glucoamylase activity staining of secretory proteins separated under nondenaturing conditions revealed one glucoamylase active protein for the Dutch strains and two active forms for the strains originating from South Africa and Siberia. Highest activities of glucoamylase can be measured at the end of the exponential growth phase for each strain. The Dutch strain CSIR 1138 secretes the highest activity. Optimum values for temperature and pH were determined and compared. By means of pulsed field gel electrophoresis and Southern analysis, the glucoamylase corresponding gene could be localized on the second of the four chromosomes of each yeast strain.

4. M.D. Bui, I. Kunze, S. Förster, T. Wartmann, C. Horstmann, R. Manteuffel & G. Kunze. 1995. Cloning and expression of an *Arxula adeninivorans* glucoamylase gene in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. (in press).

The glucoamylase gene of the yeast *Arxula adeninivorans* Ls3 has been cloned from a genomic library and sequenced. The gene could be localized on chromosome 2 from *Arxula adeninivorans* and comprises 1,875 bp. The first 16 N-terminal amino acids represent the signal sequence for entering the endomembrane system. Comparing the amino acid sequence from this glucoamylase with those of other fungal glucoamylases it is shown that the glucoamylase of the strain Ls3 has a homology to the glucoamylases from *Rhizopus oryzae* (32.6%), *Saccharomycopsis fibuligera* (23.1%), *Aspergillus niger* (22.1%), and *Saccharomyces diastaticus* (15.4%). No homology could be detected to the glucoamylase of *Schwanniomyces occidentalis.* By using the *GAL1* promoter from *Saccharomyces cerevisiae* within an autonomously replicating plasmid it was possible to express the isolated *Arxula* glucoamylase gene in *Saccharomyces cerevisiae.* The transformants secreted 95% of the enzyme into the culture media. The N-termini of glucoamylases synthesized in *Arxula adeninivorans* and *Saccharomyces cerevisiae* transformants are identical, which means that the signal sequences were cleaved at the same positions during maturation of the proteins. The highest glucoamylase activities were reached in the culture medium of *Saccharomyces cerevisiae* transformants after 36 hours of fermentation. Northern hybridization showed that the glucoamylase-transcripts were formed continuously up to 70 hours. These results reveal that the glucoamylase is expressed and secreted more rapidly in the *Saccharomyces cerevisiae* transformants than in *Arxula adeninivorans* Ls3.

5. M.D. Bui, I. Kunze, C. Horstmann, T. Schmidt, K.D. Breunig & G. Kunze. 1995. Expression of the *Arxula adeninivorans* glucoamylase gene in *Kluyveromyces lactis*. Appl. Microbiol. Biotechnol. (in press).

The glucoamylase gene of the yeast *Arxula adeninivorans* was expressed in *Kluyveromyces lactis* using the *GAP* promoter from *Saccharomyces cerevisiae* and a multicopy plasmid vector. The transformants secreted 90.1% of the synthesized glucoamylase into the culture medium. The secreted glucoamylase activities are about 20 times higher in comparison to those of *Saccharomyces cerevisiae* transformants using the same promoter. Secreted glucoamylase possesses identical N-terminal amino-acid sequences to those secreted by *Arxula adeninivorans* showing that cleavage of the N-terminal signal peptide takes place at the same site. Biochemical characteristics of glucoamylase expressed by *Kluyveromyces lactis* and *Arxula adeninivorans* are very similar.

6. R. Stoltenburg, T. Wartmann, I. Kunze & G. Kunze. 1995. Reliable method to prepare RNA from free and membrane-bound polysomes from different yeast species. BioTechniques **18:**564-568.

Some techniques exist for the preparation of total polysomes of yeast and extraction of RNA from them, but a method for the preparation of RNA from free (FP) and membrane-bound polysomes (MBP) has not been reported. We describe an improved method used in an attempt to isolate RNA after separation of FP and MBP from the yeast species *Saccharomyces cerevisiae* and *Arxula adeninivorans*. The procedure is the result of a series of experiments using different conditions for centrifugation, as well as variants of buffers with respect to detergents and pH of the extraction buffer.

7. C. Kühnel, R. Stoltenburg, I. Kunze & G. Kunze. 1995. Long-term effects of restrictive culture conditions on *Saccharomyces cerevisiae sec7* cells. Microbiol. Res. (in press).

Long-term effects of restrictive conditions on the temperature-sensitive *S. cerevisiae sec7* mutant were studied. By microscopic analysis no cell lysis could be detected of cells cultured for up to 19 days at 37°C. The optical density as well as the cell number remained constant during the whole period under restrictive conditions. However, restrictive conditions decreased the incorporation of ^{35}S-methionine into intracellular proteins in a reversible manner indicating that protein biosynthesis was inhibited whereas the cells remained alive. Northern blot experiments revealed that restrictive conditions did not markedly decrease the ratio of the mRNA levels to total RNA for the genes *TEF1*, *TEF2*, *SUC2*, and *BGL2* up to 73 hours. However the content of total RNA decreased drastically with increasing incubation times at restrictive temperature. In spite of the reduced total RNA content, cells are capable of new synthesis of mRNA under restrictive conditions which was shown by incubation of the cells in the presence of actinomycin D - an inhibitor of the mRNA synthesis. Most of the cells which survived a long-term incubation at 37°C are not able to divide and to form colonies immediately after their transfer to permissive conditions.

XX. Microbial Properties Research, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, Illinois 61604, USA. Communicated by C.P. Kurtzman.

Recent publications.

1. Kurtzman, C.P. 1994. Molecular taxonomy of the yeasts. Yeast **10**:1727-1740.

The term "yeast" is often taken as a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeasts is illustrated by their assignment to two taxonomic classes of fungi, the ascomycetes and the basidiomycetes. Subdivision of taxa within their respective classes is usually made from comparisons of morphological and physiological features whose genetic basis is often unknown. Application of molecular comparisons to questions in yeast classification offers an unprecedented opportunity to reevaluate current taxonomic schemes from the perspective of quantitative genetic differences. This review examines the impact of molecular comparisons, notably rRNA/rDNA sequence divergence, on the current phenotypically defined classification of yeasts. Principal findings include: 1) budding ascomycetous yeasts are monophyletic and represent a sister group to the filamentous ascomycetes, 2) fission yeasts are ancestral to budding ascomycetous yeasts, 3) the molecular phylogeny of basidiomycetous yeasts is generally congruent with type of hyphal septum, presence or absence of teliospores in the sexual state, and occurrence of cellular xylose.

2. Boekhout, T.[1], C.P. Kurtzman, K. O'Donnell & M. T. Smith.[1] 1994. Phylogeny of the yeast genera *Hanseniaspora* (Anamorph *Kloeckera*), *Dekkera* (Anamorph *Brettanomyces*), and *Eeniella* as inferred form partial 26S ribosomal DNA nucleotide sequences. Int. J. of Syst. Bacteriol. 44:781-786.
[1]Centraalbureau voor Schimmelcultures Yeast Division, 2628 BC Delft, The Netherlands.

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

3. Kurtzman, C.P. & C.J. Robnett. 1994. Orders and families of ascosporogenous yeasts and yeast-like taxa compared from ribosomal RNA sequence similarities, pp. 249-258. Hawksworth, D.L. (Editor) Ascomycete Systematics: Problems and Perspectives in the Nineties. Plenum Press, New York.

Extent of divergence in partial nucleotide sequences from large and small subunit ribosomal RNAs was used to assess the placement of genera among families and orders of ascosporogenous yeasts and yeast-like fungi. These data indicate the taxa comprise two orders: the *Schizosaccharomycetales* (genus *Schizosaccharomyces*) and the *Saccharomycetales* (*Endomycetales*; all genera of yeasts and yeast-like fungi except the fission yeasts). The data also suggest that certain currently accepted families are artificial. Furthermore, the rRNA sequence comparisons indicate that the ascosporogenous yeasts are not reduced forms of extant filamentous fungi.

4. Kurtzman, C.P. & C.J. Robnett. 1994. Synonymy of the yeast genera *Wingea* and *Debaryomyces*. Antonie van Leeuwenhoek **66**:337-342.

Extent of divergence in partial nucleotide sequences from large and small subunit ribosomal RNAs was used to estimate the evolutionary relationship between the genera *Wingea* and *Debaryomyces*. These data showed the monotypic genus *Wingea* to be congeneric with *Debaryomyces*, and it is proposed to transfer *W. robertsii* to *Debaryomyces*.

5. Kurtzman, C.P. 1995. Relationships among the genera *Ashbya, Eremothecium, Holleya* and *Nematospora* determined from rDNA sequence divergence. J. Industr. Microbiol. **14**:523-530.

Species of the genera *Ashbya, Eremothecium, Holleya,* and *Nematospora* were compared from extent of divergence in a 580-nucleotide region near the 5' end of the large subunit (26S) ribosomal DNA gene. The four genera are closely related and comprise a subclade of the hemiascomycetes. Because the taxa show little divergence, it is proposed that all be placed in the genus *Eremothecium*. The family Eremotheciaceae, fam. nov., is proposed.

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

The circumscription of mycelial genera among the ascomycetous yeasts has been controversial because of widely different interpretations of the taxonomic significance of their phenotypic characters. Relationships among species assigned to mycelial genera were determined from extent of divergence in a ca. 600-nucleotide region near the 5' end of the large subunit (26S) ribosomal DNA gene. Phylogenetic analyses showed that *Stephanoascus* is distinct from *Zygoascus* and that *Blastobotrys*, *Sympodiomyces* and *Arxula* represent anamorphs of the *Stephanoascus* clade. The analyses demonstrated the following teleomorphic taxa to be congeneric: *Ambrosiozyma*/*Hormoascus*, *Saccharomycopsis*/*Guilliermondella*/*Botryoascus*/*Arthroascus*, *Dipodascus*/*Galactomyces* and *Eremothecium*/*Ashbya*/ *Nematospora*/*Holleya*. Species assigned to *Dipodascus* comprise two separate clades. New taxonomic combinations are proposed which reflect the phylogenetic relationships determined.

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

The following papers were recently published.

1. E. Minárik & O. Jungová. 1995. Influence of yeast ghost preparations on the L-malic acid decomposition in wine by *Leuconostoc oenos* (in German). Mitteilungen Klosterneuburg **45**:90-92.

Yeast ghost preparations stimulate L-malic acid decomposition by *Leuconostoc oenos* cultures only insignificantly. Important preconditions for an effective course of malolactic fermentation of Lc. oenos are described.

2. E. Minárik & O. Jungová. 1995. Possibilities of activating regulated malolactic fermentation by yeast ghost preparations in wine (in Slovak). Kvasný prumysl (Prague) **41**:(8). In press.

The regulated bacterial L-malic acid decomposition in wine may be successfully achieved by non-revitalized lyophilized *Leuconostoc oenos* cultures provided that the culture is inoculated immediately after alcoholic fermentation while the wine is on the yeast sediment. In the course of acid decomposition, the wine should remain on the lees, racking should be made afterwards. The activity of *Leuconostoc oenos* may be only slightly stimulated by yeast ghost preparations.

3. F. Malík, V. Buchtová, J. Šajbidor & E. Minárik. 1995. Change in contents of some amino acids during sparkling wine fermentation (in German). Mitteilungen Klosterneuburg **45**:93-98.

Amino acid contents in sparkling wine were determined after 2, 12, and 24 months of ageing. Secondary fermentation of the cuvée had been induced by two ethanol tolerant strains of *Saccharomyces cerevisiae* and three selected dry wine yeast preparations. After 2 months an increase in concentration of most amino acids could be detected. Only proline, arginine, leucine and phenylalanine showed a decrease. After 12 months of ageing the concentration of most amino acids decreased. Proline, however, showed an increase in the sparkling wine. Increases in concentration of aspartic and glutamic acid as well as in threonine and isoleucine were found. After 24 months a decrease in the concentration of aspartic and glutamic acid, threonine, serine, proline, valine, leucine, isoleucine, lysine, arginine and methionine were detected with all 5 test yeast variants. Therefore the sum of amino acids in the finished sparkling wine is lower than in the basic cuvée.

4. F. Malík, J. Šajbidor, V. Buchtová & E. Minárik. 1995. Changes in the amount of amino acids during sparkling wine production (in German). Vitis **34**:185-188.

Changes of the amino acid content in the course of sparkling wine formation have been investigated. Two liquid and three active dry wine yeast preparations of *Saccharomyces cerevisiae* strains were used for the secondary fermentation. A decrease of almost all amino acids was observed after two months, only the histidine content was increased. The concentration of serine, glycine, alanine, and valine decreased after one year. In this phase, on the other hand, the proline, leucine, histidine contents increased. After two years the content of tyrosine, histidine and lysine as well as leucine and isoleucine in sparkling wines raised, while proline, valine and asparagine displayed a decrease compared with the initial concentration. The essential amino acid methionine was utilized in all sparkling wine samples after 24 months.

5. A. Gyozdjáková, J. Kucharská, P. Durišin & E. Minárik. 1995. Is plastoquinone$_{10}$ (PQ_{10}) the antioxidant marker of red wines? Vitis **34**: In press.

Thirty red and white grape wines originating from Western Slovakia fermented by pure yeast starter were investigated in order to find out the presence of plastoquinone$_{10}$ (PQ_{10}), d-tocopherol and superoxide dismutase (SOD) in varietal wines. It is suggested that plastoquinone$_{10}$ may be the antioxidant marker of red wines. Regular moderate red wine supplementation might thus serve as an additional source of antioxidant vitamins (B-vitamin, $PQ_{10\,ox}$) and antioxidant enzyme (SOD).

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

Recent publications.

1. Golubev W.I. 1995. Perfect state of *Rhodomyces dendrorhous (Phaffia rhodozyma).* Yeast **11**:101-110.

After mother-daughter cell conjugation, formation of long holobasidia with terminal basidiospores was observed without mycelium production in *Rhodomyces dendrorhous* (including the type strain of *Phaffia rhodozyma*) on polyol-containing media. Basidiospores are not forcibly discharged and germinate by budding. A new genus, *Xanthophyllomyces* (Filobasidiaceae, Tremellales) with a species, *X. dendrorhous,* is proposed for the teleomorphic state of *R. dendrorhous*.

2. Golubev W.I., Churkina L.G. & Seregina S.A. 1995. Intergeneric killing patterns of *Rhodotorula mucilaginosa* mycocins. Izvestiya RAN [Proc. Russian Acad. Sci.] (ser. biol.), **5** (in press).

Ustilaginaceous yeasts and yeast phase of some smuts were sensitive to *Rh. mucilaginosa* mycocins, while ascomycetous (with exception of *Hanseniaspora* spp. mainly), tremellaceous yeasts and yeast phase of Tremellales members were resistant. The mycocins produced by killers of the "carbonei" group have the narrowest spectrum action: they act against some species of the genera *Microbotryum, Rhodosporidium, Sphacelotheca, Sporidiobolus* and *Sporobolomyces roseus.* The killing pattern of *"rubra"* killers also includes species of *Bensingtonia* and *Leucosporidium,* and those of *"mucilaginosa"* and *"ukrainica"* killers comprise the species of *Entyloma* and *Kondoa* as well. Mycocins of the *"grinbergsii"* group have the broadest spectrum of action. In addition, they were active against some species of *Agaricostilbum, Sterigmatomyces* and *Tilletiopsis.* Grouping of basidiomycetous yeasts by sensitivity patterns to mycocins correlates with carbohydrate patterns and clustering of them by nucleotide sequences of rRNAs. Anamorphic yeasts having identical or very similar sensitivity patterns were crossed and teliospore formation was achieved in *Apiotrichum osvaldii, Ben. intermedia* and *Sp. tenuis.* Plasmid RNA or DNA were not detected in mycocinogenic strains of *Rh. mucilaginosa.*

The following dissertation has been presented

3. Golubev W.I. 1995. Basidiomycetous Yeasts (Taxonomy and Ecology). Senior Doctor Dissertation, 285 pp. (In Russian). Scientific Council of the Institute for Biochemistry and Physiology of Microorganisms, Pushchino, April 1995.

Introduction - "Evolution of the meaning of "yeasts".
I. Prehistory of study of basidiomycetous yeasts.
II. Morphology and cytology
- Formation of mycelial structures.
- Capsule.
- Ultrastructure of cell wall.

III. Physiological properties
- Utilization of the untraditional carbon compounds.
- Urease activity.
- Cardinal temperatures of growth.
- Vitamin requirements.
- Osmotolerance.

IV. Biochemical characteristics
- Nucleotide composition of nuclear DNA.
- Monosaccharide composition of capsular and extracellular polysaccharides.
- Coenzyme Q systems.

V. Killer toxins: mycocins and microcins
- Occurrence of the killer phenomenon.
- Assay conditions for expression of killer activity.
- Mycocin properties.
- Genetic determinants of killer phenotype.

Killing patterns of mycocins.
Microcins.
VI. Biogeography and ecology
Distribution of cryptococci in main soil types of latitudinal and altitudinal zonalities.
Yeasts from high-latitudinal regions of the Northern and the Southern Hemispheres.
Yeasts of phyllosphere.
Yeast fungi of peats.
Yeast succession in sap flows of birch.
Functions of capsule.
VII. Taxonomy and identification
Taxonomic weight of monosaccharide composition of extracellular polysaccharides.
Sensitivity to mycocins to taxonomic characteristic.
Differentiation of anamorphic ustilaginaceous and tremellaceous yeasts by glucuronate test.
VIII. Applied potential
Astaxanthin.
Ubiquinones.
Lipids. Polyases.
Extracellular polysaccharides.
Killer toxins.
Conclusions.

49 figures, 58 tables, 553 references.
Appendix 1. New taxa, combinations and revival names.
Appendix 2. The list of original yeast isolates deposited in the collections YKM, VKPM, ATCC, CBS, CCY, IFO, JCM and NRRL.

XXIII. Escola Superior de Agricultura Luiz de Queiroz, Universidade de Sao Paulo and Fermentec S/C Ltda. Rua Treze de Maio, 768 - sala 44, Ed. Sisal Center, 13400-900. Piracicaba, SP, Brasil. Communicated by H.V. Amorim.

The following is a summary of a paper presented recently at the 17th ISSY.

1. Alves, D.M.G., Basso, L.C. & Amorim, H.V. 1995. An ecological reason for succinic acid formation by yeast.

Benzoic acid, a yeast growth inhibitor, had shown promising results in fuel ethanol production with cell recycle, increasing ethanol yield by decreasing biomass and glycerol formation. However, the bacterial proliferation increased not only at the laboratory scale but mainly when benzoic acid was used in distilleries, apparently due to the reduction of succinic acid formation. The antibacterial action of succinic acid is attributed not only to the acidic effect, but mainly to the anion, in a synergetic effect with ethanol, presenting strong inhibitory action against growth of the main bacteria isolated from distilleries. An ecological function of succinic acid is suggested, since reduction of its formation causes yeast (*Saccharomyces cerevisiae*) to be less competitive in distillery environment.

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

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

1. Morais, P.B., C.A. Rosa, A. N. Hagler, & L. C. Mendonça-Hagler. 1994. Yeast communities of the cactus *Pilosocereus arrabidae* as resources for larval and adult stages of Drosophila serido. Antonie van Leeuwenhoek **66**:313-317.
2. Rosa, C.A., P.B. Morais, S.R. Santos, P.R. Peres Neto, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Yeast communities associated with different plant substrates in sandy coastal plains of Southeastern Brazil. Mycological Research **99**:1047-1054.
3. Morais, P.B., C.A. Rosa, A.N. Hagler, & L.C. Mendonça-Hagler. 1995. The yeast communities associated with the *Drosophila fasciola* subgroup (repleta group) in tropical forests. Oecologia **104**:45-51.
4. Naumov, G.I., E. Naumova, A.N. Hagler, L.C. Mendonça-Hagler, & E. Louis. 1995. A new genetically isolated population of the *Saccharomyces sensu stricto* complex from Brazil. Antonie van Leeuwenhoek **67**:351-355.
5. Morais, P.B., C.A. Rosa, S.A. Meyer, A.N. Hagler, & L.C. Mendonça-Hagler. 1995. *Candida amapae* sp. nov., an amino acid-requiring yeast from the Amazon fruit *Parahancornia amapa.* J. Industrial Microbiol. **14**:531-535.
6. Araujo, F., C.A. Soares, A.N. Hagler, & L.C. Mendonça-Hagler. 1995. Ascomycetous yeast communities of invertebrates in a southeast Brazilian mangrove ecosystem. Antonie van Leeuwenhoek **68**:91-99.

7. Morais, P.B., M.B. Martins, L.B. Klaczko, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Yeast succession in the Amazon fruit *Parahancornia amapa* as resource partitioning among *Drosophila.* Appl. Environ. Microbiol. **61**(12): (in press).
8. Hagler, A.N., L.C. Mendonça-Hagler, C.A. Rosa, & P.B. Morais. 1995. Yeasts as an example of microbial diversity in Brazil. In Oecologia Brasiliensis Vol 1. Estrutura, Funcionamento e Manejo de Ecossistemas (F. A. Esteves ed.). UFRJ, Rio de Janeiro. pp. 189-206.
9. Santos, E. A., de Oliveira, R. B., L. C. Mendonça-Hagler, & A. N. Hagler. 1995. Yeasts associated with cashew, caja, umbu, and mango fruits typical of the semiarid region of northeastern Brazil. Rev. Microbiol. Submitted for Publication.
10. Hagler, A. N., L. C. Mendonça-Hagler & J. B. Silva. 1995. Ascomycetous yeast communities in coastal forest ecosystems of southeast Brazil. ISME-7 Annais. Submitted for Publication.

The following graduate thesis were recently defended.

11. Jacqueline Abranches. M.Sc. Inst. Microbiol., UFRJ, 1995. Produção de Toxinas "Killer" e de Proteinases Extracelulares por Leveduras Tropicais.

Production of killer toxins and extracellular proteinases by tropical yeasts. Killer yeasts produce toxins that can limit the occurrence of sensitive strains by interference competition. Extracellular proteinases are widespread among the fungi for acquisition of nitrogen nutrients and may also be a defense against killer factors for some species. We have screened 961 strains of 105 yeast species for production of killer toxins and 352 strains from 82 species for production of extracellular proteinases. These yeasts were from forests (amapá fruit *Parahancornia amapa* and associated *Drosophila* from the Amazon forest; and *Drosophila* from Atlantic Rain Forest) and sand dune ecosystems (cacti *Pilosocereus arrabidae* and *Opuntia vulgaris, Ipomoea litoralis* and *I. pes-caprae* flowers, and water from tanks of the bromeliad *Neoregelia cruenta*). Killer activity was tested on YM agar adjusted to pH 4.2 with 0.003% of methylene blue with *Candida glabrata* NCYC 388 (IM-UFRJ 50083) as the sensitive strain for the general screening. The production of extracellular proteinases was tested by the hydrolysis of casein and gelatin. We found 16 killer species with *Pichia kluyveri* being the most widespread. *P. kluyveri* strain Ap 199b (IM51498) from the amapá fruit, and a *Pichia ohmeri*-like strain from cacti killed 45.8% and 64% of the species from its community, respectively. We suggest that these yeasts are amensals and not competitors. Killer yeasts were not associated with morning glory (*Ipomoea*) flowers or with water from bromeliads, probably because killer factor is more associated with ascomycetous yeasts and the yeast communities of these habitats are dominated by basidiomycetes. Extracellular proteinases were widely distributed among strains from all habitats studied and most of the proteolytic yeasts were basidiomycetous. The casein hydrolysis test had the most positive results, but additional tests should be used to estimate the proteolytic activity of the entire community. The coexistence of sensitive strains and killer yeasts may be due to their spatial separation in patches of microhabitat, or by the production of substances like extracellular proteinases that could inactivate the toxins.

12. Patrícia Valente da Silva. M.Sc. Biotecnologia Vegetal, UFRJ, 1995. Amplificação por PCR da Região ITS e Parte do 25S rDNA de Leveduras para Identificação do Género *Metschnikowia.*

PCR amplification of the ITS region and part of the 25S rDNA of yeasts for identification of the genus *Metschnikowia.* The genus *Metschnikowia* is currently composed of 10 species that form needle-shaped ascospores without any appendage. Representatives have been isolated which parasitize crustaceans, platyhelminths and diptera larvae. *Metschnikowia* cultures frequently fail to sporulate and the species are differentiated by only a few of the other tests in conventional taxonomy, making additional characterization tests important for identification. PCR amplification of the ITS region of representative species of the genera *Metschnikowia, Saccharomyces, Debaryomyces, Kluyveromyces, Torulaspora, Pichia, Issatchenkia, Yarrowia, Schizosaccharomyces, Cryptococcus* and *Rhodotorula* yielded products ranging from 400bp to about 850bp. The genera *Metschnikowia* and *Yarrowia* showed the smallest products. ITS length was capable of differentiating *Saccharomyces sensu stricto* from *S. unisporus* and *S. kluyveri.* The amplification of this region can be used as an exclusionary character complementary to the tests conventionally utilized for the characterization of yeasts. A primer MET2 specific for *Metschnikowia* was developed that is capable of detecting a deletion in the 25S rDNA region of this genus. The production of a fragment corresponding to the amplification of the ITS region of about 400bp and the detection of the deletion in the 25S rDNA region by the primer MET2 are characters capable of identifying yeasts belonging to the genus *Metschnikowia*.

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

13. Abranches J., P.B. Morais, C.A. Rosa, L.C. Mendonca-Hagler, & A.N. Hagler. 1995. The action of killer factors on tropical yeast communities. Abs. 95th Gen. Meeting Am. Soc. Microbiol. p. 364.

14. Lemos, G.A., P. Valente, D. Pimentel, A. N. Hagler, & L.C. Mendonça-Hagler. 1995. Characterization of *Saccharomyces paradoxus* and a new species of *Saccharomyces* from Brazilian ecosystems. Seventh Internat. Symp. Microbial Ecol., Santos, SP p. 215.
15. Valente, P., G.A. Lemos, F.C. Gouveia, D. Pimentel, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Length comparison of PCR-amplified ITS region in several yeast genera. Seventh Internat. Symp. Microbial Ecol., Santos, SP p. 213.
16. Nobrega, H.N., J. Abranches, P. Valente, D. Pimentel, G. Lemos, C.O. Gomes, L.C. Mendonca-Hagler, F. Fernendez, & A.N. Hagler. 1995. Leveduras associadas a roedores e marsupiais de uma area agricola do Rio de Janeiro. XVIII Cong. Bras. Microbiol., SBM, Santos SP. p. 44.
17. Abaranches, J., P.B. Morais, C.A. Rosa, R.J. Medeiros, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Toxinas killer e proteinases extracelulares como estratégias de colonizaçao de substratos terrestres tropicais por leveduras. XVIII Cong. Bras. Microbiol., SBM, Santos SP. p. 45.
18. Araujo, F.V., M.V. Motta, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Meios para isolamento seletivo de *Saccharomyces* e outras leveduras fermentativas de ambientes tropicias. XVIII Cong. Bras. Microbiol., SBM, Santos SP. p. 45.
19. de Azeredo, L.A.I., L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Estudo comparativo de métodos de extração de leveduras associadas a cana-de-açúcar. XVIII Cong. Bras. Microbiol., SBM, Santos SP. p. 44.
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23. Pimentel, D., P. Valente, G. Lemos, F.C. Gouveia, L.C. Mendonça-Hagler, & A.N. Hagler. 1995. Amplificação da região ITS do rDNA de leveduras com baixo perfil assimilativo. XVIII Cong. Bras. Microbiol., SBM, Santos SP. p. 179.

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

The following is the abstract of a paper which is in press.

1. H. Lee, C.R. Sopher & K.Y.F. Yau. In press. Induction of xylose reductase and xylitol dehydrogenase activities on mixed sugars in *Candida guilliermondii*. J. Chemical Technol. Biotechnol.

The ability of various sugars to induce xylose reductase (aldose reductase, EC 1.1.1.21) and xylitol dehydrogenase (D-xylulose reductase, EC 1.1.1.9) activities in *Candida guilliermondii* was studied. D-Xylose was found to be the best inducer of activities of both enzymes, followed closely by L-arabinose. Very low xylose reductase activity was induced by cellobiose, D-mannose, D-glucose, D-galactose, D-fructose or glycerol. With xylitol dehydrogenase, cellobiose and D-fructose caused partial induction of enzyme activity, while negligible activity was induced by D-mannose, D-glucose, D-galactose or glycerol. Several sugars were tested for the ability to repress the induction by D-xylose of xylose reductase and xylitol dehydrogenase activities in Candida guilliermondii. Enzyme activities induced on D-xylose served as controls. L-Arabinose, cellobiose and D-galactose did not repress enzyme induction by D-xylose, while D-mannose, D-glucose and D-fructose repressed enzyme induction to varying extents. Results from enzyme induction generally correlated with patterns of mixed sugar utilization, with some anomalies associated with the utilization of D-galactose and D-fructose in the presence of D-xylose. The results show that the utilization of D-xylose by *C. guilliermondii* is subject to regulation by induction and catabolite repression.

XXVI. Instituto de Investigaciones Biomédicas del CSIC, Facultad de Medicina de la U.A.M., Arzobispo Morcillo 4, 28029-Madrid, Spain. Communicated by R. Lagunas.

Recent publication.

1. E. Riballo, M. Herweijer, D.H. Wolf and R. Lagunas. 1995. Catabolite inactivation of the yeast maltose transporter occurs in the vacuole after internalization by endocytosis. 1995. J. Bacteriol. **177**:5622-5627.

The maltose transporter of *Saccharomyces cerevisiae* is rapidly degraded during fermentation in the absence of a nitrogen source. The location and mechanism of degradation of the transporter have been investigated. Using mutants defective in endocytosis, we have shown that degradation of this transporter requires internalization by endocytosis. In addition, studies of mutants defective in proteasome or vacuolar proteolysis revealed that degradation occurs in the vacuole and is independent of proteasome function. The results also revealed that degradation of the maltose transporter requires *Sec18p* and raised the question of whether in the absence of *Sec18p* activity the internalized maltose transporter is recycled back to the plasma membrane.

Obituary

In Memoriam - Dorothy M. Spencer

1933-1995

We thought that, as another long-time "scientific" couple of the ICY, it is appropriate that we extend to the yeast community our feelings of sorrow for the tragic loss of Dorothy Spencer. Instead of merely expressing our love and condolences to her husband Frank, we would rather reminisce the innumerable pleasant memories of an old brotherhood through the official ICY bulletin.

When we first met Dorothy Spencer during a meeting of many years ago, we were immediately flooded by her overflowing humanity and generosity. Thereafter we had plenty of opportunities to confirm and reinforce that first impression during the Spencers' several working visits to our lab, in many conventions around the world as well as in the course of short and long stays in their lab in London. Our children still remember her absolute availability as a splendid cook, as a tour guide or as a passionate defender against mean parents never willing to say yes. Shopkeepers near our lab in Perugia still remember that strange, decidedly non Italian, yet marvellously communicative lady with her friendliness and overwhelming smile. Not to mention our technicians who loved her dearly for her never-ending cheerfulness and roaring sympathy. Dorothy Spencer was a dedicated yeast apostle and teacher as well as a dear friend to many of us. Her passing away was more than a great loss, it leaves a void in many of our lives that will be impossible to fill. Anyone who was lucky enough to have come into contact with her remembers Dorothy's dedication to yeast research, the main reason for her move to Argentina in 1988 when the senseless redundancy movement of her native England forced her into early retirement. Dorothy chose this option in order to continue her work and teaching. Those of us who were lucky enough to have known her will never forget Dorothy's keen interest in people and her celebrated love for just about anyone, whether deserved or not. Her name was synonymous of helpfulness, generosity and unselfishness. We are all at a great loss without her and find it hard to imagine that anyone so positive could not have won the fight against the unforgiving disease which she was forced to confront and which tragically took her in July, 1995. Words cannot be expressed for what we felt for Dorothy, and we will try to remember her goodness and humanity as an inspiration.

Ann and Sandro Martini
Sezione di Microbiologia Applicata
Dipartimento di Biologia Vegetale
Università degli Studi
Perugia 06121
Italy

International Commission on Yeasts

Minutes of August 30 1995 Meeting, Edinburgh

The International Commission on Yeasts met on Wednesday August 30 during the 17th International Specialized Symposium on Yeasts (ISSY) at the Heriot-Watt University in Edinburgh, Scotland. Commissioners present were Graham Stewart, David Berry, Byron Johnson, Lex Scheffers, Peter Raspor, Graham Fleet and Sally Meyer. The topics discussed were 1) future meetings, 2) retiring and new commissioners and 3) ICY Statutes.

Graham Fleet (Australia) presented an update on the 9th International Symposium on Yeasts to be held in Sydney, Australia, August 25-30, 1996. The biggest concern is the acquisition of funds to support invited speakers. The program is nearly in place.

Two proposals were introduced for future ISSY. Peter Raspor (Slovenia) agreed to organize an ISSY in Slovenia in 1997. The topics include "Yeast Nutrition" and "Natural Habitats". Isabel Spencer-Martins (Portugal) and colleagues proposed an ISSY for 1998 on "Yeasts in the production and spoilage of foods and beverages" to be held in Portugal. (Exact location to be determined). Both proposals were readily accepted by the Commissioners in attendance.

Two Commissioners have resigned. They are Karen Oxenboll (Denmark) and A. Stenderup (Denmark). We thank them for their service to the ICY. Replacements suggested are Tom Buth Nielsen (Novo Nodisk, Denmark) and Jorgen Stenderup (Statens Seruminstitut, Denmark). It was also recommended that because of his long service on the ICY that A. Stenderup be given emeritus status on the ICY. All Commissioners in attendance accepted and agreed to these recommendations.

The question was introduced as to how many commissioners were permitted for each country. The Statutes will be reviewed to uncover this information. Also, the decision-making power and voting power of the Commissioners at the meetings of the Yeast Commission during Specialized Symposia needs to be addressed. The Statutes will be examined.

It was recommended that the Chair of the ICY write to Frank Spencer (Argentina) and John Johnston (Scotland) to express condolences on the deaths of their wives.

The meeting adjourned in time for the afternoon scientific session of ISSY-17. A special thanks to Graham Stewart for providing lunch during the meeting.

The ICY also extends the warmest thanks to David Berry and his committee for organizing the ISSY 17, which was highly rewarding, both scientifically and socially.

Correction. The name of Ana Clara Schenburg (Brazil) was inadvertently omitted from the list of Commissioners in the minutes of the meeting of the ICY 1992.

S.A. Meyer, Chair, ICY

Recent meetings

17th International specialised Symposium on Yeasts Edinburgh, Scotland, 27-3O August, 1995

The 17th International specialised Symposium on Yeasts was held at the Edinburgh Conference Centre, Edinburgh, Scotland, between the 27th and 3Oth August, 1995. The Conference was attended by over 120 delegates from 24 countries who had a stimulating series of papers presented on topics ranging from genome sequencing to the modelling of multi-phase fermentation systems. Plenary lectures were presented by Professor Stephen Oliver, UMIST, Manchester, England, and Professor G.G. Stewart, International Centre for Brewing and Distilling, Edinburgh, Scotland. In addition there were 28 other oral presentations and around 40 poster presentations. In addition to a vigorous scientific programme the delegates enjoyed receptions at the Edinburgh City Chambers and at Hopetoun House, a stately home on the banks of the Forth.

24th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts, Smolenice, Slovakia, May 17-19, 1995

The 24th Annual Yeast Conference was the third in the raw after division of the former Czechoslovakia in two states, the Czech Republic and the Slovak Republic. The Conference was attended by more than 60 scientists from both countries representing both academia and industry. The program comprised plenary lectures and posters. The plenary program was divided into three different sessions: i) Cell biology (coordinated by M. Havelková and O. Nečas), ii) Molecular biology and genetics (coordinated by J. Subík and V. Vondrejs), and iii) Ecology and biotechnology (coordinated by E. Slaviková and J. Hodan). Twenty plenary lectures were complemented by 32 poster presentations.

At a meeting of the Committee of the Commission held during the Conference it was agreed that the 25th Annual Conference on Yeasts will be organized during April 24-26, 1996, again in the Smolenice Castle near Bratislava. Its program will be focused on industrial yeasts, molecular and cell biology of yeasts and yeast enzymes. Below are listed the contributions of the 24th Annual Conference.

Plenary lectures in cell biology

O. Nečas: Molecular engines.

I. Pokorná & A. Svoboda: Progress in the investigation of the yeast cell response to pheromonal signals.

A. Svoboda: Meiosis in *Schizosaccharomyces versatilis:* The role of microtubules and nuclear structure.

V. Farkaš: Biosynthesis of yeast β-1,3-β-1,6-glucan.

V. Raclovský: Effect of Nikkomycine Z on the inhibition of *Saccharomyces cerevisiae* growth by Rylux BSU.

M. Havelková: Yeast species *Yarrowia lipolytica*.

Lectures in molecular biology and genetics

Y. Gbelská: Molecular analysis of nuclear genes of *K. lactis* participating in mitochondrial biogenesis.

M. Janitor: Studies of the nucleo-mitochondrial interactions using a mutant strain of *Saccharomyces cerevisiae*.

J. Nosek: Terminal structures of linear mitochondrial DNAs in yeasts.

J. Brozmanová: Identification of *RecA*-similar activities in the yeast *Saccharomyces cerevisiae*.

V. Vondrejs: Ubiquitinous system.

F. Cvrčková: Yeast as a model for study of cell morphogenesis.

M. Opekarová: Transport of arginine and its regulation on *Saccharomyces cerevisiae*.

Z. Storchová: The phenomenon of directed mutations in yeasts.

T. Ruml: Expression and post-translational modification of retroviral proteins in *Saccharomyces cerevisiae*.

M. Sipiczki: Genetic regulation of cytokinesis in *Schizosaccharomyces pombe*.

Lectures in ecology and biotechnology

A. Tomšíková: New trends in diagnostics of pathogenic yeasts and related diseases.

E. Sláviková: Ecological study of the yeasts isolated from the river Danube.

E. Breierová: Quantitative change of extracellular yeast polymers as a response to osmotic stress.

F. Malík: Yeasts versus chemical changes in the process of the sparkling wine production.

R. Zeman: Non-standard sources of carbon and energy in the biosynthesis of phenylacetylkarbinol in *Saccharomyces coreanus*.

Posters

N. Kolarova & M. Grešík: Effect of sugars and sugar derivatives on the galactosyltransferase activity in the yeast *Saccharomyces cerevisiae*.

N. Kolarova, I. Jančová & P. Capek: A glucose-rich extracellular glycoprotein complex of the yeast *Cryptococcus laurentii*.

N. Kolarová, P. Capek & D. Mislovicová: Characterization of the extracellular mannoprotein of the yeast *Cryptococcus laurentii*.

M. Pospíšek, Z. Palková & J. Korb: A new dsRNA virus of the yeast *Wickerhamia fluorescens*.

S. Abmann & K Sigler: Heavy metals loosen the lipidic phase of the cell membrane and inactivate membrane proteins in *Schizosaccharomyces pombe*.

L. Adamíková & L. Tomáška: Analysis of protein tyrosine kinase activity of *Saccharomyces cerevisiae* using the substrate affinity chromatography.

L. Tomáška, J Nosek & H. Fukuhara: Structure of mitochondrial telomeres in *Candida parapsilosis*.

M. Bartúněk & D. Gášková: Study of the changes of membrane potential during the action of the killer factor.

T. Ruml, I. Plačková & L Šilhánková: Mapping of genes involved in the control of the thiamine excretion in *Saccharomyces cerevisiae*.

M. Obernauerová & J. Šubík: Effect of intramitochondrial ATP and recombinant repair on mutagenicity of oflaxacine in the yeast *Saccharomyces cerevisiae*.

V. Vlčková, M. Slaninová, M. Morais and J. Brozmanová: *RAD51* of *S. cerevisiae* and *RecA* of *E. coli* proteins: A comparison of their function in DNA reparation.

M. Slaninová, V. Vlčková J.A. Henriques and J. Brozmanová: PSO4 gene of *S. cerevisiae* as a candidate for the *E. coli* RecA-similar activity in the induced mutagenesis.

M. Chovanec, V. Vlčková, G.P. Margison and J. Brozmanová: Similarity in the effects of *rad52* mutation in *S. cerevisiae* and *recA* mutation in *E. coli* in the utilization of bacterial alkyltransferase.

L. Šabová, G. Gavurníková, P. Haviernik & J. Kolarov: Two isogenes of the AAC translocator in *Saccharomyces cerevisiae* utilize different signal routes for the oxygen-activated expression.

K. Bišová & V. Vondrejs: Fusion of *Escherichia coli* spheroplasts as a substitution of the transformation of *Saccharomyces cerevisiae*.

J. Vlasák, A.P. Rojas, B. Janderová, V. Vondrejs & K. Angelis: Proliferation and induced mutagenesis of the *rad6* mutants of *Saccharomyces cerevisiae* after different wavelength UV irradiation.

A.P. Rojas, J. Vlasak, B. Janderová and V. Vondrejs: Late mutations in the rad6 mutants of *Saccharomyces cerevisiae*.

A.P. Rojas, Z. Storchová & V. Vondrejs: Death as a result of uracil deficiency in the *rad6* mutants of *Saccharomyces cerevisiae*.

A.P.Rojas & V. Vondrejs: Tumors on colonies of the rad6 mutant of *Saccharomyces cerevisiae*.

A.P. Rojas & V. Vondrejs: Use of *ade2* for estimation of the mutation time in *Saccharomyces cerevisiae*.

M. Rosenberger, J. Kaňuch & R. Hamaj: Possibilities of preparation of trehalose from yeasts.

A. Cepčeková, E. Machová & J. Šandula: Depolymerization of carboxylated β-1,3-glucan of *Saccharomyces cerevisiae*.

M. Vršanská & P. Biely: Endo-β-1,4-xylanases of the group F and G: Differences in their action on glucuronoxylan and rhodymenan.

Z. Ciesarová, J. Šajbidor, D. Smogrovičová & P. Bafrncová: Ethanol tolerance and yeast lipid composition.

Z. Ciesarová, D. Smogrovičová & Z. Dömeny: Effect of ethanol and calcium on yeast respiration.

R. Vadkertiová & E. Slaviková: Killer effect of yeasts isolated from sweet waters.

E. Stratilová, E. Breierová & R. Vadkertiová: Factors influencing the production of multiple forms of yeast polygalacturonase.

E. Stratilová, E. Breierová & R. Vadkertiová: Production an partial characterization of yeast pectolytic enzymes.

J. Šajbidor , J. Augustin & M. Dobrotová: Biodegradation an assimilation of ester-type xenobiotics by yeasts.

L. Paulová, M. Rychtera, J. Votruba & K. Melzoch: Use of yeast physiology knowledge in the control of the fed-batch cultivation of baker's yeast.

B. Janderová, B. Zikánová & H. Flegelová: Characterization of alpha-amylase from *Saccharomyces cerevisiae*.

Communicated by Peter Biely

Forthcoming meetings

International Union of Microbiological Societies Congresses 1996: 8th International Congress of Bacteriology and Applied Microbiology Division and 8th International Congress of Mycology Division, Jerusalem, Israel, August 18-23, 1996

Please contact the Secretariat if you require any information or assistance. Address all correspondence to:

IUMS Congresses'96
P.O.B. 50006
Tel Aviv 61500
Israel

Tel: 972 3 5140014
Fax: 972 3 5175674 or 972 3 5140077

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

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

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

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

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

Invitation

On behalf of the Australian Biotechnology Association, I would like to invite you to attend the 9th International Symposium on Yeasts *(ISY9)* to be held in Sydney in August 1996. The ISY is held every four years as an activity of the International Commission on Yeasts to foster interest in the science and technology of yeasts. The International Commission on Yeasts is a member of the International Union of Microbiological Societies. This will be the first time that the ISY has been held in Australia. As an added attraction, it will be held concurrently with the 10th International Biotechnology Symposium (*IBS10*). The venue for both symposia is the Sydney Convention Centre which is a custom designed complex where all the concurrent sessions, trade exhibition and poster areas are contained within the same building. The Convention Centre is located in the heart of Sydney on the foreshore of its spectacular harbour. The scientific program has been designed to cover recent advances in all aspects of yeast biology and yeast technology. Advice on content and speakers has been obtained from the vast network of councillors with the International Commission on Yeasts. The program will consist of plenary presentations, contributions to specialised symposia, and poster presentations of which several will be selected for oral communication. With the exception of plenary presentations, the scientific program has been organised as two concurrent sessions, allowing some degree of flexibility and choice. It will include 16 symposium sessions of invited speakers and 6 proffered paper sessions. In addition, opportunity will exist to attend sessions in the IBS program and there will be a substantial trade exhibition which is being held in conjunction with IBS. To encourage a strong submission of high quality posters, the International Commission on Yeasts is offering prizes of US$500 each for the two best poster presentations. The posters will be judged by an international panel on the basis of scientific merit and quality of presentation. For one of the prizes, preference will be given to younger scientists. The social program has been designed to feature some of the unique attractions of Sydney (the Opera House, the harbour, the Power House Museum) and will be

concluded with a dinner based on the theme *A taste of Australia*. The Australian Biotechnology Association is honoured to be hosting the 9th ISY and the 10th IBS. We anticipate symposia of excellence and excitement and look forward to seeing you in Australia in 1996.

Graham H. Fleet, Chair, Organising Committee, ISY9

Scientific Program

The following is an outline of the contents of the program (provisional) and names of scientists who have accepted invitations to speak at Plenary or Symposia sessions. Other invitations are pending.

Plenary presentations

1. Yeast budding - J. Pringle, U. North Carolina.
2. Molecular taxonomy - C. Kurtzman, USDA, Peoria.
3. Traditional (yeast) biotechnology - J. Friend, Burns Philp, Sydney.
4. Cryptococcal infections - T. Sorrell, Westmead Hospital, Sydney.
5. Yeasts as biotherapeutic agents - L. McFarland, Biocodex, USA.
6. Yeast genome project, the future - S. Oliver, U. Manchester
7. Wine yeasts, future directions - P. Henschke, Australian Wine Research Institute.

Symposia

Taxonomy/systematics

Speakers: T. Boekhout (The Netherlands), J. Fell (USA), A. Vaughan-Martini (Italy), J. Sugiyama (Japan), H.G. Prillinger (Austria), E. Swann (USA).

Topics: Molecular and conventional systematics of Ascomycetes and Basidiomycetes.

Ecology

Speakers: H.J. Phaff (USA), W. Middelhoven (The Netherlands), L. Mendonca-Hagler (Brazil), A.N. Hagler (Brazil)

Topics: Ecology and biodiversity; yeasts from tropical environments and plants, biodegradation.

Biochemistry and physiology

Speakers: A. Kimura (Japan), P. Piper (UK), K. Watson (Australia), J.T. Pronk (The Netherlands), K. Matsumoto (Japan), C. Leão (Portugal), J. Thevelein (Belgium), A. Goffeau (Belgium), J.P. van Dijken (The Netherlands)

Topics: Physiology and molecular biology of oxidative and heat stresses. Carbohydrate metabolism. Signal transduction, cyclic AMP and metabolic pathways. Transport of organic acids and sugars. Membrane proteins and transport.

Gene expression

Speakers: H. Bussey (Canada), I. Dawes (Australia), B. Carter (Denmark), I. Macreadie (Australia), K. Osinga (The Netherlands), M. Jagadish (Australia), P. Nagley (Australia).

Topics: Gene expression and cell walls, sporulation, mitochondria and metabolism. Foreign genes and heterologous proteins.

Food, feed and beverage yeasts

Speakers: B. Viljoen (South Africa), P. Romano (Italy), E. Johnson (USA), R. Schwan (Brazil), M. Stratford (UK), H. Takano (Japan), B. Prior (S. Africa), R. Sommer (Germany).

Topics: Ecological and metabolic diversity of food spoilage yeasts. Preservative resistant and osmotolerant yeasts. Pigments from yeasts. Yeast autolysis. Baking yeasts. Wine yeasts. Cocoa bean fermentation.

Yeasts as pathogens

Speakers: J. Edwards (USA), D. Marriott (Australia), S. Chen (Australia), M. Rinaldi (USA), D. Carter (Australia), J. Warmington (Australia), W. Meyer (Australia), J. Douglas (UK).

Topics: Pathogenesis and epidemiology of *Candida* and *Cryptococcus.* Antifungal testing and resistance. Molecular markers, PCR and molecular immunology in yeast pathogenesis. Biofilms and yeast pathogenesis.

Ethanol production.

Speakers: B. Hahn-Hägerdal (Sweden), J. du Preez (South Africa), M. Ingledew (Canada), M. Penttila (Finland)

Topics: Lignocellulosic and starch substrates for ethanol production. High gravity fermentation; Genetic improvement for immobilized yeast fermentations.

Cell structure

Speakers: G. Daum (Austria), M. Osumi (Japan).

Topics: Lipid assembly into membranes. Cell wall formation.

Education

Speakers: G. Stewart (UK), M. Miller (USA), H.J. Phaff (USA), W.A. Scheffers (The Netherlands).

Overview of the Conference

Sun Aug 25	10:00-17:00	Registration; Evening Welcome Reception (Opera House).
Mon Aug 26	8:30-10:30	Opening Plenaries
	11:00-13:00	Symposium Sessions
	14:30-16:00	Symposium Sessions
	16:30-17:30	Proffered papers
Tue Aug 27	8:30-10:30	Symposium Sessions
	11:00-13:00	Symposium Sessions
	14:30-16:00	Posters/Trade Display
	16:30-17:30	Proffered papers
	Evening	Reception (Powerhouse Museum)
Wed Aug 28	8:30-10:30	Plenaries
	11:00-13:00	Posters/Trade Display
	14:30-17:30	Excursion
Thu Aug 29	8:30-10:30	Symposium Sessions
	11:00-13:00	Symposium Sessions
	14:30-16:00	Symposium Sessions
	16:30-17:30	Proffered Papers
	Evening	Conference Dinner
Fri Aug 30	8:30-10:30	Symposium Sessions
	11:00-13:00	Plenary (wine) - Closing Ceremony - Wine Tasting

Registration fees, per delegate, in Australian dollars.

	Before April 30 96	After April 30 96
Regular *ISY9* **or** *IBS10*	$550.00	$650.00
Regular *ISY9* **and** *IBS10*	$650.00	$750.00
Student *ISY9* **or** *IBS10*	$180.00	$260.00
Student *ISY9* **and** *IBS10*	$220.00	$280.00
Day registration	$220.00	$280.00
Accompanying person	$180.00	$200.00

Registration is $100 less for members of the Australian Biotechnology Association.

Second Circular and Contact

The second and final circular, calling for abstracts, is being distributed in December 1995. For further information please contact:

9th ISY '96 Secretariat
GPO Box 128
Sydney NSW 2001 Tel: 61 2 262 2277
Australia Fax: 61 2 262 2323
Email: <tourhosts@tourhosts.com.au>

or

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Sydney NSW 2052 Tel: 61 2 385 5664
Australia Fax: 61 2 385 5931
Email: <s.debreczeni@unsw.edu.au>

Yeast Research on the Internet

YEAST on the Internet

There is a wealth of information for yeast biologist on the Internet. A good starting point is at the World Wide Web site *http://genome-www.stanford.edu/VL-yeast.html*, which is maintained by Michael Cherry <*cherry@genome.stanford.edu*>.

YEAST E-mail directory

There is an e-mail directory of all yeast biologist who have access to the Internet. If you are interested in being on this list, or interested in seeing the list, please see the instructions below. The December edition of the directory has more than 1550 names and addresses and can be obtained in a variety of ways. The latest version of the list can be obtained by anonymous FTP from *ncbi.nlm.nih.gov* in the */repository/yeast* directory. This list can also be searched by using the gopher server at *gopher.gdb.org* in the following section:

--> 14. Searching For Biologists/

--> 12. e-mail Addresses of Yeast Researchers/

In this section you can also add or correct your e-mail address if you want in on the list.

e-mail Addresses of Yeast Researchers

1. About The Yeast e-mail Directory.
2. Browse e-mail Addresses of Yeast Researchers/
3. Search e-mail Addresses of Yeast Researchers<?>
4. Retrieve the entire Yeast e-mail Addresses Directory.

--> 5. Add (or Correct) Your Address on the e-mail Directory <TEL>

This gopher server is also reachable via the *Saccharomyces cerevisiae* World Wide Web server (SGD). The URL for this server is *http://genome-www.stanford.edu/*. Please forward all corrections, additions and comments about this list to Francis Ouellette <francis@ncbi.nlm.nih.gov>.

YEAST BIOSCI/bionet newsgroup

There is a bionet/BIOSCI yeast newsgroup (you can access it via Usenet or by an electronic mail subscription) which deal with many aspects of yeast molecular biology, genetics and other related topics. If you want to subscribe to the yeast bionet newsgroup, send mail to <*biosci-server@net.bio.net*> with this message:

subscribe yeast

This will initiate your subscription. If you want to receive more information about the other BIOSCI newsgroups, send an empty message to <*biosci@net.bio.net*>. To send a contribution to the YEAST newsgroup, send email to <*yeast@net.bio.net*>. Be aware that thousands of people can then read what you send to this address. To cancel your subscription, mail to the <*biosci-server@net.bio.net*> (**NOT** <*yeast@net.bio.net*>) address with this message:

unsubscribe yeast

If you need further assistance on the BIOSCI/bionet newsgroup, send E-mail to <*biosci-help@net.bio.net*>. If all else fails, you can write to Francis Ouellette <*francis@ncbi.nlm.nih.gov*> who is the coordinator of this newsgroup.

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