ISSN 0513-5222

Y E A S T

A Newsletter for Persons Interested in Yeast

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

DECEMBER 1993 **Volume XLII, Number II**

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Notices to our readers

Format of communications

The ever increasing diversity of media and formats used to send Yeast Newsletter communications offers the advantage of flexibility and convenience. However, to facilitate the task of assembling the final copy, we ask our readers to take note of the following.

General. Whenever possible, please write references in the same format as used in the YNL, especially as to the use of capital letters. Please do not send communications by Fax.

Diskettes. If at all possible, this is the preferred method. You may use any MS-DOS compatible density (3.5" or 5.25"). The file should be either in DOS-ASCII or in WP5.1 format, with a single font size and no columns. Please enclose a printout.

E-mail. To avoid truncation of lines, please insure that each line does not exceed 78 characters in length. Please mail a printed copy to allow the restoration of correct diacritic or other symbols, italics, superscripts, or subscripts in the final copy. Please send standard ASCII (non-coded) characters or a "uuencoded" file. For e-mail users not familiar with uuencode/uudecode, these are programs that convert the entire set of extended ASCII codes (including WP5.1 commands) into e-mail transmissible material, and reconvert the coded material back at the receiving end. The e-mail package Pegassus has a built-in facility for sending uuencoded files. **Please note our new e-mail address: *lachance@julian.uwo.ca.***

Computer printouts or typewritten material. Printed or typed materials are entered by optical scanning. Whenever possible, please use 12 point (10 cpi), evenly spaced, standard characters, left justification only, and wide margins (2.5 cm or more). We have experienced considerable difficulty scanning some proportionally spaced materials, smaller fonts, or very wide text. These difficulties are exacerbated by the use of italics or boldface (underlining is preferred).

Reprints. Readers who prefer to send reprints should forward either the originals or a high quality photocopy of the same size and contrast as the original.

Thank you for your kind consideration of these matters.

Yeast Newsletter format

We are constantly working towards improving the quality of presentation of the Yeast Newsletter. The increasing volume of communications sent for inclusion, specially in the fall issue, is seen as a sign of the enthusiasm of our readers towards sharing the results of their research activities with other YNL readers. The smaller character size used in this issue allowed integral publication of all communication without doubling the cost of postage. Readers who find the legibility unacceptable should contact us regarding possible alternatives.

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

M. A. Lachance
Editor

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

Recent acquisitions:

Candida lactis-condensi (Hammer) S.A. Meyer & Yarrow

7746 ex pastry dough, Netherlands: E.S. van Reenen-Hoekstra; cond: GPYA, 25C

Filobasidiella neoformans Kwon-Chung var. *bacillispora* Kwon-Chung - CBS form 23 required.

7747 (ATCC 76108); ex seedling of olive, Australia, South Australia; T. Pfeiffer; cond: PDA, 25C; B

7748 (ATCC 76109); ex air in hollow *Eucalyptus camaldulensis,* S.Australia; T. Pfeiffer; cond: PDA, 25C; B

7749 (ATCC 76110); ex bark of *Eucalyptus camaldulensis,* Australia, New South Wales; T. Pfeiffer; cond: PDA, 25C

7750 ex bark debris of *Eucalyptus camaldulensis,* USA. California; T. Pfeiffer; cond: PDA, 25C; B

Pichia sp.

7760 ex damaged fruit of *Malus* sp. (apple), USA. West Virginia: J. Mercier; cond: GPYA, 25C

The following papers have been published recently:

1. W.I. Golubev & T. Boekhout. 1992. Dimorphism in *Itersonilia perplexans*: Yeast and hyphal phases differ in their sensitivity to mycocins produced by tremellaceous yeasts. FEMS Microbiology Letters **98**:187-190.

The monokaryotic yeast phase of the heterobasidiomycete *Itersonilia perplexans*, unlike the hyphal phase, was found to be sensitive to mycocins produced by killer strains of *Cryptococcus humicola, Cr. laurentii, Cystofilobasidium bisporidii* and *Rhodotorula fujisanense*. Both the yeast and hyphal phases were resistant to mycocins of *Cr. podzolicus, Filobasidium capsuligenum, Rhodotorula glutinis, Rh. mucilaginosa, Rh. pallida, Sporidiobolus johnsonii, Sb. pararoseus* and *Sporobolomyces alborubescens*. The different sensitivity patterns of yeast and hyphal phases are probably caused by biochemical differences in the cell walls.

2. T. Boekhout, A. Fonseca, J.-P. Sampaio, & W.I. Golubev. 1993. Classification of heterobasidiomycetous yeasts: characteristics and affiliation of genera to higher taxa of Heterobasidiomycetes. Can. J. Microbiol. **39**:276-290.

The numerous studies on morphological, physiological, biochemical, and molecular characteristics of heterobasidiomycetous yeasts discussed in this paper have changed our taxonomic understanding of these fungi. It is clear that yeasts and yeastlike growth forms can be accommodated in at least three orders of Heterobasidiomycetes, viz. Ustilaginales, Tilletiales, and Tremellales (Table 3). The possibility that other heterobasidiomycetous orders contain yeast and yeastlike growth forms cannot yet be excluded. Molecular studies have demonstrated the need for splitting or merging traditionally recognized genera. Our discussion of currently used taxonomic methods demonstrates the need for an integrated approach in the systematics of these yeasts. Table 4 summarizes the taxonomic resolution of the discussed methodologies.

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

Catalogue of Cultures 1993. The 1993 edition of the NCYC Catalogue of Cultures is now available and replaces the edition published in 1990. In an extensively revised format it gives details of over 2,500 strains, including many new accessions since 1990. The Catalogue includes large collections of genetically-defined *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Pichia angusta (Hansenula polymorpha)* and killer strains. Important references plus optimum, maximum and minimum growth temperatures are listed with individual entries. Details are also given of depositors, equivalent strain designations and habitats. Another section lists NCYC strains with special applications. Data on morphology and physiology (fermentation and assimilation tests etc.) are given in a special section which can be read into database software from the floppy disk version of the catalogue. The catalogue is available as a text file on floppy disk for Macintosh computers and IBM PCs (and PC-compatibles). A view/search facility is supplied with each version plus full instructions for hard disk installation and use. Printed Edition (£15). PC Edition 3.5in disk (£15+VAT). Mac Edition 3.5in disk (£15+VAT). An invoice will follow delivery. **Order** by writing to the address above, or by phone (0603 56122) or FAX (0603 58414). **Payment methods**: The preferred method is by cheque in pounds sterling drawn on a UK bank made payable to: The AFRC Institute of Food Research. For amounts less than £25 only payment in cash in pounds sterling is acceptable. Bank notes only are acceptable so round up to near £5. By Unesco coupons. Payment by BACS is under review and is presently only acceptable where no other method exists.

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

The following are recent publications:

1. Golubev, W.I. 1992. Killer activity in *Cryptococcus humicolus.* Abstr. VIII Int. Symp. on Yeasts (Atlanta, Aug. 23-28, 1992), 178.

Killer activity was found in *Cr. humicolus* strains including the type. It was expressed at pH values from 3 to 5.5 and the optimal pH was about 4. Killer toxins excreted had fungicidal action. They were stable against heat treatment and resistant to action of pronase E, but agitation of liquid media caused their inactivation. Column chromatography on Bio-Gel P2 indicated a small molecular mass for the *Cr. humicolus* toxin. Acid hydrolysates of active fractions contained amino acids and glucose. Plasmid-eliminating agents did not induce a loss of killer phenotype and dsRNAs were not detected in the killers. Killing patterns of the killer strains studied include not only basidiomycetous yeasts both of tremallaceous and ustilaginaceous affinity, but many ascomycetous yeasts.

2. T. Boekhout, A. Fonseca, J.-P. Sampaio & W.I. Golubev. 1993. Classification of heterobasidiomycetous yeasts: characteristics and affiliation of genera to higher taxa of Heterobasidiomycetes. Can. J. Microbiol. **39**:276-290.

See abstract in communication from CBS.

3. Golubev W.I. & Blagodatskaya, V.M. 1993. Taxonomic heterogeneity of *Pichia membranaefaciens* Hansen revealed by killer-sensitive reactions. Mikrobiologiya **62**:291-299.

23 mycocinogenic strains were found among 63 *Pichia membranaefaciens (Candida valida)* strains by cross-reaction testing on glucose-peptone agar (pH 4.5) with glycerol. They are combined in nine groups by killing patterns. Mycocins produced are thermolabile, protease-sensitive and have fungicidal action. Probably, their syntheses are coded by chromosomal genes. The strains studied are divided into two groups by sensitivity to the mycocins found. Such division coincides with differences between them in maximum temperatures of growth and succinate assimilation, and correlated with data on DNA homology. The results obtained indicate that succinate-negative strains with maximum temperature 35°C and below belong to *P. membranaefaciens (C. valida)*. Succinate-positive strains with maximum temperatures about 40°C are considered as a separate species of *Pichia punctispora* (Mélard 1910) Dekker 1931 nom. rev.

4. Golubev, W.I. & Churkina, L.G. 1993. Intrageneric killing patterns of *Rhodotorula mucilaginosa* mycocins. Izvestiya RAN (ser. biol.) **4**:550-557.

Killer strains of *Rh. mucilaginosa* were divided into several groups according to killing patterns of their mycocins produced. Most mycocins were active against *Rhodosporidium* spp. (with the exception of *Rhodosp. dacryoidum*) and the species *Rh. araucariae, Rh. fujisanensis, Rh. glutinis, Rh. graminis* and *Rh. mucilaginosa.* The species *Rh. acheniorum, Rh. ferulica, Rh. lactosa, Rh. minuta, Rh. pallida,* and *Rh. zsoltii* were insensitive to all *Rh. mucilaginosa* mycocins. In this respect the species *Rh. aurantiaca* was heterogeneous. All glucuronate- negative strains *(Rh. colostri)* were sensitive while glucuronate-positive ones were resistant. Among the yeasts that have been proposed to transfer to the genus *Rhodotorula* the species *Candida bacarum, C. fragariae, C. graminis, C. hylophila, C. ingeniosa, C. javanica, C. lignophila, C. muscorum,* and *C. sonckii* were sensitive to some *Rh. mucilaginosa* mycocins in contrast with the *C. acuta, C. auricularae, C. bogoriensis, C. buffonii, C. diffluens, C. foliorum, C. philyla, C. pustula, Cryptococcus hinnuleus, Cr. phylloplanus,* and *Torulopsis pilatii* that were resistant to all *Rh. mucilaginosa* mycocins.

5. Karamiysheva Z.N., Ksenzenko V.N., Golubev, W.I., Ratner, E.N., & Tikhomirova, L.P. 1993. Characterization of viruses from mycocinogenic strain BKM Y-2700 of *Cystofilobasidium bisporidii.* Doklady RAN **3**:376-378.

Cells of killer strain BKM Y-2700 contain icosahedral virus particles of 33-35 nm in diameter. The nucleic acids recovered from purified particles were two species of dsRNA about 1.8-1.9 and 5 kb. An AU-rich region was not detected in the dsRNA. An RNA-dependent RNA polymerase was associated with the viral particles. The molecular weight of the capsid protein was 80 kDa.

IV. Laboratoire de Microbiologie et de Technologie des Fermentations, Institut des Produits de la Vigne, INRA, 2 place Viala, 34060 Montpellier Cedex 01, France. Communicated by J.M. Salmon.

The following are recently accepted publications.

Genome of industrial yeast

1. Longo E. & F. Vézinhet. 1993. Chromosomal rearrangements during vegetative growth of *Saccharomyces cerevisiae.* Appl. Environ. Microbiol. **59**:322-326.

Improvement of industrial yeasts strains by classical genetics and molecular biology.

2. Vézinhet F., Barre P., Laurent P. & M. Valade. 1992. Introduction of flocculation into an industrial strain by transfer of a single chromosome. J. Inst. Brew., **98**:315-319.
3. Ansanay V., Dequin S., Blondin B. & P. Barre. 1993. Cloning, sequence and expression of the gene encoding the malolactic enzyme from *Lactoccocus lactis.* FEBS Lett. **332**:74-80.
4. Bidard F., Blondin B., Dequin S., Vézinhet F. & P. Barre. 1994. Cloning and analysis of a *FLO5* flocculation gene from *Saccharomyces cerevisiae.* Curr. Genet. (in press).
5. Dequin S. & P. Barre. 1993. Mixed acid-alcoholic fermentation by *Saccharomyces cerevisiae* expressing the *Lactobacillus casei* L(+)-LDH. Bio/Technology, (in press).
6. Barre P., Vézinhet F., Dequin S. & P. Barre. 1993. Genetic improvement of wine yeasts. In Wine Microbiology and Biotechnology, G.H. Fleet, editor, Harwood Academic Publishers, Chur, Switzerland, pp. 265-287.

Metabolic physiology.

7. Bely M., Salmon J.M. & P. Barre. 1994. Assimilable nitrogen addition and hexose transport activity during enological fermentations. J. Inst. Brew. (in press).
8. Delcroix A., Gnata Z., Sapis J.C., Salmon J.M. & C. Bayonove. 1994. Glycosydase activities of three enological yeast strains during winemaking: effect on the terpenol content of muscat wine. Amer. J. Enol. & Vitic. (in press).
9. Michnick S., & J.M. Salmon. 1993. Glycerol production from sugars with phosphoglycerate mutase-deficient *Saccharomyces cerevisiae* partially resistant to glucose repression. J. Indust. Microbiol. (in press).
10. Claret C., Salmon J.M., Romieu C. and A. Bories. 1994. Physiology of *Gluconobacter oxydans* during dihydroxyacetone phosphate production from glycerol. Appl. Microbiol. Biotechnol., (in press).

V. Departamento de Biotecnología, Universidad Autónoma Metropolitana, Iztapalapa, Apartado Postal 55-535, México D.F. 09340, Mexico. Communicated by M. García-Garibay and L. Gómez-Ruiz.

The following are recent papers published by our group:

1. A. Galvez, M.J. Ramírez & M. García-Garibay. 1990. Chemical composition of a mixture of single-cell protein obtained from *Kluyveromyces fragilis* and whey Proteins. Arch. Latinoamericanos de Nutrición **40**(2):252-262.

A mixture of *Kluyveromyces fragilis* biomass and coagulated whey proteins was obtained by fermentation of whole whey. This product had a chemical composition similar to washed products reported in the literature with high crude protein and low ash contents. The product had high contents of sulfur-containing aminoacids and tryptophane which are usually limiting in yeast biomass. Lysine content was inexplicably lower than the expected value being the limiting aminoacid in this case. The chemical score of the protein was 91%. From the biomass-whey proteins product a protein isolate could be recovered with a yield of 80%. The protein content of the isolate was 75% and the nucleic acids were reduced by 90.8%. The cell wall debris were also reduced considerably.

2. M. García-Garibay. 1992. Recovery of intracellular enzymes of industrial importance. Yeast β-galactosidase (in Spanish). Ciencia (Mex.). **43**(1):23-33.

The yeast lactase or β-galactosidase has been an enzyme of emerging technological importance during the last decade. There are many scientific reports concerning its production and utilization. However, literature related to downstream processing is very scarce. The lack of research is evident considering the low level of purity of commercial enzymes. This paper deals with unit operations involved in downstream processing of intracellular enzymes. It particularly focuses yeast lactase, and those techniques which have been used on industrial scale. A proposal of a process based on unit operations with high possibilities for current applications is presented.

3. P. Espinoza, E. Bárzana, M. García-Garibay & L. Gómez-Ruiz. 1992. Evaluation of *Kluyveromyces marxianus* for the production of lactase simultaneously to pectinase or inulinase. Biotechnol. Lett. **14**:1053-1058.

Five strains of *K. marxianus* were evaluated for the production of intracellular lactase, intra and extra cellular pectinase and intra and extra cellular inulinase. The strain NRRL-Y-1109 showed the highest lactase activity, but the strain CDBB-L-278 produced notably higher activities of inulinase and pectinase than the rest of the strains tested. The strain CDBB-L-278 was selected for the simultaneous production of two enzymes. Two enzymes fermentations were achieved with productions of 44% lactase and 53% pectinase, or 26% lactase and 47% inulinase compared to the single enzyme levels.

4. J.L. García, M. García-Garibay, M. Salvador & E. Galindo. 1993. A note of caution in determining glucose in molasses-based alcoholic fermentation broths by an enzymatic electrode. Biotechnology Techniques. **7**(7):453-456.

A technique for the determination of glucose as a measurement of sugars consumption in molasses-based alcoholic fermentation broths using an enzyme electrode was assessed. An electrochemical interference produced during the alcoholic fermentation was measured as glucose. An enzymatically inactive membrane discriminates between actual glucose and electrochemical interference.

Book.

5. M. García-Garibay, R. Quintero & A. López-Munguía, editors. 1993. Food Biotechnology (in Spanish). Editorial Limusa, S.A., México D.F.

The book contains 19 chapters written by 22 Mexican experts. It is divided in three parts. Part I deals with general aspects of Food Biotechnology. Part II is about the production of foodstuffs. Part III deals with the production of food additives. Several chapters deal with yeast science and technology, such as Chapter 2 which reviews genetic engineering applied to food science, Chapter 8 which deals with alcoholic beverages, Chapter 9 with indigenous fermented foods, Chapter 11 with single-cell protein and Chapter 18 with the production of microbial enzymes. The Book will be sold in all Latin America, Spain, U.S.A., and The Philippines.

Book Chapter.

6. M. García-Garibay, L. Gómez-Ruiz & E. Bárzana. 1993. Single-Cell Protein. Yeasts and Bacteria. In Macrae, R.K. Robinson & M.J. Sadler, editors. ENCYCLOPAEDIA OF FOOD SCIENCE, FOOD TECHNOLOGY AND NUTRITION. Academic Press, London. 1993.

VI. Instituto de Investigaciones Biomédicas del CSIC, Arturo Duperier 4, 28029 Madrid, Spain. Communicated by J.M. Gancedo.

The following papers have been published recently.

1. Blázquez M.A., Lagunas R., Gancedo C. & Gancedo J.M. 1993. Trehalose-6-phosphate, a new regulator of yeast glycolysis that inhibits hexokinases. FEBS Lett. **329**:51-54.

Trehalose-6P competitively inhibited the hexokinases from *Saccharomyces cerevisiae*. The strongest inhibition was observed upon hexokinase II,with an inhibition constant of 40 micromolar, while in the case of hexokinase I the inhibition constant was 200 micromolar. Glucokinase was not inhibited by trehalose-6P up to 5 mM. This inhibition appears to have physiological significance, since the intracellular level of trehalose-6P during growth on glucose was about 0.2mM. Hexokinases from other organisms were also inhibited while glucokinases were unaffected. The hexokinase from the yeast *Yarrowia lipolytica* was particularly sensitive to trehalose-6P inhibition; when assayed with 2 mM fructose an apparent inhibition constant of

5 micromolar was calculated. Two *S. cerevisiae* mutants with abnormal levels of trehalose-6P exhibited defects in glucose metabolism. It is concluded that trehalose-6P play an important role in the regulation of the first steps of yeast glycolysis, mainly through the inhibition of hexokinase II.

2. Blázquez M.A. & Gancedo C. 1993. Identification of extragenic suppressors of the *cif1* mutation in *Saccharomyces cerevisiae.* Curr. Genet. (In press).

The *cif1* mutation of *S. cerevisiae* causes inability to grow on glucose and related fermentable carbon sources. We have isolated two different suppressor mutations that allow growth on glucose of yeasts carrying the *cif* mutation. One of them, *sci1-1,* is recessive and caused inability to grow on non-fermentable carbon sources and to derepress fructose-1,6-bisphosphatase. The other suppressor mutation, *SCI2-1,* is dominant and diminished the capacity to phosphorylate glucose or fructose. The *SCI2-1* mutation decreased sporulation efficiency by 70% in heterozygosis and by more than 90% in homozygosis. In a *CIF1* background, cells carrying the mutation *SCI2-1* accumulated trehalose during the logarithmic phase of growth and hyperaccumulated it during the stationary phase. Genetic tests showed that *SCI2* was either allelic or closely linked to *HXK2*.

3. Amigo L., Moreno E. & Lagunas R. 1993. *In vivo* inactivation of the yeast plasma membrane ATPase in the absence of exogenous catabolism. Biochim. Biophys. Acta **1151**:83-88.

Yeast plasma membrane ATPase is inactivated up to 80% in the absence of catabolism of exogenous nutrients (exogenous catabolism). This inactivation, that is not accompanied by a decrease in the cellular content of ATPase, is due to an irreversible decrease of the V_{max} and does not require protein synthesis. The inactivated enzyme maintains the ability to be regulated by fermentable sugars but shows important alterations in the characteristics of this regulation. Upon addition of glucose, the V_{max} of the inactivated enzyme increases as well as its K_i for vanadate but, in contrast to the normal enzyme, its affinity for ATP or its pH optimum do not increase. It is concluded that in the absence of exogenous catabolism an irreversible modification of the yeast plasma membrane ATPase takes place that affects several of its kinetic properties.

4. Lagunas R. 1993. Sugar transport in *Saccharomyces cerevisiae.* FEMS Microbiol. Rev. **104**:229-242.

In this paper the characteristics of the transport systems for glucose, galactose and maltose are reviewed. It is concluded that the rate of sugar uptake in yeast cells is controlled by changes in the affinity of the corresponding transporters as well as by an irreversible inactivation that affects their maximal velocity. The mechanisms involved in these regulatory processes are at present unknown.

VII. Departamento de Bioquimica, Facultad de Medicina, U.A.M., Instituto de Investigaciones Biomedicas del CSIC, Arturo Duperier 4, 28029 Madrid, Spain. Communicated by F. Portillo.

The following is the summary of a paper recently submitted for publication to the Journal of Biological Chemistry.

1. Eraso, P. & Portillo, F. Molecular mechanism of regulation of yeast plasma membrane H^+-ATPase by glucose: Interaction between domains and identification of new regulatory sites.

The carboxyl-terminus of yeast plasma membrane H^+-ATPase is an autoinhibitory domain and its effect is counteracted by modification of the enzyme triggered by glucose metabolism (Portillo et al., FEBS Lett., 247, 381-385, 1989). To identify interacting domains involved in this regulation we have performed intragenic suppressor analysis. A double mutation at the carboxyl-terminus (S911AT912A) results in no activation of the ATPase by glucose and lack of yeast growth on this sugar (Portillo et al., FEBS Lett. 287, 71-74, 1991). Random *in vitro* mutagenesis of this mutant ATPase gene, transformation into yeast and selection for growth on glucose resulted in 29 revertants. Six corresponded to full revertants of the initial double mutation. Fourteen suppressor (second-site) mutations, six of them isolated two or three times, are located within three functional domains of the enzyme. Four mutations (A165V, V169ID170N, A350T and A351T) are localized at the cytoplasmic ends of predicted transmembrane helices 2 and 4; six mutations (P536L, A565T, G587N, G648S, P669L and G670S) map within the proposed ATP binding domain and the other four substitutions (P890opa, S896F, R898K and M907I) are located at the carboxyl-terminus. These results demonstrate the interaction, direct or indirect, between these three domains far apart in the linear sequence of the ATPase. All the second-site mutations caused constitutive activation of the ATPase in the absence of glucose metabolism. Second-site mutations at the carboxyl-terminus were close to S899 and suggested phosphorylation of this amino acid during glucose activation. Accordingly, the introduction of a negative charge, in a S899D mutant constructed by site-directed mutagenesis, partially mimics the glucose effect on the ATPase.

VIII. Molecular and Population Genetics Group, Research School of Biological Sciences, The Australian National University, P.O. Box 475, Canberra, ACT 2601, Australia. Communicated by R. Maleszka.

The following manuscript is in press in Gene.

1. M. Skrzypek[1] & R. Maleszka. A gene homologous to that encoding UDP galactose-4-epimerase is inducible by xylose in the yeast *Pachysolen tannophilus.*
[1]Department of Biochemistry, University of Kentucky, Lexington, KY 40506, USA

Using DNA sequencing, we have identified a fragment of genomic DNA from *Pachysolen tannophilus* that is homologous to the *GAL10* gene in yeasts and *galE* gene in bacteria, both encoding the UDP galactose-4-epimerase (EC 5.1.3.2). This gene (designated *PTGAL10*) is equally efficiently inducible by D-galactose (a hexose) and D-xylose (a pentose). The encoded protein shows the highest similarity to the homologous *Kluyveromyces lactis* protein. This includes the N-terminal domain that is not present in homologous polypeptides from other organisms.

IX. Dipartimento di Biologia Vegetale, Università degli Studi di Perugia, Borgo 20 Giugno 74, 06122 Perugia Italy. Communicated by A. Martini.

Dr. Maurizio Ciani from this department is presently working on a NATO fellowship in the laboratory of Dr. Linda Bisson of the Department of Viticulture & Enology of the University of California at Davis. Dr. Gianluigi Cardinali is spending one year as a postdoctoral fellow in the laboratory of Dr. Cornelis Hollenberg at the Institut für Mikrobiologie of the University of Dusseldorf in Germany. The following papers have been published recently.

1. Martini A. 1992. Biodiversity and conservation of yeasts. Biodiv. Conser. **1**:324-333.

2. Vaughan Martini A.E., A. Martini & G. Cardinali. 1993. Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces.* Antonie van Leeuwenhoek **62**:145-156.

3. Martini, A. 1993. Origin and domestication of the wine yeast *Saccharomyces cerevisiae.* J. Wine Res. **4**:165-176.

4. Diriye F.U., G. Scorzetti & A. Martini. 1993. Methods for the separation of yeast cells from the surfaces of processed, frozen foods. Int. J. Food Microbiol. **19**:27-37.

5. Palpacelli V., Ciani M. & G. Rosini. 1991. Activity of different "killer" yeasts on strains of yeast species undesirable in the food industry. FEMS Microbiol. Lett. **84**:75-78.

6. Ciani M., V. Palpacelli & G. Rosini. 1991. Sparkling wine production by cell-recycle fermentation. Biotechnol. Lett. **13**:533-536.

7. Rosini G. & M. Ciani. 1993. Influence of sugar type and level on the malate metabolism of immobilized *Schizosaccharomyces pombe* cells. Am. J. Vit. Enol. **44**:113-117.

X. Departamento de Microbiología, Escuela Técnica Superior de Ingenieros Agrónomos y de Montes (ETSIAM), Universidad de Córdoba, Spain. Communicated by J. Ramos.

The following are papers recently accepted for publication:

1. Luyten, K.,[1] Koning, W.,[1] Tesseur, I.,[1] Ruiz, M.C., Ramos, J., Cobbaert, P.,[1] Thevelein, J.M.,[1] & Hohmann, S.,[1] 1993. Disruption of the *Kluyveromyces lactis GGS1* gene causes inability to grow on glucose and fructose is suppressed by mutations that reduce sugar uptake. Eur. J. Biochem. In press.
[1]Laboratorium voor Moleculaire Celbiologie, Katholieke Universiteit Leuven, Flanders, Belgium.

In the yeast *Saccharomyces cerevisiae* the *GGS1* gene is essential for growth on glucose or other readily fermentable sugars. *GGS1* is the same gene as *TPS1* which was identified as encoding a subunit of the trehalose-6-phosphate synthase/phosphatase complex and it is allelic to the *fdp1, byp1, glc6* and *cif1* mutations. Its precise function in the regulation of sugar catabolism is unknown. We have cloned the *GGS1* homologue from the distantly related yeast *Kluyveromyces lactis*. The *KlGGS1* gene is 74% and 79% identical at the nucleotide and amino acid sequence level, respectively, to the *S. cerevisiae* counterpart. We also compared the sequence with the partly homologous products of the *S. cerevisiae* genes *TPS2* and *TSL1* which code for the larger subunits of the trehalose synthase complex and with a *TSL1* homologue, *TPS3*, of unknown function. Multiple alignment of these sequences revealed several particularly well conserved elements. Disruption of *GGS1* in *K. lactis* caused the same pleiotropic phenotype as in *S. cerevisiae,* i.e. inability to grow on glucose or fructose and strongly reduced trehalose content. We have also studied short-term glucose-induced regulatory effects related to cAMP and cAMP-dependent protein kinase, i.e. the cAMP signal, trehalase activation, trehalose mobilization and inactivation of fructose-1,6-bisphosphatase. These effects occur very rapidly in *S. cerevisiae* and are absent in the *Scggs1* mutant. In *K. lactis* all these effects were much slower and largely unaffected by the

Klggs1 mutation. On the other hand, glucose strongly induced pyruvate decarboxylase and activated the potassium transport system in *K. lactis* and both effects were absent in the *Klggs1* mutant. Addition of glucose to galactose-grown cells of the *Klggs1* mutant caused, as in *S. cerevisiae*, intracellular accumulation of free glucose and of sugar phosphates and a rapid drop of the ATP and inorganic phosphate levels. Glucose transport kinetics were the same for the wild type and the *Klggs1* mutant in both derepressed cells and in cells incubated with glucose. We have isolated phenotypic revertants of the *Klggs1* mutant for growth on fructose. The suppressors that we characterized had, to different extents, diminished glucose uptake in derepressed cells but cells incubated in glucose showed very different characteristics. The suppressor mutations prevented deregulation of glycolysis in the *Klggs1* mutant but not the accumulation of free glucose. The mutants with higher residual uptake activity showed partially restored induction of pyruvate decarboxylase and activation of potassium transport. These observations further support the idea that unregulated influx of sugar into glycolysis, at least during the transition from the derepressed to the repressed state, is causing the inability of *ggs1* mutants to grow on fermentable sugars. Thus, *GGS1* is involved in a novel regulatory mechanism of the initiation of glycolysis and its importance is not restricted to *S. cerevisiae*.

2. Ramos, J., Alijo, R., Haro, R.,[1] Rodríguez-Navarro, A.[1] 1993. *TRK2* is not a low-affinity potassium transporter in *Saccharomyces cerevisiae*. J. Bacteriol. In press.

[1]Departamento de Microbiología, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Spain.

TRK1 and *TRK2* encode proteins involved in K^+ uptake in *Saccharomyces cerevisiae*. A kinetic study of Rb^+ influx in *trk1 TRK2*, *trk1 TRK2^D*, and *trk1 trk2* mutants reveals that TRK2 shows moderate affinity for Rb^+. K^+-starved *trk1Δ TRK2* cells show a low-affinity component accounting for almost the total Vmax of the influx, and a moderate-affinity component, exhibiting a very low Vmax. Overexpression of TRK2 in *trk1Δ TRK2^D* cells increases the Vmax of the moderate-affinity component, and this component disappears in *trk1Δ trk2Δ* cells. In contrast, the low-affinity component of Rb^+ influx in *trk1Δ TRK2* cells is not affected by mutations in *TRK2*. Consistent with the different levels of activity of the moderate-affinity Rb^+ influx, *trk1Δ TRK2* cells grow slowly in micromolar K^+, *trk1Δ TRK2^D* cells grow rapidly, and *trk1Δ trk2Δ* cells fail to grow. The existence of a unique K^+ uptake system composed of several proteins is also discussed.

XI. Laboratoire de Biologie végétale et biotechnologie, Faculté des Sciences et Techniques, 2, rue de la Houssinière, Université de Nantes, F44072 Nantes Cédex 03. Communicated by L. Simon.

The following papers are recently published or in press.

1. L. Simon, C. Caye-Vaugien, and M. Bouchonneau. 1993. Relation between pullulan production, morphological state and growth conditions in *Aureobasidium pullulans:* new observations. J. Gen. Microbiol. **139**:979-985.

Aureobasidium pullulans is a polymorphic microfungus that produces extracellular pullulan in culture media with a life-cycle involving blastospores, hyphae, chlamydospores and intermediate forms. The form(s) responsible for polysaccharide synthesis is still uncertain. In order to clarify the relation between pullulan synthesis and the different morphological forms, we carried out studies under different culture conditions, varying nutrients, incubation time, aeration and agitation. The extracellular polysaccharides were analysed by enzymic and acidic hydrolysis and thin-layer chromatography. The number of residues in repeating units was established. The results are reported in relation to the cell density and the percentage of the different morphological types. The best conditions for producing polysaccharides, particularly pullulan, were determined. The results suggest that swollen cells and chlamydospores are the forms that produce extracellular polysaccharides. When significant amounts of chlamydospores are present, authentic pullulan is always observed whatever the culture conditions. The swollen cells are responsible for the synthesis of another polysaccharide capable of being transformed into pullulan; the hyphae do not play a role in extracellular polysaccharide biosynthesis.

2. L. Simon, G. Billon-Grand[1] and C. Caye-Vaugien. In press. The relationship between coenzyme Q system and morphological states in Aureobasidium pullulans. J. Gen. Appl. Microbiol.

[1]Laboratoire de Biologie, Section levures, Institut de Chimie et Biologie moléculaire et cellulaire, 43 Boul. du 11 november 1918, F 69622 Villeurbanne, France.

The coenzyme Q system of *Aureobasidium pullulans* has been investigated under different cultural and environmental conditions and its variation has been analysed in relation to conidial, hyphal and "resting form" biomass. The coenzyme Q system depends not only on culture age and studied strain but also on induced morphological states. The coenzyme Q system of resting forms (major Q10 and Q10 H_2)involved in pullulan production appears very different from those of hyphal forms (minor Q9 and major Q10 H_2).

XII. Department of Microbiology, University of Horticulture and Food, Somloi ut 14-16, Budapest H-1118, Hungary. Communicated by T. Deak.

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

1. Deak T. 1993. Simplified techniques for identifying foodborne yeasts. Int. J. Food Microblol. **19**:15-26.

2. Deak T. and L.R. Beuchat. 1993. Comparison of the SIM, API20C and ID32 systems for the identification of yeasts isolated from fruit juice concentrates. J. Food Protection **56**:585-592.

3. Deak T. and L.R. Beuchat. 1993. Evaluation of indirect conductance method for the detection of yeasts in laboratory media and apple juice. Food Microbiol. **10**:255-262.

4. Deak T. and L.R. Beuchat. Yeasts associated with fruit juice concentrates. Submitted to J. Food Protection.

5. Deak T. and L.R. Beuchat. Comparison of conductimetric and traditional plating techniques for detecting yeasts in fruit juices. Submitted to J. Appl. Bacteriol.

XIII. All-Russian Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, 1 Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova.

We would like to thank our co-authors Zinaida Azbukina (Institute of Biology, Vladivostok), Nadezhda Bur'yan (Institute Magarach, Yalta), Matti Korhola (ALKO Research Labs, Helsinki), Hilkka Turakainen (University of Helsinki), Corinne Michels (City University of New York), Claude Gaillardin (1.N.R.A., Paris-Grignon), Anita Panek, Allen Hagler and Leda Mendonça-Hagler (Universidade Federal do Rio de Janeiro), Edward Louis (Oxford University, U.K.) for fruitful cooperation during our visits in their labs. We will be glad to cooperate with zymologists studying natural polymorphism, taxonomy and evolution of yeasts. Our address until the end of 1994 is: Departamento de Microbiologia, Universidad de Cordoba, Escuela Tecnica Superior de Ingenieros Agronomos, Apartado 3048, 14080 Cordoba, España. Fax: (34 57) 218563.

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

1. G.I. Naumov, M. Korhola, E.S. Naumova, D.R. Beritashvili & R. Lantto. 1990. The molecular karyotyping of the biological species *S. cerevisiae, S. paradoxus* and *S. bayanus.* Dokl. Biol. Sciences **311**:212-216.

2. G. Naumov, H. Turakainen, E. Naumova, S. Aho & M. Korhola. 1990. A new family of polymorphic genes in *Saccharomyces cerevisiae:* α-galactosidase genes *MEL1-MEL7*. Mol. Gen. Genet. **224**:119-128.

3. G.I. Naumov, E.S. Naumova & M. Korhola. 1990. Identification of the *Saccharomyces sensu lato* yeasts on the basis of pulsed-field gel electrophoresis of chromosomal DNAs. In: Isolation, identification and storage of micromycetes and other microorganisms. Proceedings of the Conference. (pp. 98-101). Institute of Botany of Lithuanian Academy of Sciences, Vilnius (in Russian).

4. G. Naumov, E. Naumova, H. Turakainen, P. Suominen & M. Korhola. 1991. Polymeric genes *MEL8, MEL9* and *MEL1O* - new members of α-galactosidase gene family in *Saccharomyces cerevisiae.* Curr. Genet. **20**:269-276.

5. E.S. Naumova, G.I. Naumov, C.A. Michels & D.R. Beritashvili. 1991. Chromosomal DNA identification in yeast *Saccharomyces bayanus* and *S. pastorianus.* Dokl.Biol. Sciences **316**:58-61.

6. G.I. Naumov, E.S. Naumova & C.A. Michels. 1991. Identification of a functional α-glucosidase gene in natural mutants of *Saccharomyces cerevisiae* and *S. paradoxus* that do not ferment maltose. Dokl. Biol. Sciences **316**:78-81.

7. G.I. Naumov, E.S. Naumova, T.V. Chernookova, T.K. Skorikova, N.I. Bur'yan & N.G. Sarishvili. 1991. Killer-yeasts in champagne production. Soviet Agricul. Sciences **2**:33-34.

8. G. Naumov, E. Naumova & M. Korhola. 1992. Genetic identification of natural *Saccharomyces sensu stricto* yeasts from Finland, Holland and Slovakia. Antonie van Leeuwenhoek **61**:237-243.

9. G.I. Naumov, E.S. Naumova, R.A. Lantto, E.J. Louis & M. Korhola. 1992. Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus:* electrophoretic karyotypes. Yeast **8**:599-612.

10. G.I. Naumov, E.S. Naumova, H. Turakainen & M. Korhola. 1992. A new family of polymorphic metallothionein-encoding genes *MTH1(CUP1)* and *MTH2* in *Saccharomyces cerevisiae.* Gene **119**:65-74.

11. E. Naumova, G. Naumov, Ph. Fournier, Huu-Vang Nguyen & C. Gaillardin. 1993. Chromosomal polymorphism of the yeast *Yarrowia lipolytica* and related species: electrophoretic karyotyping and hybridization with cloned genes. Curr. Genet. **23**:450-454.

12. G.I. Naumov, E. Naumova, Z.M. Azbukina, M. Korhola & C. Gaillardin. 1993. Genetic and karyotypic identification of *Saccharomyces* yeasts from Far East Asia. Cryptogamie Mycol. **10**:85-93.

13. G.I. Naumov. 1993. Phytopathogenic fungi Ustilaginales as prospective object for zymology and biotechnology. Biotechnologia **3**:2-3 (in Russian).

14. E.S. Naumova, G.I. Naumov & M. Korhola. 1993. Molecular karyotyping of different genetic lines of yeast *Saccharomyces cerevisiae.* Biotechnologia **5**:2-5 (in Russian).

15. E.S. Naumova, T.I. Chernookova, T.K. Skorikova, V.I. Kondratieva, N.I. Bur'yan & G.I. Naumov. 1993. Breeding of champagne strains on the base of interspecies hybridization *Saccharomyces cerevisiae* x *S. bayanus.* Biotechnologia **7**:8-13 (in Russian).

16. H. Turakainen, G. Naumov, E. Naumova & M. Korhola. 1993. Physical mapping of the *MEL* gene family in *Saccharomyces cerevisiae.* Curr. Genet. **21** (in press).

17. G. Naumov, E. Naumova & C. Gaillardin. 1993. Genetic and karyotypic identification of wine *Saccharomyces bayanus* yeasts isolated in France and Italy. Syst. Appl. Microbiol. **16** (in press).

18. G.I. Naumov, E.S. Naumova, L.C. Mendonça-Hagler & A.N. Hagler. 1993. Taxogenetics of *Pichia angusta* and similar methylotrophic yeasts (Minireview). Ciencia e Cultura **45** (in press).

XIV. Department of Applied Microbiology, Lund Institute of Technology, Lund University, P.O. Box 124, S-22100 Lund, Sweden. Communicated by B. Hahn-Hägerdal.

The following is a report on the current activities of my department on yeast.

Xylitol production with *S. cerevisiae* transformed with the gene for the enzyme xylose reductase from *P. stipitis* has been investigated with different cosubstrates, different ratios of xylose to cosubstrate and different levels of oxygenation (1, 2). On-line analysis for following ethanol fermentation of lignocellulose hydrolysates has been developed (3). It comprises microdialysis sampling, column liquid chromatography and an alcohol biosensor. A rapid chromatographic method for the production of preparative amounts of xylulose has been developed (4). The performance of yeasts and bacteria in two different non-detoxified lignocellulose hydrolysate have been compared (5). In an interlaboratory study the influence of media components and detoxification on the performance of ethanol-producing microorganisms in a xylose-rich acid hydrolysate was compared (6). In *Pichia stipitis* an alternative oxidase has been identified (7). It is hypothesised that in addition to the dual cofactor utilisation of the enzyme xylose reductase the presence of the alternative oxidase prevents xylitol formation during xylose fermentation in the yeast *Pichia stipitis.* From *Pichia stipitis* a short chain xylitol dehydrogenase has been cloned (8). The enzyme is a member of the medium chain alcohol dehydrogenase family. Two reviews on xylose and xylulose fermenting yeasts have recently been prepared (9,10).

References:

1. Carlsen H., Hallborn J., Gorwa M-F., & Hahn-Hägerdal B. 1993. Bioconversion of xylose to xylitol with recombinant *Saccharomyces cerevisiae* harbouring genes for xylose metabolism from *Pichia stipitis.* Proceedings ECB **6** (in press).
2. Meinander N., Hallborn J., Keränen S., Ojamo H., Penttilä M., Walfridsson M., & Hahn-Hägerdal B. 1993. Utilisation of xylose with recombinant *Saccharomyces cerevisiae* harbouring genes for xylose metabolism from *Pichia stipitis*. Proceedings ECB **6** (in press).
3. Buttler T., Gorton L., Jarskog H., Marko-Varga G., Hahn-Hägerdal B., Meinander N., & Olsson L. Monitoring of ethanol during fermentation of a lignocellulose hydrolysate by on-line microdialysis sampling, column liquid chromatography and an alcohol biosensor. (submitted for publication).
4. Olsson L., Linden T., & Hahn-Hägerdal B. A rapid chromatographic method for the production of preparative amounts of xylulose. Enzyme Microb. Technol. (accepted for publication).
5. Olsson L. & Hahn-Hägerdal B. 1993. Fermentative performance of bacteria and yeasts in lignocellulose hydrolysates. Process Biochem. **28**:249-257.
6. Hahn-Hägerdal B., Jeppsson H., Olsson L., & Mohagheghi A. An interlaboratory comparison of the performance of ethanol-producing microorganisms in a xylose-rich acid hydrolysate. Appl. Microbiol. Biotechnol (accepted for publication).
7. Jeppsson H., Alexander N J., & Hahn-Hägerdal B. Cyanide insensitive respiration in the xylose fermenting yeast *Pichia stipitis* CBS 6054. Biosource Technol. (accepted for publication).
8. Persson B., Hallborn J., Walfridsson M., Hahn-Hägerdal B., Keränen S., Penttilä M., & Jörnwall H (1993). Dual relationships of xylitol and alcohol dehydrogenases in families of two protein types. FEBS Letter **324**:9-14.
9. Hahn-Hägerdal B., Hallborn J., Jeppsson H., Olsson L., Skoog K., & Walfridsson M. 1993. Pentose fermentation to ethanol. In "Bioconversion of forest and agricultural plant residues", Ed. J. Saddler, CAB International, Wallingford, United Kingdom. pp 231-290.
10. Hahn-Hägerdal B., Jeppsson H., Skoog K., & Prior BA. Biochemistry and physiology of xylose fermenting yeasts. Enzyme Microb. Technol. (accepted for publication).

XV. VTT, Biotechnical Laboratory, P.O. Box 202, FIN-02151 Espoo, Finland. Communicated by M.-L. Suihko.

The following papers have been published since my last report.

1. Suihko, M.-L., Home, S. & Linko, M. 1993. Wort sugars, yeast sugar uptake and beer quality. Monatsschrift fr Brauwissenschaft **46**(5):185-192.
2. Suihko, M.-L., Vilpola, A. & Linko, M. 1993. Pitching rate in high gravity brewing. J. Inst. Brew. **99**:341-346.

XVI. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.C. du Preez.

The following papers have recently appeared or are in press:

1. Kock, J.L.F. and A. Botha. 1993. Acetic acid - a novel source of cocoa butter equivalents and gamma-linolenic acid. South African J. Science **89**:465.
2. Botha, A. and J.L.F. Kock. 1993. The distribution and taxonomic value of fatty acids and eicosanoids in the Lipomycetaceae and Dipodascaceae. Antonie van Leeuwenhoek **63**:111-123.
3. Botha, A. and J.L.F. Kock. 1993. Application of fatty acid profiles in the identification of yeasts. Int. J. Food Microbiol. **19**:39-51.

4. Botha, A., J.L.F. Kock, M.S. van Dyk, D.J. Coetzee, O.P.H. Augustyn and P.J. Botes. 1993. Yeast Eicosanoids. IV. Evidence for prostaglandin production during ascosporogenesis by Dipodascopsis tóthii. Syst. Appl. Microbiol. **16**:159-163.

5. Pretorius, E.E., F. Spaaij, G. Weber and J.P. van der Walt. 1993. *Myxozyma monticola* sp. nov. (Candidaceae), a new species from South African soil. Syst. Appl. Microbiol. **16**:261-265.

6. Meyer, P.S., J.C. du Preez, and S.G. Kilian. 1993. Selection and evaluation of astaxanthin-overproducing mutants of *Phaffia rhodozyma.* World J. Microbiol. Biotechnol. **9**:514-520.

7. Kilian, S.G., B.A. Prior and J.C. du Preez. 1993. The kinetics and regulation of D-xylose transport in *Candida utilis.* World J. Microbiol. Biotechnol. **9**:357-360.

8. Meyer, P.S. and J.C. du Preez. 1993. Effect of acetic acid on astaxanthin production by *Phaffia rhodozyma.* Biotechnol. Lett. **15**:919-924.

Low concentrations of acetic acid decreased the growth rate of and astaxanthin production by *Phaffia rhodozyma* on glucose, with growth completely inhibited by 2 g acetic acid/l. Using H2SO4 for pH control after sugar depletion caused a decline in the biomass concentration, whereas using acetic acid as titrant resulted in an increase in the biomass with a high astaxanthin content of 1430 µg/g cells. An extended culture with a continuous glucose feed failed to maintain a high astaxanthin content.

9. Meyer, P.S. and J.C. du Preez. 1993. Astaxanthin production by a *Phaffia rhodozyma* mutant on grape juice. World J. Microbiol. Biotechnol. **10** (in press).

During fermenter cultivation of *Phaffia rhodozyma* on a grape juice medium, the presence of glucose initially delayed fructose utilization, although fructose was consumed before glucose depletion. Total pigment and astaxanthin production were growth associated and reached maximum values of 15.9 µg/ml and 9.8 µg/ml, respectively, after depletion of the carbon source. The cellular total pigment and astaxanthin content increased during the stationary growth phase due to a decrease in biomass, reaching final values of 2120 µg/g and 1350 µg/g, respectively, without the volumetric concentration in the culture changing. The final cell yield was 0.33 g/g sugar utilized. High sugar concentrations in shake-flasks as well as oxygen limitation decreased the astaxanthin content of the cells. Addition of yeast extract to a grape juice minimal medium markedly increased the maximum specific growth rate, total pigment and astaxanthin content of the cells. An excess of ammonia decreased the intracellular astaxanthin content, which reached a maximal value in cultures with no residual glucose or ammonia.

10. Meyer, P.S., B.D. Wingfield and J.C. du Preez. 1994. Genetic analysis of astaxanthin-overproducing mutants of *Phaffia rhodozyma* using RAPDs. Biotechnol. Techniques **8**:1-6 (in press).

Simple and reproducible DNA fingerprints from a naturally occurring *Phaffia rhodozyma* strain as well as from astaxanthin-overproducing mutants were produced with a single arbitrary primer using PCR. Between 3 and 5 major DNA fragments were produced. These ranged in size from 0.7 to 2 kilobase pairs (kb). Some bands were present in all the *P. rhodozyma* strains while others were observed only in individual mutants.

11. Van Zyl, C., B.A. Prior, S.G. Kilian and E.V. Brandt. 1993. Role of D-ribose as a cometabolite in D-xylose metabolism by *Saccharomyces cerevisiae.* Appl. Environ. Microbiol. **59**:1487-1494.

12. Van Eck, J.H., B.A. Prior and E.V. Brandt. 1993. The water relations of growth and polyhydroxy alcohol production by ascomycetous yeasts. J. Gen. Microbiol. **139**:1047-1054.

13. Van Zyl, P.J., S.G. Kilian and B.A. Prior. 1993. Physiological responses of *Zygosaccharomyces rouxii* to osmotic stress. Appl. Microbiol. Biotechnol. **39**:235-241.

14. Van der Walt, J.P., E.A. Brewis and B.A. Prior. 1993. A note on the utilization of aliphatic nitriles by yeasts. Syst. Appl. Microbiol. **16**:330-332.

15. Albertyn, J., S. Hohmann and B.A. Prior. 1993. Characterization of the osmotic-stress response in *Saccharomyces cerevisiae:* osmotic stress and glucose repression regulate glycerol-phosphate dehydrogenase independently. Curr. Genet. (in press).

XVII. Biochemisches Institut der Universität Freiburg, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany. Communicated by H. Holzer.

Manuscript in preparation:

1. M. Destruelle, H. Holzer & D.J. Klionsky.[1] Identification and characterization of a novel yeast gene: the *YGP1* gene product is a highly glycosylated secretory protein that is synthesized in response to nutrient limitation.

[1]Department of Microbiology, University of California, Davis, California, 95616.

In this study we investigated the expression pattern of a new yeast gene, *YGP1* (Yeast Newsletter, Vol.XLII, No.I, 1993) and the possible role of its gene product, gp37. The gene is expressed at a basal level during logarithmic growth. Northern-blot studies with RNA isolated from the yeast strain SEY6210 at different time points during the growth-phase revealed an increasing expression of *YGP1* by entering the stationary phase. The construction of a hybrid-gene with the promotor sequences from *YGP1* fused to the coding region of *SUC2* allowed a quantitative analysis of the expression of *YGP1* in the dependence of several nutrients. The concentration of gp37 was measured as the activity of secreted invertase. When the glucose concentration in the medium falls below 1%, the *YGP1* gene is derepressed and the gene product, gp37, is synthesized at levels up to fifty-fold above the basal amount. Derepression also results from depletion of nitrogen and phosphate suggesting a general response to nutrient deprivation. When stationary phase cells depleted of glucose are shifted to a medium with high glucose (3%), gp37 is degraded within 6-8 hours as shown by Western analysis with a polyclonal antiserum raised against an oligopeptide from the protein. GP37 is therefore subject to catabolite inactivation. Gp37 is a highly glycosylated, secreted protein with a high homology to Sps100p, the putative gene product of *SPS100* (Law and Segall, Mol. Cell. Biol (1988) **8**:912-922). The authors suggested a possible role for Sps100p in the formation of the spore wall. A yeast strain that has a chromosomal disruption of the *YGP1* gene and is therefore not able to synthesize gp37 displays an increased sensitivity to zymolyase digestion of the cell wall. These results suggest that the *YGP1* gene product gp37 may be involved in cell survival during stationary phase and may be a component of the cell wall.

XVIII. AG Hefegenetik, Institut für Pflanzengenetik und Kulturpflanzenforshung, Correnstr. 3, D(0)-4325 Gatersleben, Germany. Communicated by G. Kunze.

The following are publications recently published or accepted, and a recently defended thesis.

1. Kunze, G., Kunze,I., Barner, A., & Schulz, R. 1993. Genetical and biochemical characterization of *Saccharomyces cerevisiae* industrial strains. Fresenius J. Anal. Chem. **346**:868-871.

Different brewing and wine yeast strains could be characterized by means of different genetical and biochemical techniques like pulsed-field gel electrophoresis, DNA-finger printing and one-dimensional SDS-polyacrylamide gel electrophoresis of secretory proteins. By combination of all three methods it was possible to distinguish each yeast strain from each other. Especially pulsed-field gel electrophoresis was the appropriate method to distinguish the strains.

2. Kunze,G. & Kunze, I. 1993. Characterization of *Arxula adeninivorans* strains from different habitats. Antonie van Leeuwenhoek (in press).

Some *Arxula adeninivorans* strains selected from wood hydrolysates in Siberia, from soil in South Africa and from maize silage and soil in The Netherlands were compared. DNA-fingerprinting, pulse field gel electrophoresis as well as analysis of secretory proteins have been chosen to describe the similarities among the strains. Combination of the three methods allowed identification of each strain. Strains from the same origin show extensive similarities. The results of the DNA-fingerprints indicate that the strain isolated in Siberia belongs to the group of strains originated from South Africa. However, it differed in the molecular weight of the third chromosome and in the pattern of secretory proteins from the South African isolates.

2. Forster, S. 1993. Characterization of *Arxula adeninivorans* mutants. Thesis (Diplomarbeit).

Arxula adeninivorans harbours a glucoamylase gene which can be induced by growth on maltose or starch media. The glucoamylase protein produced is secreted. Mutagenesis experiments with N-Methyl-N'-Nitro-Nitrosoguanidine (NG) were performed using the cys^-lys^- auxotrophic strain G704. Mutant cells were enriched by nystatin and colonies unable to grow on starch medium were selected. Six potential glucoamylase deficient strains (A1-A6) were characterized. With the exception of A4 and A6 they showed altered colony morphology. While colonies of the wild type have smooth surfaces, colonies of the mutant strains have rough surfaces and are partly intricated. All of them tend to form much more pseudomycelia

than the wild type. The strains A3, A4 and A6 do not express glucoamylase; we could measure neither intracellular nor extracellular activity. The other three strains possibly reverted to the wild type genotype. Interestingly, A1, A2, and A5 retained their altered colony morphology.

XIX. Laboratory of Research & Development, Bodegas Castel Pujol, Cesar M. Gutierrez 2556, 12400 Montevideo, Uruguay. Communicated by F. M. Carrau.

Recently accepted publication.

1. F.M. Carrau, E. Neirotti,[1] & O. Giola. 1993. Stuck Wine Fermentations: Effect of Killer/Sensitive Yeast Interactions. J. Ferment. Bioeng. **76**: in press.
[1]Laboratory of Microbiology, Faculty of Science, University of the Republic, Tristan Narvaja 1674, Montevideo, Uruguay.

Mixed-strain fermentations were carried out with different proportions of killer/sensitive yeasts. It was observed that stuck and sluggish fermentations due to killer/sensitive strain interaction depended on: a. the killer/sensitive yeast proportion at the start of fermentation, b. the concentration of nitrogen source in the grape must, and c. the presence of bentonite during fermentation. The effect of the addition of active carbon and commercial yeast cell walls to sluggish fermentation media was also studied.

XX. Department of Biology, Carleton University Ottawa, Ontario KIS 5B6, Canada. Communicated by B.F. Johnson.

Recent publication

1. B.F. Johnson, I. Curran,[1] and T. Walker.[1] 1993. Medium-induced fragility of *Schwanniomyces*. Antonie van Leeuwenhoek, in press.
[1]National Research Council, Ottawa, Ontario KIA OR6.

Schwanniomyces occidentalis has attracted interest because of its ability to metabolize starch and similar complex carbohydrates. Studies have been undertaken, mostly using defined media, to ascertain conditions for optimal production and secretion of hydrolytic enzymes. Here we demonstrate the fragility of *Schw. occidentalis* in many defined media. We especially examined viability in YNB (Yeast Nitrogen Base) plus 1% glucose. Without phosphate supplementation, viability was routinely very low at stationary phase (usually less than 37%), whereas viability of stationary-phase cultures in phosphate-supplemented YNB usually exceeded 97%. The negative implications of having many, presumably permeabilized, dead cells present in assays for secretion of enzymes by living yeast cells are discussed.

XXI. Institute of Enology and Viticulture, Yamanashi University, Kitashin, 1-13-1, Kofu, 400, Japan. Communicated by S. Goto.

The following papers were recently published.

1. Goto, S., Kitano,K., & Shinohara, T. 1992. Utilization of KHR killer as genetic marker for purity test of starter yeast during fermentation of grape musts. J. Ferment. Bioeng. **73**:70-73.

An excellent wine yeast, *Saccharomyces cerevisiae* W3, which had KHR killer, was added as a starter yeast into grape must and behavior of the starter strain and wild yeasts was investigated during fermentation by using KHR killer as a genetic marker. The KHR killer was detected only in the strain W3 and not other wine and wild yeast strains. Accordingly, the frequency of starter yeast W3 was monitored throughout the fermentation of grape musts by using KHR killer. W3 was discriminated efficiently from wild yeasts during fermentation by KHR killer activity and proved to lead the fermentation as a dominant yeast until their fermentation.

2. Kishimoto, M., Shinohara, T., Soma, E., & Goto, S. 1993. Selection and fermentation properties of cryophilic wine yeasts. J. Ferment. Bioeng. **75**:451-453.

Two cryophilic strains, YM-84 and YM-126, were selected by a double-layer agar fermenting technique from 100 strains of the wine yeast, *Saccharomyces cerevisiae.* The viability (specific growth rate) and fermentability of the two selected strains at low temperatures (7 and 13 C) were superior to those to wine yeast. strains W3 and OC-2, indicating the usefulness of the two strains as cryophilic wine yeasts. Experiments using the two selected strains at intermediate temperatures (22 and 30 C) showed that their fermentation ceased prematurely and their ethanol yields were reduced.

3. Kishimoto, M., Shinohara, T., Soma, E., & Goto, S. 1993. Identification and enological characteristics of cryophilic wine Yeasts. J. Brew. Soc. Japan. **88**:708-713.

Identification and enological characteristics of cryophilic wine yeast, YM-84 and YM-126, which were selected by a double-layer agar fermenting technique, were investigated. The two strains were identified as *Saccharomyces cerevisiae* (biotype, *S. uvarum*), and estimated to be homothallic and diploid strains. These two strains were also subjected to laboratory-scale fermentation tests using Koshu grape must. Their fermentability at low temperature (7-10 C) were superior to those of wine yeast strains W3 and OC-2. At intermediate temperature (22 and 25 C), however, their fermentation ceased prematurely and their

ethanol yields were reduced. In addition, these strains produced large amounts of higher alcohols (HA), b-phenethyl alcohol (PheOH) and b-phenethyl acetate (PheOAc) at low temperature. Especially at 10 C fermentation, YM-84 produced about 1.7 times as much HA and about 10 times as much PheOH and PheAc as OC-2. These strains also produced more malic acid and less acetic acid than OC-2 at low temperature. These suggested that the two strains of YM-84 and YM-126 were suitable for low temperature fermentation in white wine making.

4. Kishimoto, M., Oshida, A., Shinohara, T., Soma, E., & Goto, S. 1993. Effect of temperature on ethanol productivity and resistance of cryophilic wine yeasts. J. Gen. Appl. Microbiol. in press.

Effects of incubation temperature and ethanol concentration on cell viability of two strains of cryophilic wine yeasts, YM-84 and YM-126, which showed good fermentability at low temperatures (7 and 13 C), but prematurely ceased fermentation and reduced ethanol yields at intermediates (22 ed 30 C), were compared with those of mesophilic wine yeasts W3 and OD-2. The cryophilic wine yeasts, YM-84 and YM-126, showed certain cell viability in the presence of 10% ethanol at 8 C, the same as the mesophilic wine yeasts, W3 and OC-2. At 25 C, however, viable cell numbers of these cryophilic wine yeasts were decreased, and this tendency became more remarked with the presence of ethanol. These results expound that the two strains of YM-84 and YM-126 produced low amounts of ethanol in the fermentation process at intermediate temperatures. Comparison of the composition of fatty acid in cells, YM-84 and YM126 showed high proportions of myristic acid to fatty acid, in addition to high ratios of C16 fatty acids to C18 fatty acids. These phenomena suggest that these strains have different adaptation mechanisms against temperatures. Five strains of cryophilic wine yeasts selected in our culture collection showed the same fermentation activity at the low temperature of 7 C as did YM-84 and YM-126, and at 28 C their fermentation ceased prematurely and their ethanol yields were reduced. So from these results, it can be conclude that good fermentability at low temperatures accompanied by production of low amounts of ethanol at intermediate temperatures is one of the fermentation characteristics observed among cryophilic wine yeasts.

5. Kishimoto, M., Soma, E., & Goto, S. 1993. Classification of cryophilic wine yeasts based on electrophoretic karyotypes, GC content and DNA similarity. J. Gen. Appl. Microbiol. in press.

Seven strains of cryophilic wine yeasts, two strains of mesophilic wine yeasts and two strains of bottom fermenting brewer's yeasts were investigated for their utilization of sugars, viability of ascospores in hybrid constructed between cryophilic wine yeast and mesophilic wine yeast, electrophoretic karyotypes, GC content of DNA and DNA similarity in photobiotin microplate-hybridization. Six strains of cryophilic wine yeasts were identified as *Saccharomyces uvarum* and one strain of cryophilic wine yeast was identified as *S. chevalieri* according to THE YEASTS (1970) based on mainly sugar utilization. However, these cryophilic wine yeasts were similar to the type strain of S. bayanus IFO 1127 in electrophoretic karyotype and GC content. In DNA hybridization, the cryophilic wine yeasts also showed high DNA similarity values to the type strain of *S. bayanus* and low DNA similarity values to the type strain of *S.cerevisiae* IFO 10217. Accordingly, these seven strains of cryophilic wine yeasts were classified to *S. bayanus.* Additionally, another eight strains of *S. bayanus,* classified on the basis DNA similarity, showed good fermentability at low temperature accompanied by production of low amounts of ethanol at intermediate temperatures, the same as cryophilic wine yeasts. These results suggest that the fermentation characteristics are specific for *S. bayanus.* Two strains of mesophilic wine yeasts showed high DNA similarity values to the type strain of *S. cerevisiae.* Two strains of brewer's yeasts showed intermediate fermentation characteristics of cryophilic wine yeast and mesophilic wine yeast, and are considered to be a hybrid between *S. cerevisiae* and *S.bayanus* in DNA hybridization experiments.

6. Yanagida, F., Kamata, T., Shinohara, T., & Goto, S. 1993. Identification of lactic acid bacteria isolated from red wine-making process. J. Brew. Soc. Japan **88**:238-244.

Red wines were made from Muscat Bailey A and Cabernet Sauvignon grapes, respectively, in 1990. Twenty-six strains of lactic acid bacteria were isolated from experimental red wine-making process. Isolated strains were examined in regard to their morphological and physiological characters, and identified. Twelve strains of *Lact. plantarum* were isolated from the first stage of wine-making process. Eight strains of *Leuc. oenos* were isolated from the latter term of wine making process. The six other bacterial strains isolated were classified as belonging to the genus *Lactobacillus* and *Leuconostoc,* including one strain of *L. reuteri,* one strain of *Leu. mesenteroides,* two strains of *Lactobacillus* sp., and two strains of *Leuconostoc* sp. Growth temperature, initial pH and alcohol tolerance were different according to the isolates. Decomposition rate of L-malic acid by the isolates ranged from zero to 41%.

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

The following papers have been published recently.

1. O.E. Vuorio, N. Kalkkinen[1] & J. Londesborough. 1992. Cloning of two related genes encoding the 565 kDA and 123 kDA subunits of trehalose synthase from the yeast *Saccharomyces cerevisiae.* Eur. J. Biochem. **216**:849-861.

[1]University of Helsinki, Institute of Biotechnology, Helsinki, Finland.

Preparations of intact trehalose synthase contain three polypeptides with molecular masses of 56, 102 and 123 kDa. We have cloned the genes *TSS1* and *TSL1* coding for the 56- and 123-kDa subunits, respectively. These genes are located on chromosomes 11 (*TSS1*) and XIII (*TSL1*). The TSS1 gene was found to be identical with *CIF1,* a gene required for normal growth on glucose. The product of the entire *TSS1* gene exhibits 37% identity with a 502-amino-acid stretch from the middle of the *TSL1* product. Disruption of the *TSS1* gene in yeast eliminates both trehalose 6-phosphate synthase (Tre6P synthase) and trehalose 6-phosphate phosphatase (Tre6Pase) activities, and reintroduction of this gene restores these activities. Transformation of *Escherichia coli* with *TSS1* increases its Tre6P synthase activity. Specific proteolytic degradation of the 123-kDa polypeptide from the N-terminus greatly influences the Tre6P synthase activity, decreasing its inhibition by phosphate and activatability by fructose 6-phosphate but has little effect on the Tre6Pase activity. These results suggest that this N-terminal part confers regulatory properties upon the Tre6P synthase activity.

2. A.E.I. Vainio, H.T. Torkkeli, T. Tuusa, S.A. Aho, B.R. Fagerström, M.P. Korhola. 1993. Cloning and expression of *Hormoconis resinae* glucoamylase P cDNA in *Saccharomyces cerevisiae.* Curr. Genet. **24**:38-44.

A cDNA coding for glucoamylase P of *Hormoconis resinae* was cloned using a synthetic oligonucleotide probe coding for a peptide fragment of the purified enzyme and polyclonal anti-glucoamylase antibodies. Nucleotide-sequence analysis revealed an open reading frame of 1848 base pairs coding for a protein of 616 amino-acid residues. Comparison with other fungal glucoamylase amino-acid sequences showed homologies of 37-48%. The glucoamylase cDNA, when introduced into *Saccharomyces cerevisiae* under the control of the yeast *ADC1* promoter, directed the secretion of active glucoamylase P into the growth medium.

XXIII. Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604. Communicated by C.P. Kurtzman.

The following are publications recently accepted or published.

1. Kurtzman, C.P. & C.J. Robnett. 1993. Orders and families of ascosporogenous yeasts and yeastlike taxa compared from ribosomal RNA sequence similarities. Proc. First Intl. Workshop on Ascomycete Systematics. In press.

The type species from all known genera of cultivatable ascomycetous yeasts and yeastlike fungi were compared from extent of divergence in partial nucleotide sequences from large and small subunit ribosomal RNAs. The data indicate the taxa comprise two orders - the Schizosaccharomycetales and the Saccharomycetales (Endomycetales). The comparisons demonstrate that certain currently accepted families are artificial. Furthermore, the rRNA sequence comparisons show that the ascosporogenous yeasts and yeastlike taxa are not reduced forms of extant filamentous fungi, but that they represent a phylogenetically homogeneous clade distinct from the fission yeasts, Euascomycetes, Taphrinales, and Protomycetales.

2. Kurtzman, C.P. 1993. Systematics of the ascomycetous yeasts assessed from ribosomal sequence divergence. Antonie van Leeuwenhoek **63**:165-174.

Extent of divergence in partial nucleotide sequences from large and small subunit ribosomal RNAs was used to estimate genetic relationships among ascomycetous yeasts and yeastlike fungi. The comparisons showed four phylogenetically distinct groups comprised of the following taxa: Group 1. The budding yeasts *Saccharomyces, Saccharomycopsis, Debaryomyces, Metschnikowia, Saturnospora,* and *Lipomyces,* and the yeastlike genera *Ascoidea, Cephaloascus, Dipodascus, Dipodascopsis,* and *Galactomyces;* Group 2. *Eremascus, Emericella* and *Ceratocystis;* Group 3. *Taphrina* and *Protomyces;* Group 4. *Schizosaccharomyces.* Because of the genetic relationships indicated by sequence analysis, Group I taxa are retained in the order Endomycetales, and *Schizosaccharomyces* is retained in the Schizosaccharomycetales Prillinger et al. ex Kurtzman.

XXIV. Institut für Biologie IV (Mikrobiologie) der Rheinisch-Westfäl, Technischen Hochschule Aachen, 5100 Aachen, Germany. Communicated by K. Wolf.

The following have been accepted for publication.

1. B. Schäfer, B. Wilde, D.R. Massardo, F. Manna, L. Del Giudice, & K. Wolf. A mitochondrial group I intron in fission yeast encodes a maturase and is mobile in crosses. Curr. Genet., in press.

The open reading frame in the first intron of the mitochondrial gene encoding subunit I of cytochrome c oxidase encodes a maturase (Merlos-Lange *et al.,* 1987) and stimulates homologous recombination in *Escherichia coli* (Manna *et al.,* 1991). In this paper, we demonstrate that this intron is mobile in crosses, indicating that it encodes an endonuclease. This is the first report on an intron which possesses mobility and acts as a maturase.

2. A. Coblenz & K. Wolf. The role of glutathione biosynthesis in heavy metal resistance in fission yeast *Schizosaccharomyces pombe.* FEMS Microbiol. Rev., in press.

Plants and the fission yeast *Schizosaccharomyces pombe (S. pombe)* synthesize small calcium-binding peptides, called phytochelatins in response to calcium. Derived from glutathione (GSH: γ-Glu-Cys-Gly), they have the general structure $(\gamma\text{-Glu-Cys})_n$Gly, where n is from 2 to 11. In order to study the biosynthesis of phytochelatins, we used the mutagen N-methyl-N nitro-N-nitrosoguanidine (MNNG) to select mutants with a lowered GSH content. GSH-deficient mutants show a Cd-sensitive phenotype, whereas resistance to Cu is only slightly influenced. These Cd-sensitive mutants contain between 2 to 15% of the wild-type GSH level. For three mutants a lowered activity of γ-glutamylcysteine-synthetase was measured. One of the mutants was transformed to Cd-resistance and the complementing fragment analyzed further. The complementing fragment hybridized with chromosome III. In the transformants, GSH-content was restored up to wild-type levels, whereas the activity of γ-glutamylcysteine-synthetase was significantly increased compared with the wild-type. Possible mechanisms for Cd-resistance in the transformants are discussed.

3. D.R. Massardo, F. Manna, B. Schäfer, K. Wolf & L. Del Giudice. Complete absence of mitochondrial DNA in the petite-negative yeast *Schizosaccharomyces pombe* leads to resistance towards the alkaloid lycorine. Curr. Genet., in press

The petite-negative yeast *Saccharomyces cerevisiae* can be efficiently and completely converted to respiratory-deficient cytoplasmic petite mutants by intercalating drugs. Rho° petites from *Schizosaccharomyces pombe* could only be obtained in strains carrying a nuclear mutation. In this paper we report the efficient isolation of *rho °* mutants in a *Schizosaccharomyces pombe* strain containing a mitochondrial mutator mutation. We also show that the alkaloid lycorine is able to differentiate between cells containing defective mitochondrial DNA *(mit⁻)* and those lacking mitochondrial DNA completely *(rho °). Rho °* cells are resistant to the alkaloid, whereas *mit⁻* and wild-type cells show the same sensitivity.

4. R. Buschges, G. Bahrenberg, M. Zimmermann & K. Wolf. NADH:Ubiquinone oxidoreductase in obligate aerobic yeasts. Yeast, in press

The strictly aerobic yeasts *Candida pinus, Cryptococcus albidus, Rhodotorula minuta, Rhodotorula mucilaginosa,* and *Trichosporon beigelii* possess mitochondrial NADH dehydrogenases with significant features of the NADH:ubiquinone oxidoreductase (complex I). These species show in all growth phases and under standard cultivation conditions, NADH dehydrogenases of approximately 700 kDa, which are sensitive to rotenone, a specific inhibitor of this complex. Identical results were obtained with the weakly fermenting *Candida pinus*. The facultatively fermenting yeasts *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* do not possess the 700 kDa-complex and are insensitive to rotenone. In *Saccharomyces cerevisiae,* a rotenone-insensitive NADH-dehydrogenase of about 100-600 kDa is detected only in stationary phase cells. As in *Neurospora crassa,* upon incubation of the obligately aerobic yeast *Rhodotorula mucilaginosa* with chloramphenicol, an intermediate NADH dehydrogenase of approximately 350 kDa was formed, which was insensitive to rotenone.

XXV. Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA. Communicated by G.B. Kohlhaw.

The following recently published papers reflect our ongoing efforts in the area of transcriptional regulation.

1. J.Y. Sze, M. Woontner, J.A. Jaehning, & G.B. Kohlhaw. 1992. In vitro transcriptional activation by a metabolic intermediate: activation by Leu3 depends on α-isopropylmalate. Science **258**:1143-1145.

In the absence of the leucine biosynthetic precursor α-isopropylmalate (α-IPM), the yeast LEU3 protein (LEU3p) binds DNA and acts as a transcriptional repressor in an in vitro extract. Addition of α-IPM resulted in a dramatic increase in Leu3p-dependent transcription. The presence of α-IPM was also required for Leu3p to compete effectively with another transcriptional activator, GAL4/VP16, for limiting transcription factors. Therefore, the addition of α-IPM appears to convert a transcriptional repressor into an activator. This represents an example in eukaryotes of direct transcriptional regulation by a small effector molecule.

2. J.Y. Sze & G.B. Kohlhaw. 1993. Purification and structural characterization of transcriptional regulator Leu3 of yeast. J. Biol. Chem. **268**:2505-2512.

The transcriptional regulatory protein Leu3 of *Saccharomyces cerevisiae* was enriched approximately 70-fold above wild type level in yeast cells carrying a pGAL1-LEU3 expression vector. Sustained overproduction of Leu3 following induction by galactose required elevated intracellular levels of α-isopropylmalate, a leucine pathway intermediate known to act as transcriptional co-activator. Starting with galactose-induced cells, the Leu3 protein was purified about 3,500-fold (i.e. 245,000-fold over wild type level) by a procedure that included treatment of the cell-free extract with polyethylenimine, fractionation with ammonium sulfate, heat treatment, and DNA affinity chromatography. Highly purified preparations still showed two protein bands when subjected to polyacrylamide electrophoresis under denaturing conditions. Their apparent molecular sizes were about 104,000 and 110,000 kDa. The smaller of these values was very close to the maximum molecular weight obtained previously for Leu3 protein translated in vitro in a rabbit reticulocyte lysate. (The molecular weight deduced from the open reading frame of the LEU3 gene is 100,162). Both protein bands reacted with antibodies raised against different portions of the Leu3 molecule and were, therefore, likely to represent two forms of Leu3. Treatment with calf intestinal phosphatase quantitatively converted the slower moving band into the faster moving one. Conversion was prevented by inorganic phosphate, a phosphatase inhibitor. These experiments showed that the two bands very likely correspond to phosphorylated and nonphosphorylated forms of Leu3. Phosphorylation did not appear to affect the DNA binding function of Leu3, but (indirect) effects on the activation function or effects on the modulation by α-isopropylmalate have not been ruled out. Electrophoretic mobility shift assays were used to estimate the apparent dissociation constants of the two specific Leu3-DNA complexes routinely seen in these assays. The values obtained were 1.1 and 2.6 nM. Finally, using size exclusion chromatography, native Leu3 protein was shown to have dimeric structure, irrespective of the state of phosphorylation.

3. J.Y. Sze, E. Remboutsika, & G.B. Kohlhaw. 1993. Transcriptional regulator Leu3 of *Saccharomyces cerevisiae:* separation of activator and repressor functions. Mol. Cell. Biol. **13**:5702-5709.

The Leu3 protein of *Saccharomyces cerevisiae* binds to specific DNA sequences present in the 5' noncoding region of at least five RNA polymerase II-transcribed genes. Leu3 functions as a transcriptional activator only when the metabolic intermediate α-isopropylmalate is also present. In the absence of α-isopropylmalate, Leu3 causes transcription to be repressed below basal levels. We show here that different portions of the Leu3 protein are responsible for activation and repression. Fusion of the 30 C-terminal residues of Leu3 to the DNA-binding domain of the Gal4 protein created a strong cross-species activator, demonstrating that the short C-terminal region is not only required but also sufficient for transcriptional activation. Using a recently developed Leu3-responsive in vitro transcription assay as a test system for repression (J. Sze, M. Woontner, J. Jaehning, and G.B. Kohlhaw, Science **258**:1143-1145, 1992), we show that mutant forms of the Leu3 protein that lack the activation domain still function as repressors. The shortest repressor thus identified had only about 15% of the mass of the full-length Leu3 protein and was centered on the DNA-binding region of Leu3. Implications of this finding for the mechanism of repression are discussed.

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

The following are the abstracts of papers recently published or in press.

1. Halász, A., Szakács-Dobozi, M., Vigyázó-Vámos, L., & Szalma-Pfeiffer, I. 1991. Investigation of proteases from baker's yeast. Food Technol. Int. Europe 1991. pp. 191-196.

To summarise, cell-free extract of baker's yeast was separated by preparative isoelectric focusing (IF) and the different protease activities (A, B, Y) were determined. Main protease B activity was found in the range pI 4.77-5.66, the protease Y fractions covered the range pI 6.12-6.61, while the peak pI 7.87-8.20 seemed to be protease A. The fractions were investigated by SDS gel electrophoresis. Many of them contained bands with similar molecular weights. Rabbit antisera produced against the three main proteases were used to explore changes in the intracellular protease activity determined by the Anson method. The affinity of the polyspecific antisera against the antigens was not influenced whether the enzymes were in their active form or inactivated. Increase in protease activity in the exponential growth phase in comparison with the inoculum stage is not only a result of enzyme activation but also caused by de novo synthesis of protease A and B. Activity changes in the transient phase from exponential to the stationary stage are dominantly caused by changes of the active form in case of 0.1 per cent glucose concentration. At higher glucose content, however, de novo synthesis of B also has a role. Activity changes at constant glucose concentration and different aeration intensities are a result of enzyme activation as enzyme concentrations are constant. Proteases can be found in inactive forms (zymogens) in cell-free yeast extract. According to Saheki and Holzer (1975) and Holzer (1974, 1975) the activation of proteinase A, B and C (Y) at acidic pH is due to proteolysis of specific inhibitors. Yeast protease inhibitors are very specific as each inhibits only one type of protease and has no effect on proteolytic enzymes of non-yeast origin.

2. Halász, A., Tawfik, N., F., & Szalma-Pfeiffer, I. 1992. The strategy of efficient production of brewer's yeast biomass. Acta Alimentaria **21**:137-144.

The effect of preaeration, addition of unsaturated fatty acids and the most important fermentation parameters - sugar concentration and aeration level on brewer's yeast production - was investigated in synthetic and malt germ medium. Increased aeration level (500 lh^{-1}) has

a beneficial effect on growth rate, but the lower intensity resulted in higher protein content. Yeast growth at different maltose concentrations underline the effect of glucose on brewer's yeast, causing catabolite repression of the respiratory enzymes. Supplementation of fermentation broth with linseed oil caused a drastic decrease in lag period. The unsaturated oil is co-utilized which results in a higher growth rate at 0.5°% linseed oil concentration. Preaeration of the inoculum is also effective in the reduction of lag period without any effect on the growth rate.

3. Halász, A. & Baráth, A. 1993. The effect of yeast proteases on gluten proteins.

Low lysine and methionine content of wheat proteins could be balanced with yeast supplementation and protein biological value of white bread or pasta products might increase dramatically. In our experiments we used spray dried baker's yeast at 3 % and 6% resp. Despite the fact that yeast powder did not contain viable cells dough consistency decreased significantly. The changes may be explained with the remaining proteolytic activity of yeast added. Addition of inactive dry yeast increases not only protein content and biological value but results in better dough consistency. The effect of baker's yeast proteolytic enzymes on wheat gluten were investigated by determination of protein solubility and S-S bonds. Gluten and yeast crude enzyme solution were mixed in proportion as generally used for dough leavening (500 wheat flour and 20 g pressed yeast). The proteolytic effect was investigated at 30°C and 50°C after 10, 30 and 60 minutes incubation time. Results are shown in the following table.

Time (min)	g soluble protein/100 g gluten 30°C	50°C
10	0.09±0.01	0.08±0.01
30	0.24±0.04	0.24±0.01
60	0.44±0.05	0.52±0.02

Changes in disulfide bonds were investigated during dough leavening and after 60 min incubation significant increase could be detected.

4. Halász A. & Szakács-Dobozi, M. 1993. Proteolytic enzyme activity of *S. cerevisiae* baker's yeast and *S. carlsbergensis* brewer's yeast. Acta Alimentaria, in press.

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

5. Halász, A., Baráth, A., Szalma-Pfeiffer, I., & Bruschi, C. 1993. Regulation of methionine biosynthesis and its distribution between main yeast protein fractions. Presented at the 16th Int. Spec. Symp. on yeasts, Aug. 23-26, 1993, Arnhem, The Netherlands.

Methionine biosynthesis in *S. cerevisiae* is regulated by two different systems, one involves S-adenosylmethionine as an external signal, the other methionine (SURDIN-KERJAN *et al.*, 1976). In sulfate uptake, the first step of sulfate assimilation pathway two permeases participate. Regulation of the synthesis of both permeases is under the control of exogenous methionine or S-adenosylmethionine (BRETON & SURDIN-KERJAN, 1977). Results of HALÁSZ *et al.* (1984) show that methionine biosynthesis in yeast is also influenced by oxygen transfer rate during the yeast propagation. In the present work we investigated the effect of glucose concentration aeration intensity and supplementation of yeast extract on methionine content and distribution in four yeast strains: *Saccharomyces cerevisiae* 1395, *Saccharomyces pastorianus* CBS 1503, *S. cerevisiae* CB 67 an CB 89. Yeast were grown in batch culture on synthetic medium. The effect of glucose was investigated at two levels: 0.1% and 1%; aeration intensities were 100 lh^{-1} and 500 lh^{-1} resp. Methionine content of the whole yeast biomass and its distribution among the different protein fractions (according to OSBORNE) were determined and expressed as mg/dry whole cell and mg/g protein CBS 1503 and CBS 1395 strains showed similarity in methionine distribution, main part of the whole methionine content was in the water soluble protein fraction. Highest methionine concentrations (% of prot.) were found in the acid soluble and 0.1 N NaOH soluble fractions. In respect of sulfate uptake, glucose effect and sensitivity towards aeration intensity the investigated strains are different.

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

Recent publications.

1. A. Riaublanc, R. Ratomahenina, P. Galzy & M. Nicolas. 1993. Peculiar properties of lipase from *Candida parapsilosis* (Ashford) Langeron and Talice. JAOCS, **70**:497-500.

2. D. Vivier, R. Ratomahenina, G. Moulin & P. Galzy. 1993. Study of physico-chemical factors limiting the growth of *Kluyveromyces marxianus.* J. Ind. Microbiol. **11**:157-161.

3. Drider D., Chemardin P., Arnaud A. & Galzy P. 1993. Isolation and characterization of the exocellular β-glucosidase of *Candida cacaoi*: possible use in carbohydrates degradation. Lebensm. Wiss. u. Technol. (CHE) **26**:198-204.

4. Blondeau K., Boutur O., Boze H., Moulin G. & Galzy P. 1993. Influence of culture conditions on the production of heterologous interleukin 1β by *Kluyveromyces lactis.* Biotechnol. Techniques **7**:609-614.

XXVIII. Escola Sup. Agr. Luiz de Queiroz, University of São Paulo, and Fermentec S/C Ltda, Rua Treze de Maio, 768 - Sala 153, Edificio Sisal Center, 13400-900 Piracicaba SP, Brasil. Communicated by H.V. Amorim.

The following paper was presented at a conference recently.

1. L.C. Basso, A.J. de Oliveira, V.F.D.M. Orelli, A.A. Campos, C.R. Gallo, & H.V. Amorim. 1993. Dominance of wild yeast over industrial yeast strains evaluated by karyotyping technique. 5° Congresso Nacional da Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil (August 22-26 1993).

Industrial yeast strains were followed up in several distilleries by means of the karyotyping technique (separation of the intact chromosomal DNAs by pulsed-field gel electrophoresis - TAFE). The results showed that the starter yeast was absent after a 40 days period and the dominant yeasts were typical for each distillery, comprising several different strains. One same strain could be found in different distilleries. Only JA-1 strain, selected from the fermentation industry, was able to maintain itself all over the period of the process.

XXIX. Department of Genetics, L.K. University, H-4010 Debrecen, P.0. Box 56, Hungary. Communicated by M. Sipiczki.

The following papers have been published recently.

1. Benko, Z. & Sipiczki, M. 1993. Caffeine tolerance in *Schizosaccharomyces pombe:* physiological adaptation and interaction with theophylline. Can. J. Microbiol. **39**:551-554.

Caffeine at concentrations of 8 mM or higher inhibited cell propagation and killed a fraction of the population. Cell inactivation increased incrementally with increasing concentrations. The survivors developed tolerance by physiological adaptation that enabled them to propagate in the presence of the drug, but the tolerance could easily be lost if the cells grew in the absence of caffeine for a few generations. Theophylline was found to diminish the toxic effect of caffeine. Possible mechanisms for the observed cellular response and its implications for studies of the effects of these drugs in eukaryotes are discussed.

2. Molnár, M. & Sipiczki, M. 1993. Polyploidy in the haplontic yeast *Schizosaccharomyces pombe:* construction and analysis of strains. Curr. Genet. **24**:45-52.

The fission yeast *Schizosaccharomyces pombe* has a haplontic life cycle in which the diplophase is confined to the zygote. Through the use of one- and two-step protoplast fusions we show that the ploidy can be increased up to pentaploid. The polyploid fusion products are rather unstable and segregate cells of lower ploidies by gradual loss of chromosomes during mitotic divisions. The polyploid cells conjugate normally but are prone to arrest at various stages of meiosis (1-, 2- and 3-spored asci,

binucleate spores) and/or produce inviable, most probably aneuploid, spores. Marker segregation in the complete tetrads indicates the multiple association of homologous chromosomes. In tetra- and penta-ploid meiosis, multispored (6- to 7-spored) asci are also produced, probably by postmeiotic division of the nuclei.

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

Recent publications.

1. Scheffers, W.A. 1992. Aerobic and anaerobic carbohydrate metabolism in yeasts. Proceedings of the COMMET Course on microbial contaminants, Helsinki 1991 and 1992, ed. by M. Korhola & V. Backström, Foundation for Biotechnical and Industrial Fermentation Research **7**:19-36.

2. Scheffers, W.A. Contaminants in baker's yeast. 1992. Proceedings of the COMMET Course on microbial contaminants, Helsinki 1991 and 1992, ed. by M. Korhola & V. Backström, Foundation for Biotechnical and Industrial Fermentation Research **7**:217-228

3. Weusthuis, R.A., H. Adams, W.A. Scheffers, & J.P. van Dijken. 1993. Energetics and kinetics of maltose transport in *Saccharomyces cerevisiae:* a continuous culture study. Appl. Environ. Microbiol. **59**:3102-3109.

In *Saccharomyces cerevisiae,* maltose is transported by a proton symport mechanism, whereas glucose transport occurs via facilitated diffusion. The energy requirement for maltose transport was evaluated with a metabolic model based on an experimental value of Y_{ATP} for growth on glucose and an ATP requirement for maltose transport of 1 mol.mol^{-1}. The predictions of the model were verified experimentally with anaerobic, sugar-limited chemostat cultures growing on a range of maltose-glucose mixtures at a fixed dilution rate of 0.1 h^{-1}. The biomass yield (grams of cells.gram of sugar^{-1}) decreased linearly with increasing amounts of maltose in the mixture. The yield was 25% lower during growth on maltose than during that on glucose, in agreement with the model predictions. During sugar-limited growth, the residual concentrations of maltose and glucose in the culture increased in proportion to their relative concentrations in the medium feed. From the residual maltose concentration, the in situ rates of maltose consumption by cultures, and the K_m of the maltose carrier for maltose, it was calculated that the amount of this carrier was proportional to the in situ maltose consumption rate. This was also found for the amount of intracellular maltase. These two maltose-specific enzymes therefore exert high control over the maltose flux in *S. cerevisiae* in anaerobic, sugar-limited, steady-state cultures.

4. Boekhout, T., M. Renting, W.A. Scheffers & R. Bosboom. 1993. The use of karyotyping in the systematics of yeasts. Antonie van Leeuwenhoek **63**:157-163.

The use of electrophoretic karyotyping in systematics of yeasts is discussed. New data are provided on the karyotypes of the medically important fungi *Hortaea werneckii, Filobasidiella* (= *Cryptococcus*) *neoformans,* and *Malassezia species. Hortaea werneckii* has twelve to eighteen bands of chromosomal DNA, ranging in size between 500 and 2300 kb. The karyotypes of *Filobasidiella neoformans* consist of seven to fourteen bands of chromosomal DNA. The varieties *neoformans* and *bacillispora* cannot be separated by their karyotypes, and no obvious correlation was found with serotypes, geography or habitat. All strains of *Malassezia pachydermatis* studied have similar karyotypes consisting of five bands, whereas in *M. furfur,* four different karyotypes are prevalent. However, each of these karyotypes is stable.

5. Teunissen, A.W.R.H., J.A. van den Berg, & H.Y. Steensma. 1993. Physical localization of the flocculation gene *FLO1* on chromosome I of *Saccharomyces cerevisiae.* Yeast **9**:1-10.

The genetics of flocculation in the yeast *Saccharomyces cerevisiae* are poorly understood despite the importance of this property for strains used in industry. To be able to study the regulation of flocculation in yeast, one of the genes involved, *FLO1,* has been partially cloned. The identity of the gene was confirmed by the non-flocculent phenotype of cells in which the C-terminal part of the gene had been replaced by the URA3 gene. Southern blots and genetic crosses showed that the *URA3* gene had integrated at the expected position on chromosome I. A region of approximately 7 kb in the middle of the *FLO1* gene was consistently deleted during propagation in *Escherichia coli* and could not be isolated. Plasmids containing the incomplete gene, however, were still able to cause weak flocculation in a non-flocculent strain. The 3' end of the *FLO1* gene was localized at approximately 24 kb from the right end of chromosome l, 20 kb centromere proximal to *PHO11.* Most of the newly isolated chromosome I sequences also hybridized to chromosome VIII DNA, thus extending the homology between the right end of chromosome I and chromosome VIII to approximately 28 kb.

6. Steensma, H.Y., G. Barth, & C. de Virgilio. 1993. Genetic and physical localization of the acetyl-coenzyme A synthetase gene *ACS1* on chromosome I of *Saccharomyces cerevisiae*. Yeast **9**:419-421.

The *ACS1* gene, encoding acetyl-coenzyme A synthetase, was mapped genetically at the left arm of chromosome between *pURA3* and *PYK1* at 19 and 28 cM respectively. Comparison with the physical map defined a recombinational 'hot-spot' in this region in addition to the one between *CDC24* and *PYK1.*

7. Teunissen, A.W.R.H. E. Holub, J. van der Hucht, J.A. van den Berg, & H.Y. Steensma. Sequence of the open reading frame of the *FLO1* gene from *Saccharomyces cerevisiae.* Yeast **9**:423-427.

The cloned part of the flocculation gene *FLO1* of *Saccharomyces cerevisiae* (Teunissen, A.W.R.H., van den Berg, J.A. and Steensma, H.Y. (1993)). Physical localization of the flocculation gene *FLO1* on chromosome I of *Saccharomyces cerevisiae* (Yeast, in press) has been sequenced. The sequence contains a large open reading frame of 2685 bp. The amino acid sequence of the putative protein reveals a serine- and threonine-rich C-terminus (46%), the presence of repeated sequences and a possible secretion signal at the N-terminus. Although the sequence is not complete (we assume the missing fragment consists of repeat units), these data strongly suggest that the protein is located in the cell wall, and thus may be directly involved in the flocculation process.

8. van Heusden, G.P.H., W.J. de Koning, Q.J.M. van der Aart, J.A. van den Berg, & H.Y. Steensma. 1993. The nucleotide sequence of a 2.1 kb fragment from chromosome VI of *Saccharomyces cerevisiae* identifies a $tRNA^{Gly}$ gene, part of a delta element and a palindromic sequence. Yeast **9**:1107-1110.

The nucleotide sequence was determined of a 2.1 kb DNA fragment located at approximately 35 kb to the right of the centromere of chromosome VI from *Saccharomyces cerevisiae.* Analysis revealed the presence of a $tRNA^{Gly}$ gene, part of a delta element and a remarkable palindromic sequence. The longest open reading frame found encodes a putative protein of 195 amino acids. Although the fragment was isolated by hybridization to a human diacylglycerol kinase cDNA, no evidence was obtained for the presence of a gene encoding diacylglycerol kinase.

9. Hensing, M.C.M., R.J. Rouwenhorst, W.A. Scheffers, & J.P. van Dijken. 1993. Production and localization of inulinase in *Kluyveromyces* yeast. In: A. Fuchs (ed.) Inulin and inulin-containing crops. Elsevier, Amsterdam.

The production of inulinase (EC 3.2.1.7) by *Kluyveromyces marxianus* was studied in continuous and fed-batch cultures. In sucrose-limited chemostat cultures growing on mineral media the highest inulinase activity in these cultures was 52 U mg^{-1} dwt. The inulinase activity in these cultures decreased from 52 U mg^{-1} dwt at low growth rates to 2 U mg^{-1} dwt at high growth rates. This suggests that the inulinase production is negatively controlled by the residual sugar concentration. In high-cell-density (> 100 g dwt l^{-1}) fed-batch cultures up to 70,000,000 units of supernatant inulinase were produced at a 100-litre scale. In *K. marxianus* part of the inulinase is secreted into the culture fluid and another part is retained in the cell wall. In order to explain the difference in localization both enzymes were purified to homogeneity. Denaturing gel electrophoresis of endo-H-treated supernatant and cell-wall inulinase showed both enzymes to consist of a 64-kDa polypeptide. The degree of glycosylation was 27-37% (w/w). Non-denaturing gel electrophoresis showed the supernatant and cell-wall inulinase to differ in size, due to a difference in subunit aggregation. The enzyme present in the culture fluid was a dimer and the enzyme retained in the cell wall a tetramer. The amino-terminal end of inulinase was determined and compared with the amino terminus of the functionally related invertase from *Saccharomyces cerevisiae.* No homology was found in the first 20 residues except for the first one, serine, indicating that invertase and inulinase are different enzymes.

10. Scheffers, W.A. & J.P. van Dijken. 1993. Metabolic compartmentation in yeasts. Programme and abstracts of the 16th International Specialized Symposium on Yeasts (ISSY 16), Arnhem, The Netherlands, August 23-26, 1993, 170 pp.

About 200 participants from 26 countries contributed 110 abstracts. Copies of the book may still be obtained by transferring Dfl.60 to: Yeast Physiology Group, c/o Dr. Scheffers, at the address above.

XXXI. Vetrogen Corporation, 1200 Wonderland Road, Bldg 9 - Unit 1, London, Ontario N6L 1A8, Canada. Communicated by D.V. Mohandas.

The following paper is to be presented in the 16th symposium on Biotechnology for Fuels and Chemicals, Oak Ridge National Laboratory, Tennessee, USA May 9-13 1994.

1. D.V. Mohandas, D.W. Whelan & C.J. Panchal. 1994. Development of xylose-fermenting yeasts fro ethanol fermentation at high acetic acid concentrations.

Xylose-fermenting yeasts *C. shehatae* and *P. stipitis* do not grow in media containing acetic acid concentrations of 0.2% or more. Most acid hydrolysates of wood and spent sulfite liquor from paper industry contain acetic acid concentrations of about 0.4%. We have developed strains of these yeasts which are capable of growing and fermenting xylose at these higher acetic acid concentrations. By adapting these cultures to increasing concentrations of acetic acid, we generated stable mutants in both organisms which grow well in the presence of at least 0.8% acetic acid. These mutants were compared with their wild type parents for their ability to grow on, and ferment xylose in (i) defined media containing high acetic acid concentrations (ii) acid hydrolysates of aspen and (iii) hardwood spent sulphite liquor (HSSL). In all instances the mutants showed higher efficiency with respect to growth, fermentation time and production of ethanol. *P. stipitis* mutant could grow and ferment xylose in media containing 60% HSSL, while the wild type parent failed to do so at 20% HSSL. The same mutant was also able to grow and ferment xylose at pH 4.0 and 0.4% acetic acid concentration. [supported in part by The Ministry of Energy, Mines and Resources, Canada].

XXXII. Instituto de Microbiologia, CCS, Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, 21941, Brasil. Communicated by A.N. Hagler and L.C. Mendonça-Hagler.

The following papers, whose abstracts were given in the December 1992 YNL, have now been published.

1. Morais, P.B., A.N. Hagler, C.A. Rosa, L.C. Mendonça-Hagler, A.N. Hagler & L.B. Klaczka. 1992. Yeasts associated with *Drosophila* in tropical forests of Rio de Janeiro, Brazil. Can. J. Microbiol. **38**:1150-1155.
2. Rosa, C.A., A.N. Hagler, R. Monteiro, L.C. Mendonça-Hagler, & P.B. Morais. 1992. *Clavispora opuntiae* and other yeasts associated with the moth *Sigelgaita* sp. in the cactus *Pilosocereus arrabidae* of Rio de Janeiro, Brazil. Antonie van Leeuwenhoek **62**:267-272.
3. Naumov, G.I., E.S. Naumova, L.C. Mendonça-Hagler, & A.N. Hagler. 1992. Taxogenetics of *Pichia angusta* and similar methylotrophic yeasts. (Minireview) Ciencia e Cultura **44**:397-400.
4. Mendonça-Hagler, L.C., A.N. Hagler, & C.P. Kurtzman. 1993. Phylogeny of *Metschnikowia* species estimated from partial rRNA sequences. Int. J. Syst. Bacteriol. **43**:368-373.
5. Guimaraes, V.F., M.A. Araujo, L.C. Mendonça-Hagler & A.N. Hagler. 1993. *Pseudomonas aeruginosa* and other microbial indicators of pollution in fresh and marine waters of Rio de Janeiro, Brazil. Environ. Toxicol. Water Qual. **8**:313-322.

XXXIII. Centro de Investigación y de Estudios Avanzados del I.P.N., Km 9.6 del Libramiento Norte Carretera, Irapuato-Leon A.P. 629, Irapuato, Guanajuato, México. Communicated by J. Ruiz-Herrera.

Our group is interested on the structure of the cell wall, and on dimorphism in several fungal models. Our working models are *Candida albicans, Yarrowia lipolytica, Ustilago maydis* and *Mucor rouxii.* Our most recent publications are the following.

1. Ruiz-Herrera, J. 1992. Fungal Cell Wall. Structure, Synthesis and Assembly. CRC Press. Boca Raton, Fl, USA. 248 pp.
2. Cano-Canchola, C., Sosa, L., Fonzi, W., Sypherd, P., & Ruiz-Herrera, J. 1992. Developmental regulation of *CUP* gene expression through DNA methylation in *Mucor* spp. J. Bacteriol. **174**:362.
3. Diaz, S., Zinker, S. & Ruiz-Herrera, J. 1992. Alterations in the cell wall of *Saccharomyces cerevisiae* induced by the alpha sex factor or a mutation in the cell cycle. Antonie van Leeuwenhoek **61**:269-276.
4. Morales, M. & Ruiz-Herrera, J. 1993. Critical evaluation of the ultrastructure of sporangiophores from *Phycomyces blakesleeanus* (Zygomycetes) at stages I and IV. Cryptogamic Botany. **2**:273-282.
5. Guevara-Olvera, L., Calvo-Mendez, C. & Ruiz-Herrera, J. 1993. The role of polyamine metabolism in dimorphism of *Yarrowia lipolytica.* J. Gen. Microbiol. **193**:485-493.

6. Reyna-Lopez, G. & Ruiz-Herrera, J. 1993. Polyamines and the phorogenesis of Mucorales. Exptl. Mycol. **17**:79-89.

7. Salcedo-Hernandez, R. & Ruiz-Herrera, J. 1993. Isolation and characterization of a mycelial cytochrome aa3 deficient mutant, and the role of mitochondria in dimorphism of *Mucor rouxii*. Exptl. Mycol. **17**:142-154.

8. Martinez-Pacheco, M. & Ruiz-Herrera, J. 1993. Differential compartmentation of ornithine decarboxylase in cells of *Mucor rouxii.* J. Gen. Microbiol. **139**:1387-1394.

9. Ruiz-Herrera, J. & Bartnicki-Garcia, S. 1993. Regulation of chitin synthetase in Mucorales. In: Chitin Enzymology, Muzzarelli, R.A.A., ed. European Chitin Society, Lyon and Ancona. pp. 35-45.

10. Cubero, B., Ruiz-Herrera, J., Reyna, G. & Cerda-Olmedo, E. 1993. Isolation of fungal mutants resistant to chitin synthetase inhibitors. In: Chitin Enzymology, Muzzarelli, R.A.A., ed., European Chitin Society, Lyon and Ancona. pp. 67-74.

11. Cubero, B., Ruiz-Herrera, J. & Cerdá-Olmedo, E. 1993. Chitin synthetase mutants of *Phycomyces blakesleeanus.* Mol. Gen. Genet. **240**:9-16.

12. Ruiz-Herrera, J. 1993. Dimorphism in *Mucor* species. In: Fungal Dimorphism, Vanden Bossche, H, Odds, F., & Kerridge, D., eds. Plenum Press, New York, London, pp. 257-265.

13. Ruiz-Herrera, J. 1993. La quitina. Investigación y Ciencia (Spanish edition of Scientific American) No. 202 (July): 42-49.

14. Dubon, F., Gozalbo, D., Rico, H., Sentandreu, R. & Ruiz-Herrera, J. 1992. Synthesis of chitin microfibrils by chitosomes from *Candida albicans* (Deuteromyretes). Cryptog. Bot. In press.

15. Salcedo-Hernandez, R., Escamilla, E. & Ruiz-Herrera, J. Organization and regulation of the mitochondrial oxidative pathway in *Mucor rouxii.* J. Gen. Microbiol. In press.

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

The following paper whose abstract appeared in the June issue of the YNL has now been published.

1. Lachance. M.A. 1993. *Metschnikowia agaveae* sp.nov., a heterothallic haploid yeast from blue agave. Can. J. Microbiol. **39**:562-566.

The following paper has been published recently.

2. Lachance, M.A. 1993. *Kluyveromyces:* systematics since 1970. Antonie van Leeuwenhoek **63**:95-104.

The taxonomy of *Kluyveromyces* has been the object of intense study since van der Walt's (1970) monograph. This is an account of the major developments and the classification to be adopted in the 4th edition of THE YEASTS, A TAXONOMIC STUDY. The guiding principles that will be followed in eventual revisions of the genus are presented.

Addendum: Ming Zhan will soon be submitting his Ph.D. thesis on the phylogeny of rRNA sequences in *Kluyveromyces*, and we are preparing manuscripts dealing with this topic. The results confirm the prediction that the genus is polyphyletic, but do not provide an easy answer to its natural classification.

Obituaries

Anthony H. Rose

(1930-1993)

The life of Anthony H. Rose, Tony to his friends, had many facets, ranging over university academic, scientist, scientific administrator and prolific writer. He was also a lover of art, music and football (soccer) and a keen squash player.

Tony graduated with first class honours in Applied Biochemistry from the University of Birmingham in 1950, completing a Ph.D. in 1954 and obtaining a D.Sc. in 1969. His early work on the growth of yeast and lactic acid bacteria stimulated an interest in microbial physiology which was to be a feature of his whole scientific career. Following two years at the Institute of Microbiology at Rutgers University, National Service as an Education Officer in the RAF and a post with the National Research Council of Canada in Ottawa, he held successive appointments at Heriot-Watt University and the University of Newcastle-upon-Tyne before moving to the then newly established University of Bath in 1968 as Professor of Microbiology, a post he held until his death. At Bath he completed two tours of duty as Head of the School of Biological Sciences and was Dean of Science from 1988 to 1989. As a university teacher he excelled and generations of students will recall his highly interactive style of delivery, usually without notes and always authoritative. His early writing include valuable textbooks on industrial microbiology and three editions of arguably the best text every written on microbial physiology.

As a research scientist his interests were always directed towards the physiology of the yeast *Saccharomyces cerevisiae* and his success in his chosen field is amply illustrated by the publication of over 200 original papers and reviews. This yeast is the most useful of microorganisms being "the work horse" of the baking, brewing, distilling, and enology industries with which he had a close affiliation as advisor and consultant. Tony actively promoted research on yeast, as a model eukaryote, long before it became the fashionable organism of the molecular biologist, founding the British Yeast Group in 1976. His research on the structure of the plasma membrane, which lead to "the dial a membrane" concept has greatly increased our knowledge of the basic facts of ethanol tolerance and have found practical application in brewing, distilling and wine-making fermentation systems. With J.S. Harrison he edited the definitive three volumes of "The Yeasts" in 1969 and an enlarged second edition is currently being produced, the publication of the final volume (Volume 6) being scheduled for 1994. One of his recent engagements was to deliver the Kathleen Barton-Wright Lecture earlier this year on the topic "Yeasts, the oldest of man's cultivated plants, continue to present problems".

As a writer he excelled and few contemporaries would or could claim to match his general skills and turn of phrase. In addition to the many publications mentioned above, he edited an eight volume series on Economic Microbiology and established the excellent series of reviews "Advances in Microbial Physiology" with J.F. Wilkinson in 1967. With D.W. Tempest and G. Morris succeeding Wilkinson as co-editor, this series has now reached volume 35. In this area alone he will be a hard act to follow.

Tony was an active member of a number of scientific societies and will be remembered particularly for his work in and for the Society for General Microbiology as a Member of the Council from 1967 to 1971 and International Secretary from 1971 to 1974. In this latter post he was founding father of the Federation of European Microbiological Societies which brought together the various societies representing microbiology throughout Europe. He served as Secretary General of the Federation for the period 1974 to 1980. He was a British representative on the International Yeast Commission for over a decade and presented the opening Plenary Lecture at the Vth International Yeast Symposium in 1980.

Tony was also a family man, married to Jane on St. Aidan's Day in 1957, and many fortunate friends and colleagues have enjoyed the warm hospitality offered in their family home in Bath. Tony and Jane greatly valued the time they were able to spend in their retreat in the Dordogne where he had a study established in a converted pig sty. As ardent francophiles they had planned to spend more time there following their retirement at the end of this year. His next writing project was to have been a single volume on the yeasts which would have brought together a

professional life time of experience on his favourite microorganism. Tony lived life to the full. His sudden death a few weeks before his 63rd birthday has robbed the scientific world of a great character who will be severely missed.

C.M. Brown
Heriot-Watt University, Edinburgh, Scotland with additions by G.G. Stewart, London, Ontario

Aurora Brunner

(-1993)

Aurora Brunner, Professor at the Institute of Cellular Physiology, the National Autonomous University of Mexico, died on August 18, 1993. She was one of those who contributed much to the development of mitochondrial genetics in yeast. She was also one of the few experts of Kluyveromyces lactis genetics. Aurora Brunner started her work on yeast in 1967-69 in the laboratory of H. de Robichon Szulmajster, Gif-sur-Yvette, France. Later she collaborated with many people of the yeast research community: to name a few, J. R. Mattoon, of Johns Hopkins University, D.E. Griffiths of the University of Warwick, P. A. Whittaker, of St. Patrick's College, Maynooth, L. de Giudice, of the International Laboratory of Genetics and Biophysics in Naples, N. C. Martin, of the University of Texas, Dallas A. Tzagoloff at Columbia University. In recent years, she was collaborating with one of us (Peña) on potassium transport in K. lactis. In 1993, she had to interrupt her stay at the Curie Institute, Orsay, France, hit by a cancer in an early age. Always full of modesty, she has been a most helpful hand and mind for everyone in her field. She will remain in the memory of all those who knew her.

Antonio Peña,
Instituto de Fisiologia Celular
Universidad Nacional Autonoma de México

Hiroshi Fukuhara,
Curie Institute
Orsay, France.

International Commission on Yeasts

I wish to offer a special thanks to Dr. W. A. Scheffers, Kluyver Laboratory of Biotechnology, Technical University, Delft, The Netherlands and the other members of the organizing committee for their efforts in presenting ISSY-16 in Arnhem, The Netherlands, August 23-26, 1993. It was a superb meeting, both scientifically and socially. Approximately 200 people attended and benefited from the excellent scientific presentations and social events.

Minutes of the meeting of the ICY held at ISSY-16, August 25, 1993

The commissioners of the ICY in attendance at ISSY-16 met informally during lunch at ISSY-16, August 25, 1993. Graham Fleet, W. A. Scheffers, M. Korhola, Peter Raspor, J.F.T Spencer, Byron Johnson, and Sally Meyer were present. Graham Fleet gave an update of the next International Symposium on Yeasts, ISY-9, to be held in Sydney, Australia in August, 1996. The site has been selected and plans are in progress for the scientific program.

We were reminded that Dr. D. Berry is organizing the next specialized yeast meeting, ISSY-17, which will be in at Heriot-Watt University, Edinburgh, Scotland, August 27-Sept 1, 1995. The title is: Yeast Growth & Differentiation: Biotechnological & Genetic Aspects. Also, the 7th International Congress of the Mycology Division, IUMS, will be in Prague, Czech Republic, July 3-8, 1994.

Sally Meyer announced that a letter dated 8 September, 1992 was received from Professor P. Galzy with the following recommendations: that Guy Moulin replace P. Galzy, Michelle Mallie replace J.M. Bastide and Éveline Guého replace H. Heslot as commissioners from France.

Other new commissioners were proposed: Dr. Neville Pamment, University of Melbourne, Dept. Chemical Engineering, Parkville, Victoria, Australia as a commissioner from Australia and Dr. Maria Kopecka, Masaryk University, Brno as a commissioner from the Czech and Slovak Yeast Group. These new commissioners were tentatively approved. (The actual vote takes place at the next general meeting of the commissioners which will be at the ISY-9 in Sydney, Australia, August 1996.)

Peter Raspor treated the commissioners to a 'yeast product' from Slovenia to compliment the lunch. Our appreciation is noted. The meeting adjourned in time for the afternoon scientific session.

Sally A. Meyer, Chair, ICY

Forthcoming meetings

Fourth European Congress of Cell Biology. June 26 - July 1, 1994, Prague, Czech Republic.

The 4th European Congress of Cell Biology will be held in Prague, Czech Republic, June 26 to July 1, 1994. **To receive additional information, contact:**

Secretariat
Dr. Zdeněk Drahota
Institute of Physiology
CS 142 20 Prague 4
Czech Republic

M. Kopecká

7th International Symposium on the Genetics of Industrial Microorganisms, GIM 94, Montréal, Canada, June 26 - July 1, 1994

The 7th International Symposium on the Genetics of Industrial Microorganisms will be held at the Palais des Congrès, Montreal, Québec, Canada on June 26-July 1, 1994. Nineteen symposia will cover a broad range on topics of interest in all aspects of the genetics of industrial microorganisms. A satellite workshop on plasmid diversity will be held June 24 to 26 1994. The deadline for receipt of abstracts is January 28 1994, for advance registration April 15 1994, and for reservation of hotel accommodation May 9 1994. **The advance program, registration bulletin, and call for abstracts are available from:**

Nicole Léger, Symposium Manager, GIM 94
National Research Council Canada
Ottawa Ontario, Canada K1A OR6

Tel. 613 993 9431
Fax. 613 957 9828
Telex: 053 3145

Seventh International Congress of Bacteriology and Applied Microbiology Division & Seventh International Congress of Mycology Division of IUMS. July 3-8, 1994, Prague.

The 7th International Congress of the Bacteriology and Applied Microbiology Division and the 7th International Congress of the Mycology Division of the International Union of Microbiological Societies will be held in Prague, Czech Republic, July 3 to 8, 1994. **To receive additional information including the 2nd circular, contact:**

Secretariat, IUMS Congresses '94.
Institute of Microbiology,
Vídeňská 1083
CS-142 20 Prague 4
Czech Republic

Tel./Fax. (+42 2) 471 32 21

Fifth International Mycological Congress, August 14-21, 1994, Vancouver, B.C. Canada

The Fifth International Mycological Congress (IMC 5) will be held on the campus of the University of British Columbia (UBC), Vancouver, British Columbia, Canada, August 14 through August 21, 1994. A comprehensive scientific programme is planned, with congress symposia, contributed symposia, poster sessions, and discussion groups. Also, there will be pre- and post-congress field trips. Deadlines: Advance registration and abstracts Feb 28 1994; preregistration, late abstracts, and request for accommodation June 14 1994; **The preliminary program, registration forms, and call for abstracts are available from:**

IMC5 Congress Secretariat,
c/o Venue West Conference Services
645 - 375 Water Street
Vancouver, B.C., Canada V6B 5C6

Telephone: (604) 681-5226
FacSimile: (604) 681-2503

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

Provisional outline of the scientific program: Nuclear dynamics. Regulation of gene expression. Post-transcriptional processes. Signal transduction pathways. Membrane transport. Metabolic regulation. Yeast in biotechnology. Satellite symposium: it is planned to hold a satellite Workshop on Yeast Genome Sequencing after the Conference. The conference Centre is a modern building, overlooking the river Tagus in a famous area of the city of Lisbon, in the Junqueira/Belem-Jerónimos Monastery, historical quarter. The registration fee includes participation in all scientific sessions, congress documentation, free buffet lunches and dinner, tea/coffee between sessions and official social program. Special fees for students will be considered. Hotels of different categories have been reserved. Lisbon has frequent flight connections with all major cities. **Contact**:

Dr. Claudina Rodrigues Pousada, XVII CYGMB
Laboratorio de Genetica Molecular
Instituto Gulbenkian de Ciencia
Ap. 14 - 2781 OEIRAS Codex, Portugal

Fax: 351 1 443 16 31

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

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

For additional information please contact:

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Telex: INRAAVI 432.870 F

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

Ninth International Symposium on Yeasts 1996

As decided by the Commissioners of the ICY at their meeting during the 8th International Symposium on Yeasts, Atlanta, the 9th International Symposium on Yeasts will be held in Sydney, Australia, 1996 in conjunction with the International Biotechnology Congress. Planning of the meeting is in progress.
Contact:

Graham Fleet
Department of Food Science and Technology
University of New South Wales
P.0. Box 1, Kensington NSW 2033, Australia.

Brief News Items

Change of address: Tibor Deak

After two years at the Department of Food science and Technology of the University of Georgia, I have now returned to the following address:

Dr. Tibor Deak
Department of Microbiology,
University of Horticulture and Food
Somloi ut 14-16, Budapest H-1118
Hungary

Phone: 36 1 267 1390
Fax: 36 1 267 1304

Change of address: R. H. Haines

I have recently returned from Palo Alto to the following address:

Dr. R. H. Haines
Department of Biology
York University
4700 Keele Street
Toronto, Ontario M3J 1P3
Canada

Fax 416 736 5698

Publications of interest

from the International Mycological Institute (CAB International), Wallingford, Oxon OX10 8DE, United Kingdom

1. Reynolds, D.R. & J.W. Taylor, eds. 1993. The Fungal Holomorph. Mitotic, meiotic and pleiomorphic speciation in fungal systematics. 350 pp. ISBN 0 85198 865 2, £40.00, USD$76.00 (Americas only).

2. Hawkesworth, D.L., ed. 1991. The biodiversity of microorganisms and invertebrates: its role in sustainable agriculture. 300 pp. ISBN 0 85198 722 2. £40.00, USD$76.00 (Americas only).

For more information on these and other publications or on the IMI itself, contact CAB International at the address above, or one of the following: 845 North Park Avenue, Tucson Az 85719 USA (North America); P.O. Box 11872, 50760 Kuala Lumpur, Malaysia (Asia); Gordon Street, Curepe, Trinidad & Tobago (Caribbean and Latin America).