

Y E A S T

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S.C. Jong, Rockville, Maryland, USA	28
M.Th. Smith, Delft, The Netherlands	28
J. Brouwer, Leiden, The Netherlands	29
M. Vancanneyt, Gent, Belgium	29
J. Ramos Cordoba, Spain	30
C.J. Panchal, London, Ontario, Canada	30
F. Spaaj, Tübingen, Germany	30
W.I. Golubev, Pushchino, USSR	31
E. Minárik, Bratislava, Czechoslovakia	31
H. Prillinger, Tulln, Austria	31
A. Vaughan Martini, Perugia, Italy	34
J.A. Barnett, Norwich, England	34
R. Maleszka & G.D. Clark-Walker, Canberra, Australia	35
R.J. Thornton & S.B. Rodriguez, Palmerston North, New Zealand	35
R.T. Moore, Coleraine, N.Ireland	36
S. Fogel, Berkely, California, USA	37
W.M. Ingledew, Saskatoon, Saskatchewan, Canada	38
S. Goto, Kofu, Japan	39
J.L. Ochoa, La Paz, BCS, Mexico	39
W.T. Starmer, Syracuse, New York, USA	40
Y. Yamada, Shizuoka, Japan	40
A.D. Panek, Rio de Janeiro, Brazil	40
J.F.T. Spencer, Tucuman, Argentina	41
E.A. Johnson, Madison, Wisconsin, USA	42
P. Romano & G. Suzzi, Sede di Reggio Emilia, Italy	42
J.P. van der Walt, Bloemfontein, South Africa	43
N. van Uden, Oeiras, Portugal	43
M. Sipiczki, Debrecen, Hungary	45
M. Korhola, Helsinki, Finland	46
O. Bendová, Prague, Czechoslovakia	47
S.A. Meyer, Atlanta, Georgia	48
W.A. Scheffers, Delft, The Netherlands	48
F. Radler, Mainz, Germany	50
M.C. Pignal, Villeurbanne, France	50
M. Miranda, Davis, California, USA	51
P. Venkov, Sofia, Bulgaria	51
R. Lagunas, Madrid, Spain	52
Recent meetings	53
Forthcoming meetings	55
Brief news items	56

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EDITORIALS

Subscription rates

Although every effort has been made to maintain the subscription rate of the Yeast Newsletter to a minimum, the steady rise in postage and printing costs is forcing us to introduce a modest increase. The 1991 surface mail rate will be US\$6.00. The airmail supplement will remain unchanged at US\$4.00. The effects of the new *goods and services* tax imposed by the Canadian government over our production costs are difficult to anticipate at this time, but further adjustments may be necessary in the near future.

Format of contributions

Our special thanks to the several readers who have taken advantage of the convenience of sending their contribution to this issue by electronic mail or as ASCII files on MS-DOS 5 $\frac{1}{4}$ inch diskettes. We are of course very happy to continue receiving typewritten contributions by regular mail. We are still experiencing problems with the quality and reliability of FAX transmissions, and request that this medium be used only in emergencies or in conjunction with regular mail. Please note that our correct FAX number is (519) 661 3935.

Kluyveromyces researchers

A group of approximately 100 international researchers interested mostly in the genetics and various other aspects of the biology of Kluyveromyces lactis is gaining momentum. Participants are based mostly in Europe and have held workshops in Italy, France, and the Netherlands. Several members are long time readers of the Yeast Newsletter, and the group has discussed the possibility of using the Yeast Newsletter as their official vehicle of information exchange. We welcome this proposal, and take this opportunity to invite all Kluyveromyces workers each to subscribe on an individual basis.

ISY 1992, Atlanta

The next general International Symposium on Yeasts to be organized under the auspices of the International Yeast Commission will be hosted by Dr. S.A. Meyer, at Georgia State University, Atlanta, Georgia. Commission members are hopeful that the Atlanta meetings will match the considerable success of the meetings of the eighties held in London, Montpellier, and Perugia. Please consult the *Forthcoming Meetings* section of the Yeast Newsletter for further information. Dr. Meyer soon will be sending announcements to all Yeast Newsletter readers, who in turn are urged to bring the information to the attention of other colleagues who might be interested in participating.

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

M. A. Lachance
Editor

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

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

Name	ATCC No.	Depositor & Strain	Significance & Reference
<u>Cryptococcus neoformans</u> var. <u>gatti</u>	76108 76109	D.H. Ellis B32.6M AB34.3	Human pathogen (J. Clin. Microbiol. 28:1642-1644, 1990)
<u>Metschnikowia hawaiiensis</u>	65058 65059	M.A. Lachance 87-2203.2; CBS 7433 87-2167.2; CBS 7432	Isolated from morning glory and associated flies, Hawaii Island (Int. J. Syst. Bacteriol. 40:415-420).
<u>Saccharomyces cerevisiae</u>	76062 76063 76064 76101 76102 76103	A. Tzagoff E103 E103/UT/T1 W303/ATP10 E250 E250/V6/T1 W303-KG02	Mutation in a yeast nuclear gene. (Mol. Cell. Biol. 10:4221, 1990)
<u>Schizosaccharomyces pombe</u>	76046	SAGO 1	For production of SCP from hydrocarbon feedstock (Folia Microbiol. 34: 112-119, FEMS Microbiol. Lett. 57:151-154, 1989)
<u>Trichosporon cutaneum</u>	66835	T. Ueno YTR1;AKU4864	Production of dihydroxyphenyl acetic acid (Agric. Biol. Chem. 51:947-948).

We have published a new catalogue, "ATCC Catalogue of Yeasts, 18th edition, 1990". This 230 page reference catalogue lists over 4,000 yeast strains available from the ATCC. The catalogue provides strain descriptions which include genotypes, source of isolation, literature references, media formulae, special applications, and more. New to the catalogue is a section listing overlapping continuous genomic clones (contigs), including chromosome maps, from Saccharomyces cerevisiae.

This publication is available free-of-charge in the USA, for \$5 in Canada and Mexico, and for \$9 elsewhere. Copies can be obtained from:

ATCC Sales & Marketing
12301 Parklawn Dr.
Rockville, MD 20852 - U.S.A.

Telephone 1(800)638-6597 or (301)881-2600; Fax (301)231-5826

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

New acquisitions.

- Brettanomyces naardenensis Kolfschoten & Yarrow 7540, from soft drink, South Africa, R.L. Steyn.
Bullera miyagiana Nakase et al. 7526 = JCM 7536, T, from Abies firma at Sendai in Japan, T. Nakase (J. Gen. Appl. Microbiol. 36:33-39, 1990)
Candida milleri Yarrow 7541, from bread, Morocco, Rabat, L. Arpent
Candida taiwanensis Lee et al. 7530 = CCRC 21917, T, from black-soybean sauce mash, Taiwan, CCRC
Cryptococcus elinovii Golubev 7528 = H1, from waste water, decomposes phenol, H. J. Rehm (Ehrhardt & Rehm, Appl. Microbiol. Biotechnol. 21:32-36, 1985)
Cryptococcus gilvescens Chernov & Bab'eva 7525 = VKM Y-2748 + KBP-2779, T, from tundra soil in USSR, VKM (Mikrobiologiya 57:1031-1034, 1988)
Filobasidiella neoformans var. bacillispora 7523, from bark debris of Eucalyptus camaldulensis in South Australia, D.H. Ellis (J. Clin. Microbiol. in press)
Kurtzmanomyces tardus Giménez-Jurado et al. 7421 + IGC 4529, T, from contaminated demineralized water in Portugal, N. van Uden (A.V. Leeuwenhoek 58:129-135, 1990)
Lipomyces japonicus van der Walt et al. 7549, 7550, from uncultivated soil, South Africa, J.P. van der Walt
Lipomyces kononenkoae Nieuwdorp et al. 7543, from soil, South Africa, Kwa-Mbonambi State Forest Reserve, J.P. van der Walt.
Lipomyces starkeyi Lodder & Kreger-van Rij 7542, 7544, 7545, from soil, South Africa, J.P. van der Walt
Metschnikowia hawaiiensis Lachance et al. 7432 = ATCC 65059 = NRRL Y-17272 = UWO(PS) 87-2167-2, T, from flower of morning glory Ipomoea acuminata, 7433 = ATCC 65058 = UWO(PS)87-2203.2, from fly Drosophila floricola in Hawaii, M.-A. Lachance (Int. J. Syst. Bacteriol. 40:415-420, 1990)
Mysozyma udenii Spaaij et al. 7439, T, from roots of Mangifera indica, USA, Florida, H. Spaaij (System. Appl. Microbiol. 13:182-185, 1990)
Rhodotorula glutinis (Fres.) Harrison 7538, from atmosphere, Poland, decomposes phenol, catechol and hydroquinone, R. Kocwa-Haluch

- Saccharomyces cerevisiae 7524 = USM12, K2 neutral strain carrying a M dsRNA encoding for resistance to K2 toxin, B.D. Wingfield (Yeast 6:159-169, 1990)
- Sporobolomyces lactophilus Nakase CBS 7527 = JCM 7595, T, from branch of Abies firma in Japan, T. Nakase (Trans. Mycol. Soc. Japan 31:159-167, 1990.
- Zygozma smithiae van der Walt et al. 7407, T, 7408, from rass of Crossotarsus externedentatus in Macaranga capensis, South Africa, Natal, J.P. van der Walt (Antonie van Leeuwenhoek 58:95-98, 1990)

Publications.

1. Nakase, T.¹, A. Takematsu¹, M. Itoh¹ and T. Boekhout. 1990. Conspicuity of Bullera derxii, Bullera sinensis and Bullera alba var. lactis. J. Gen. Appl. Microbiol. 36:209-213.
¹Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 DC Delft, The Netherlands.

We confirmed that B. sinensis and B. alba var. lactis had similar physiological characteristics. Bullera derxii had physiological characteristics similar to both taxa. These three differed physiologically from B. alba in their inability to assimilate lactose. On the basis of our observations, we conclude that B. derxii, B. sinensis and B. alba var. lactis represent one and the same species. Bullera sinensis has priority over the other two taxa.

2. Smith, M.Th., Yamazaki, M., & Poot, G.A. 1990. Dekkera, Brettanomyces and Eniella: electrophoretic comparison of enzymes and DNA-DNA homology. Yeast 6:299-310.

III. Laboratory of Molecular Genetics, Department of Biochemistry, Gorlaeus Laboratories, Leiden University, P.O.Box 9502, 2300RA Leiden, The Netherlands. Communicated by J. Brouwer.

Recently we have started our research on differential DNA excision repair in Saccharomyces cerevisiae. We study the removal of UV induced photo-products from transcriptionally active and inactive DNA. We were able to show that pyrimidine dimers are preferentially removed from the active MAI α locus as compared to the inactive HML α locus. Furthermore it was found that several UV-sensitive rad mutants (rad7, rad9, rad16, rad24) are specifically deficient in the removal of DNA damage from inactive DNA. Finally we have showed that the difference in repair between active and inactive DNA presumably can only be repaired in the G₂ phase of the cell cycle (mitotic). Recently we have cloned the gene that complements the repair deficiency of rad16 mutants.

Publications:

1. Terleth, C., van Sluis, K., & van de Putte, P. 1989. Differential repair of UV damage in Saccharomyces cerevisiae. Nucl. Acids Res. 17:4433-4439.
2. Terleth, C. Schenk, P., Poot, R., Brouwer, J., & van de Putte, P. 1990. Differential repair of UV damage in rad mutants of Saccharomyces cerevisiae: a possible function of G₂ arrest upon UV irradiation. Mol. Cell. Biol. 10:4678-4684.
3. Terleth, C., Waters, R., Brouwer J., & van de Putte, P. 1990. Differential repair of UV damage in Saccharomyces cerevisiae is cell cycle dependent. EMBO J. 9:2899-2904.
4. Terleth, J., van de Putte, P., & Brouwer, J. In press. New insights in DNA repair: preferential repair of transcriptionally active DNA. Mutagenesis. Review.

IV. Laboratorium voor Microbiologie en microbiele Genetica, Rijksuniversiteit Gent, 9000 Gent, Belgium. Communicated by M. Vancanneyt.

The following is an abstract of a recently accepted paper.

1. Vancanneyt, M., Pot, B., Hennebert, G.¹, & Kersters, K.. In press. Differentiation of Yeast Species Based on Electrophoretic Whole-Cell Protein Patterns. System. Appl. Microbiol.
¹Mycothèque de l'Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

One-dimensional polyacrylamide gel electrophoretic whole-cell protein patterns of 112 isolates of 20 different yeast species, mainly belonging to the genus Candida, were compared by numerical analysis. This technique allowed to evaluate taxonomic relationships at the species level. Qualitative differences between the protein electrophoregrams of different species enabled to confirm anamorph/teleomorph relations and to detect synonymy between species. The high reproducibility allowed to evaluate quantitative differences found at the infraspecific level. Inter- and intrageneric relationships could be deduced after comparison with other taxonomic criteria. The construction of a database of protein fingerprints is therefore feasible and will be a useful tool for routine identification of yeasts.

V. Department of Microbiology, E.T.S.I. Agronomos, University of Cordoba, Cordoba, Spain. Communicated by J. Ramos.

The following is an abstract of a recently accepted paper.

1. Ramos, J., Haro, R., Rodríguez-Navarro, A. 1990. Regulation of potassium fluxes in Saccharomyces cerevisiae. Biochim. Biophys. Acta. In press.

To investigate the regulation of K^+ fluxes in Saccharomyces cerevisiae the dependence of K^+ efflux and Rb^+ influx on $[K^+]_i$, pH_i , $[Na^+]_i$, membrane potential, cell volume, and turgor pressure were studied in cells with different K^+ contents. By decreasing the cell volume with osmotic shocks and the cellular pH with butyric acid the following was found. (1) The K^+ efflux induced by uncouplers decreases simultaneously with the decrease of the K^+ content of the cell, but the process was insensitive to $[K^+]_i$, pH_i , cell volume and turgor pressure. The internal presence of Na^+ inhibited this K^+ efflux. (2) The increase of the V_{max} of Rb^+ influx observed in low- K^+ cells is due to the decrease of the pH_i and probably mediated by the increase of the activity of the plasma membrane ATPase. The V_{max} is independent of $[K^+]_i$, $[Na^+]_i$, cell volume and turgor pressure. (3) The decrease in the K_m of Rb^+ influx observed in low- K^+ cells does not depend directly on $[K^+]_i$, cell volume or turgor pressure. If Na^+ is present, $[Na^+]_i$ might be directly involved in the regulation of the K_m .

VI. Vetro-Gen Corporation, 1200 Wonderland Road S., Bldg 7/2, London, Ontario N6L 1A8, Canada. Communicated by C.J. Panchal.

The following book has appeared recently.

1. Panchal, C.J. (ed.). 1990. Yeast Strain Selection. Marcel Dekker, Inc., New York and Basel. ISBN 0-8247-8276-3.

Content:

1. Kurtzman, C.P. - Culture Collections as Sources of Strains for Industrial Uses.
 2. Lachance, M.A. - Yeast Selection in Nature
 3. Bilinski, C.A. & N. Marmiroli - Classical Approaches to Yeast Strain Selection
 4. Casey, G.P. - Yeast Selection in Brewing
 5. Subden, R.E. - Wine Yeast: Selection and Modification
 6. Nagodawithana, T.W. & N.B. Trivedi - Yeast Selection for Baking
 7. Finkelman, M.A.J. - Yeast Strain Development for Extracellular Enzyme Production
 8. Panchal, C.J. & F.C. Almeida Tavares - Yeast Strain Selection for Fuel Ethanol Production
 9. Klein, R.D. and P.G. Zaworski - Transformation and Cloning Systems in Non-Saccharomyces Yeasts
 10. Das, R.C. & D.A. Campbell - Host Cell Control of Heterologous Protein Production in Saccharomyces cerevisiae
 11. Panchal, C.J. - Future Prospects
-

VII. Institut Biologie I, Lehrstuhl f. Spezielle Botanik/Mykologie, Auf der Morgenstelle 1, 7400 Tübingen, Germany. Communicated by F. Spaaij.

The following paper has been published recently.

1. Spaaij, F., Weber, G., van der Walt, J.P. & Oberwinkler, F. 1990. Myxozyma udenii sp. nov. (Candidaceae), a new yeast isolated from the rhizosphere of Mangifera indica. System. Appl. Microbiol. 13:182-185.

Two strains of an undescribed, soil-borne species of the genus Myxozyma were recovered. A description of the new species, Myxozyma udenii, and a key to the genus, is given.

The following paper has been submitted for publication:

2. Spaaij, F., Weber, G., Roeijmans, H., van Eijk G.W., & Oberwinkler, F. Fellomyces horovitziae sp. nov., a new basidiomycetous yeast species isolate from a Xenasmatella basidiocarp.

A new species of the genus Fellomyces, F. horovitziae is proposed. The delimiting characters are discussed and a key to the genus is provided.

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

Recently I have found that the type strain of Candida quercuum Nakase (J. Gen. Appl. Microbiol. 17:469, 1971) forms long filamentous outgrowths from the vegetative cells. This property is characteristic of the genus Mastigomyces Imshenetsky et Kriss. On this basis I propose a new combination, Mastigomyces quercuum (Nakase) Golubev.

The following papers have been published recently.

1. Golubev, W.I., & Kuznetsova, L.B. 1989. Production and killing patterns of mycocins produced by basidiomycetous yeast Cryptococcus laurentii (Kuff.) Skinner. Mikrobiologiya 58:980-984.

Three killer strains of Cr. laurentii were found among yeasts belonging to the genera Cryptococcus, Phaffia, Sporobolomyces and Trichosporon. One of the killer strain produces a mycocin with a narrow killing pattern (mainly against some Cryptococcus spp.). The two other killers (including the type strain of Cr. laurentii) produce mycocins which are active against the majority of Cryptococcus spp., some organisms belonging to the orders Atractiellales, Filobasidiales and Tremellales as well as their related asporogenous basidiomycetous yeasts. Ascomycetous yeasts, basidiomycetous yeasts related to the Ustilaginales and smut fungi are insensitive to mycocins produced by Cr. laurentii.

2. Bab'eva, I.P., Reshetova, I.S., Blagodatskaya, V.M. & Galimova, L.M. 1989. new yeast species, Candida odintsovae. Mikrobiologiya 58:631-634.

The new species is closest in its properties to Pichia rebaulensis and Candida maritima but differs from them in GC-content and/or in physiological properties. The type strain is BKM Y-2025.

3. Golubev, W.I. 1990. Capsules. In: The Yeasts, 2nd Edition, Ed. by A.H. Rose and J.S. Harrison. Acad. Press, London. Vol. 4, 175-198.

Content: Introduction. Morphology and fine structure. Culture conditions promoting capsule formation. Decapsulation and chemical composition. Functions. Conclusion.

IX. Research Institute for Viticulture and Enology, 83311 Bratislava, Matúškova 25, Czechoslovakia. Communicated by E. Minárik.

The following is the abstract of a paper presented in the 14th ISSY, in Smolenice, Czechoslovakia, September 1990.

1. Minárik, E. 1990. Occurrence and importance of Zygosaccharomyces sp. in winemaking.

Osmophilic Zygosaccharomyces bailii (Lindner) Guilliermond are very seldom isolated from primary habitats in vineyards, grape must or young grape wines. Out of more than 4,000 isolated yeast strains in different viticultural regions of Czechoslovakia, only 4 strains of this species could be found and identified. On secondary habitats in winery, Z. bailii may be, however, frequently isolated in the final stage of wine production, viz. wine bottling, first of all on the filling time and/or in bottled wines with residual sugar. Reasons of this occurrence of Z. bailii on secondary habitats are dealt with. Efficient measures preventing secondary fermentation and/or haze formation of sweet natural grape wines are elucidated. Technological properties of fructophilic Z. bailii (chemotolerance, tolerance to low fermentation temperature and other unfavourable fermentation conditions, e.g. osmotolerance), are underlined. The position of Z. bailii in winemaking is compared with Saccharomyces sp. Technological consequences are illustrated.

- X. Raiffeisen - Bioforschung - Tulln, Reitherstraße 21-23, A-3430 Tulln, Austria. Communicated by H. Prillinger.

The following is a summary of theses and papers in press or recently published by H. Prillinger, Ch. Dörfler, and G. Laaser, working at the University of Regensburg, on the systematics and evolution of higher fungi and the significance of yeast stages in the Basidiomycetes. Relevant references are given below.

The existence of yeasts in the Homobasidiomycetes Collybia tuberosa and C. cookei and the occurrence of microbotryoid, ustilaginoid, and tremelloid yeasts in different species of Homobasidiomycetes (Prillinger 1988, Prillinger & al. 1989) prompted the idea to investigate basidiomycetous yeasts and yeasts states from Homo- and Heterobasidiomycetes comparatively. Two hundred and five yeasts and yeast states of Basidiomycetes and presumed relatives were investigated on the basis of the carbohydrate (neutral sugars) patterns of purified cell walls (Dörfler & al. 1986, Dörfler 1990), urease-activity, diazonium blue B reaction, the production of extracellular amyloid compounds (EAC), fermentation of carbohydrates (Laaser 1989), and ubiquinone data. Taxa formed clusters with affinities to clustering leading to the Protomyces (Prillinger & al. 1990a), Microbotryum (Prillinger & al. 1990b), Ustilago (Prillinger & al. 1990c), Dacrymyces, and Tremella types (Prillinger & al. 1991). The different types correspond well with 5S rRNA clusters known from

the literature (e.g. Walker & Doolittle 1983, Gottschalk & Blanz 1985, Blanz & Unseld 1988).

The Protomyces type comprises all the investigated Protomyces (4) and Taphrina (33) strains. Presence of rhamnose in the cell wall differentiates the Protomyces type from the Microbotryum, Ustilago, Dacrymyces, and Tremella types. Predominant quantities of glucose, moderate amounts of mannose, small amounts of galactose, and the absence of xylose also characterize the cell walls of Protomyces and Taphrina yeast stages. Members of the Protomyces type exhibit affinities to both the Ascomycetes and the Basidiomycetes. A two layered cell wall (TEM: von Arx & al. 1982) and a negative DBB-reaction are common with all ascomycetous yeasts. On the other hand the presence of urease, production of extracellular amyloid compounds, and the primary and secondary structure of 5S rRNA (Gottschalk & Blanz 1985, Walker 1985) reflect a relationship closer to the Basidiomycetes. The ancestral nature of the Protomyces type is substantiated by the host spectra of the investigated Protomyces and Taphrina strains (Protomyces: Apiaceae (2), Cichoriaceae (2); Taphrina: Polypodiaceae (2), Betulaceae (9), Fagaceae (1), Salicaceae (3), Ulmaceae (1), Rosaceae (6), Aceraceae (2), Anacardiaceae (1)). Interestingly, Taphrina isolates from ferns exhibit a remarkable variability. For example, the cell walls of T. vestergrenii (from Dryopteris) contain significant amounts of fucose, a cell wall sugar which differentiates the Microbotryum type from the other types.

To clarify the relationship of Protomyces and Taphrina to the Endomycetales, 21 strains of different ascomycetous yeast genera (mostly type strains) have been included in this investigation. Surprisingly the qualitative cell wall carbohydrate pattern of these genera displayed a remarkable homogeneity. Two groups could be detected: group 1 contains only mannose and glucose and is characteristic for: Kloeckera apiculata, Kluyveromyces polysporus, Saccharomyces cerevisiae, Saccharomycodes ludwigii, Torulasporea delbrueckii, Zygosaccharomyces rouxii, Ambrosiozyma monospora, Arthroascus javanensis, Citeromyces matritensis, Pachysolen tannophilus, Dekkera bruxellensis, Feniella nana, Lodderomyces elongisporus, Metschnikowia reukauffii, Schwannomyces occidentalis, Wickerhamia fluorensis, and Wingea robertsiae. Significant quantities of galactose, as well as mannose and glucose are characteristic for group 2: Wickerhamiella domercgiae, Octosporomyces octosporus, Lipomyces starkeyi, and Schizosaccharomyces pombe. Molecular and biochemical data (cell wall carbohydrate, 5S rRNA, ubiquinone (Q-9, Q-10), urease activity) position the Schizosaccharomycetales, the Protomycetaceae, and Taphrinaceae in the phylogenetic vicinity of the Basidiomycetes (e.g. genera of the Microbotryum-type) and of fruit-body forming Ascomycetes (e.g. Ophiostoma species).

Consistent with the close relationship between Protomyces and Taphrina indicated by cell wall analysis and 5S rRNA sequence data (Walker 1985, Gottschalk & Blanz 1985), the ascus of Taphrina is regarded as the "siphonal" germination stage of a chlamydospore (Prillinger 1987). This "siphonal" germ tube acts as a meiosporangium, where evolution from an indeterminate number of meiotic nuclei in the case of Protomyces (polykaryotic) to a single meiotic nucleus represented by Taphrina species becomes obvious (Prillinger 1984).

Thirty-one strains clustering within the Microbotryum type comprise the phragmobasidial smut fungi of dicotyledonous hosts (Microbotryum, Sphacelotheca), the phragmobasidial Rhodosporeidium and Leucosporidium species including some anamorphic Rhodotorula species, which lack an oxidative degradation of myo-inositol, the genera Sporobolomyces and Sporidiobolus, the Septobasidiales and some simple septate Auriculariales e.g. Agaricostilbum, Platyglöea. The main characteristics of the Microbotryum type are (1) the absence of EAC, (2) the dominance of mannose, the absence of xylose, and commonly the presence of fucose as cell wall constituents, and (3) a positive DBB-reaction and splitting of urea. Four Ustilago species parasitic on dicotyledonous hosts were transferred to the genus Microbotryum (M. scabiosae, M. scorzonerae, M. cordae, M. vinosum) on the basis of cell wall carbohydrate composition, production of rhodotorulic acid, and 5S rRNA sequence data from the literature (Gottschalk & Blanz 1985, Prillinger & al. 1990b). The predominance of mannose in the cell wall (otherwise only known from ascomycetous yeasts), a type A secondary structure of 5S rRNA (Gottschalk & Blanz 1985), and a simple unifactorial mating system in all parasitic smut species (Whitehouse 1951, Zambettakis 1977, 1978a,b) suggest that the Microbotryum type might be ancestral relative to the Ustilago type. An evolution of simple ("siphonal") holobasidia from "pseudotrivial" phragmobasidia has been discussed (Prillinger 1987).

Fifty eight strains clustering within the Ustilago type comprise the phragmobasidial smut fungi which parasitize monocotyledonous hosts (Ustilago s.str., Sporisorium, Moesziomyces, Schizonella, Farysia), the Tilletiales (Tilletia, Entyloma), the Exobasidiales, the Cryptobasidiales (Microstroma), and some anamorphic yeasts (Tilletiopsis, Sterigmatomyces). The main characteristics of the Ustilago type are (1) the absence of EAC, (2) the dominance of glucose, the presence of galactose and small amounts of mannose (absence of xylose) in the cell wall of yeast states, and (3) a positive DBB reaction and urease activity. The predominance of glucose in the cell wall (similar to hyphal states of Homobasidiomycetes), a type B secondary structure of the 5S rRNA, and the partial occurrence of the complex heterobifactorial (A factor: extracellular function, few, commonly two alleles; B factor: intracellular function, multiple alleles) mating system in parasitic smut (U. maydis, U. filiformis) species (Puhalla 1968, Prillinger 1982, Prillinger & al. 1989) suggest that the Ustilago type is derived in comparison with the Microbotryum type.

The concept of the "yeast basidium" as a simple basidiomycetous meiosporangium is introduced to clarify the close phylogenetic relationship of Graphiolales and Ustilaginales s. str. (pseudotrivial, phragmobasidial smuts of monocots) detected by methods of molecular systematics (e.g. cell wall sugars, 5S rRNA sequences, absence of EAC, ferrichromes). The close relationship of the Tilletiales (especially Entyloma species) with the Exobasidiales, as indicated by the cell wall carbohydrate pattern and 5S rRNA sequences, gives support to the concept that simple holobasidia (e.g. Microstroma, Exobasidium) have evolved via "siphonal" (Prillinger 1987) germination tubes of chlamydospores (Tilletia, Entyloma). The plastic simple holobasidium at the beginning of the ontogeny in Entyloma and Tilletia reappears but in a less plastic form at the end of the ontogeny in the more advanced Exobasidium species. This was further corroborated by the infrequent occurrence of transversely septate phragmobasidia in Exobasidium karstenii.

The 5 strains clustering within the Dacrymyces type comprise the species Dacrymyces stillatus, D. variisporus, Calocera cornea, Trichosporon dulcitum, and Sebacina penetrans. The 26 strains assigned to the Tremella type included strains in the genus Mrakia, the Filobasidiaceae (Cystofilobasidium, Filobasidium), Tremellaceae (Tremella, Trimorphomyces), Carcinomycetaceae (Christiansenia, Carcinomyces), the anamorphic basidiomycetous yeasts Phaffia, Cryptococcus, and the imperfect ballistosporogenous genus Bullera.

The Dacrymyces and Tremella type are well differentiated from the Protomyces, Microbotryum, and Ustilago type by the presence of xylose in the cell wall of yeasts or yeast states. Although the Dacrymyces and Tremella type seemed to be closely related judging from a similar cell wall carbohydrate pattern and 5S rRNA

ribosomal sequences, both types are distinct based on the production of extracellular amyloid compounds. The presence of EAC is characteristic for all members of the *Tremella* type.

Although morphology of meiosporangia reflects a high degree of variation within genera of the *Tremella* type (e.g. *Sterigmatosporidium*, *Cystofilobasidium*, *Tremella*, *Christiansenia*, *Carcinomyces*), a close phylogenetic relationship becomes obvious from a comparative investigation of the unicellular, coccid (yeast) growth forms (Oberwinkler 1977) of these organisms, using characters of molecular systematics. Two phylogenetically distinct types of holobasidia became evident. A simple holobasidium characteristic for the Filobasidiaceae can be deduced from "siphonal" germination states (Prillinger 1987) of chlamydospores (e.g. *Mrakia*, *Cystofilobasidium*). On the other hand, a complex holobasidium has evolved from the longitudinally septate *Tremella* basidium via a partially septate holobasidium like *Syzygospora* to the complex holobasidia of *Christiansenia*, *Carcinomyces*, and the bulk of Homobasidiomycetes (Oberwinkler & Bandoni 1982). The close relationship of *Sebacina penetrans* and the Dacrymycetales indicated by the investigated yeasts states and the lack of representatives with a partially compartmented basidial apex within the Dacrymycetales further suggest, that the basidia of the Dacrymycetales may also be simple holobasidia (Prillinger & al. 1991).

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3. Laaser, G. 1989. Vergleichende systematische Studien an Basidiomycetenhefen unter besonderer Berücksichtigung der Hefestadien. *Bibliotheca Mycologica* 130:1-325.
4. Prillinger, H. 1982. Zur genetischen Kontrolle und Evolution der sexuellen Fortpflanzung und Heterothallie bei Chitinpilzen. *Z. Mykol.* 48:297-324.
5. Prillinger, H. 1984. Zur Evolution von Mitose, Meiose und Kernphasenwechsel bei Chitinpilzen. *Z. Mykol.* 50:267-352.
6. Prillinger, H. 1987. Yeasts and anastomoses: their occurrence and implications for the phylogeny of Eumycota. In Rayner, A.D.M. & al. (eds). *Evolutionary biology of the fungi*. BMS Symp. Vol. 12. Cambridge University Press, Cambridge. pp. 355-377.
7. Prillinger, H. 1988. Are there yeasts in Homobasidiomycetes? In de Hoog, G.S. & al. (eds.). *The expanding realm of yeast-like fungi*. Elsevier Sci. Publ., Amsterdam. pp. 33-59.
8. Prillinger, H., Altenbuchner, J., Schulz, B., Dörfler, Ch., Forst, Th., Laaser, G., & Stahl, U. 1989. *Ustilago maydis* isolated from Homobasidiomycetes. *Proc. Appl. Plant Mol. Biol.* (Braunschweig Symposium Nov. 1988) Technische Universität, Braunschweig. pp. 408-425.
9. Prillinger, H., Dörfler, Ch., Laaser, G., Eckerlein, B. & Lehle, L. 1990a. Ein Beitrag zur Systematik und Entwicklungsbiologie Höherer Pilze: Hefe-Typen der Basidiomyceten. Teil I: Schizosaccharomycetales, *Protomyces*-Typ. *Z. Mykol.* 56:219-250.
10. Prillinger, H., Dörfler, Ch., Laaser, G. & Lockau, W. 1990b. Ein Beitrag zur Systematik und Entwicklungsbiologie Höherer Pilze: Hefe-Typen der Basidiomyceten. Teil II: Microbotryum-Typ. *Acta Botanica* 103: (in press).
11. Prillinger, H., Dörfler, Ch., Laaser, G. & Hauskä, G. 1990c. Ein Beitrag zur Systematik und Entwicklungsbiologie Höherer Pilze: Hefe-Typen der Basidiomyceten. Teil III: Ustilago-Typ. *Z. Mykol.* 56:251-278.
12. Prillinger, H., Laaser, G., Dörfler, Ch. & Ziegler, K. 1991. Ein Beitrag zur Systematik und Entwicklungsbiologie Höherer Pilze: Hefe-Typen der Basidiomyceten. Teil IV: Dacrymyces-Typ, Tremella-Typ. *Sydowia* 53: (in press).

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- Zambettakis, Ch. 1978b. La sexualité chez les Ustilaginales. Troisième Partie. Rev. Mycol. 42:113-142.

XI. Dipartimento di Biologia Vegetale, Sezione Microbiologia Applicata, Borgo 20 Giugno 74, I-06100 Perugia, Italy. Communicated by A. Vaughan Martini

The following chapters have been published recently:

1. Vaughan Martini, A. & Martini, A. 1990. A proposal for correct nomenclature of the domesticated species of the genus *Saccharomyces*. In: C. Cantarelli & G. Lanzarini (eds.) "Biotechnology Application in Beverage Production". Elsevier, Amsterdam-London. pp. 1-16.
2. Martini, A. & Vaughan Martini, A. 1990. Grape must fermentation: past and present. In: J.F.T. Spencer & D.M. Spencer (eds.) "Yeast Technology". Springer-Verlag, Berlin. pp. 105-123.
3. Rosini, G. 1990. Killer yeasts: notes on properties and technical use of the character. In: C. Cantarelli & G. Lanzarini (eds.) "Biotechnology Application in Beverage Production". Elsevier, Amsterdam-London, pp. 41-48.

The following papers have recently been published.

4. Vaughan, Martini A., Rosini, G., & Martini, A. 1988. Killer sensitivity patterns as a tool for the fingerprinting of strains within the yeast species *Kluyveromyces lactis* and *Kluyveromyces marxianus*. Biotechnol. Techn. 2:293-296.
5. Vaughan Martini, A. & Rosini, G. 1989. Killer relationships within the yeast genus *Kluyveromyces*. Mycologia. 81:317-321.
6. Rosini, G., Ciani, M., & Palpacelli, V. 1989. Oxygen consumption as a measure of yeast starter culture vitality for the wine industry. Biotechnol. Techn. 3:223-226.
7. Rosini, G., & Ciani, M. 1990. Selection of strains of *Saccharomyces cerevisiae* of "Sagrantino D.O.C." for their wine-making properties: preliminary results. Ital. J. Food Sci. 2:151-158.

The following paper, whose abstract appeared in the June 1989 issue, has recently been published.

8. Vaughan Martini, A. 1989. *Saccharomyces paradoxus* comb. nov., a newly separated species of the *Saccharomyces sensu stricto* complex based upon nDNA/nDNA homologies. System. appl. Microbiol. 574:119-122.

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

The following publications have appeared recently.

1. Barnett J.A., Payne, R.W.¹ & Yarrow, D.². Photomicrographs by L. Barnett. *Yeasts: Characteristics and Identification*. Second Edition
¹Statistics Department, Rothamsted Experimental Station, Harpenden, England
²Yeast Division, Centraalbureau voor Schimmelcultures, Delft, Netherlands

This new edition includes: (a) Descriptions of 597 species with information on the results of 91 tests, growth temperatures, effect of cycloheximide on growth, DNA base composition, (v) cell morphology, sexual reproduction, 870 photomicrographs (about 200 show ascospores), and further features updated from 1983 edition.

ISBN 0-521-35056-5, 1024 pp., £125.00

2. Barnett, J.A., Payne, R.W. & Yarrow, D. Yeast Identification: PC Program, Version 2.

Based on data from the above book. This program identifies yeasts, selects yeasts with chosen characteristics. For use in research, industry, medical mycology, is available for IBM PC and compatibles with MS/PC-DOS.

After entering the results, lists can be obtained of the following. All species with a matching set of characteristics, yeasts with characteristics that most nearly match, with a statement of the characteristic(s) that differ, further tests necessary to complete identification. The program can allow for mistakes in results, display description of a selected species, select yeasts with particular characteristics.

Improvements to the previous (1987) version include the ability to list differences between 2 species or between an unknown and any other species, and calculation of probabilities of identification.

Price £125.00 or US\$210 (US)

Enquiries: J.A. Barnett, 36 Le Strange Close, Norwich NR2 3PW, UK.

3. Sims, A.P. & Barnett, J.A. In press. Levels of activity of enzymes involved in anaerobic utilization of sugars by six yeast species: observations towards understanding the Kluyver effect. FEMS Microbiol. Lett.

XIII. 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 and G.D. Clark-Walker.

The following articles have been accepted recently.

1. Skelly, P.J. and Maleszka, R. 1991. Distribution of mitochondrial intron sequences among 21 yeast species. Curr. Genet. (in press)

The mitochondrial and nuclear genomes of 21 species belonging to 12 genera have been tested for the presence of sequences similar to 7 *S.cerevisiae* mitochondrial introns (*Sc* *cox1.1,2,3,4,5c*, *Sc* *cob.4*, and *ScLSU.1*) and one *Kluyveromyces lactis* mitochondrial intron (*Kl* *cox1.2*). Some introns, (*Sc* *cox1.4*, *Sc* *cob.4*, *ScLSU.1* and *Kl* *cox1.2* - all group I type), are widely distributed and are found in species with either basidiomycete or ascomycete affinities. The distribution is suggestive of recent sequence transfer between species. The remaining *S.cerevisiae* introns cross react with an additional species but with no set pattern. Pulsed field gel electrophoresis studies confirm that none of the tested mitochondrial introns cross react with nuclear rDNA. These introns are therefore mitochondria specific. Seven strains of *K.lactis* exhibit striking variability in intron content. In contrast to all mitochondrial introns tested, two intron of nuclear genes (the *K.lactis* actin gene and the *S.cerevisiae* RP298 gene) are not detected beyond their source species.

2. Skelly, P.J. & Clark-Walker, G.D. In press. Polymorphism in tandemly repeated sequences of *Saccharomyces cerevisiae* mitochondrial DNA. J. Mol. Evol.

3. Skelly, P.J. & Clark-Walker, G.D. In press. Sequence rearrangements at the ORI7 region of *Saccharomyces cerevisiae* mitochondrial DNA. J. Mol. Evol.

XIV. Microbiology and Genetics Department, Massey University, Palmerston North, New Zealand. Communicated by R.J. Thornton and S.B. Rodriguez.

The following publications have been accepted or have appeared since our last communication.

1. Rodriguez, S.B. & Thornton, R.J. In press. Factors affecting the utilization of L-malate by five genera of yeast. FEMS Microbiol. Lett.

2. Rodriguez, S.B., Amberg, E., Thornton, R.J., & McLellan, M.R. 1990. Malolactic fermentation in Chardonnay: growth and sensory effects of commercial strains of *Leuconostoc oenos*. J. Appl. Bact. 68:139-144.

3. Rodriguez, S.B. & Thornton, R.J. 1989. A malic acid dependent mutant of *Schizosaccharomyces malidevorans*. Arch. Microbiol. 152:564-566.

4. Wedlock, D.N., James, A.P. & Thornton, R.J. 1989. Glucose-negative mutants of *Pachysolen tannophilus*. J. Gen. Microbiol. 135:2019-2026.

5. Wedlock, D.N. & Thornton, R.J. 1989. A hexokinase associated with catabolite repression in *Pachysolen tannophilus*. J. Gen. Microbiol. 135: 2013-2018.

6. Thornton, R.J. & Bunker, A. 1989. Characterization of wine yeasts for genetically modifiable properties. J. Inst. Brew. 95:181-184.

7. Wedlock, D.N. & Thornton, R.J. 1989. Transformation of a glucose negative mutant of *Pachysolen tannophilus* with a plasmid carrying the cloned hexokinase P II gene from *Saccharomyces cerevisiae*. *Biotechnol. Lett.* **11**: 601-604.
 8. Smart, R.E., Thornton, R.J., Rodriguez, S.B., & Young, J.E. (eds.). 1988. Proceedings of the Second International Symposium for Cool Climate Viticulture and Oenology. 11-15 January 1988; Auckland, New Zealand: New Zealand Society for Viticulture and Oenology.
 9. Thornton, R.J. 1987. An introduction to the genetics of industrial yeasts. In: *Industrial Yeast Genetics*. Foundation for Biotechnical and Industrial Fermentation Research. Korhola, M., and Nevalainen (eds.). Helsinki, Finland **5**:11-25.
 10. Rodriguez, S.B. 1987. A system for identifying spoilage yeast in packaged wine. *Am. J. Enol. Vitic.* **38**:273-276.
 11. Thornton, R.J. & Rodriguez, S.B. 1988. Commercial scale deacidification of grape juice for winemaking by *Schizosaccharomyces malidevorans* mutant #11. Proceedings of the 4th Annual Seminar of the New Zealand Society for Viticulture and Oenology: Innovations in Viticulture and Oenology. N.K. McCullum and I.D.G. Milne (eds.). Masterton, New Zealand p. 1-6.
 12. Rodriguez, S.B. & Thornton, R.J. 1988. Rapid utilisation of malic acid by a mutant of *Schizosaccharomyces malidevorans*. Proceedings of the 2nd International Symposium for Cool Climate Viticulture and Oenology. Smart, R.E., Thornton, R.J., Rodriguez, R.S., and Young, J.E. (eds.). Auckland, New Zealand, pp. 313-315.
 13. Rodriguez, S.B., Amberg, E., Arnink, K.J., Thornton, R.J. & McLellan, M.R. 1988. Complexity in Chardonnay wines: effects of malo-lactic fermentation and barrel fermentation. Proceedings of the 2nd International Symposium for Cool Climate Viticulture and Oenology. Smart, R.E., Thornton, R.J., Rodriguez, R.S., and Young, J.E. (eds.). Auckland, New Zealand. p. 287.
 14. Thornton, R.J., Wedlock, D.N. & Clark, T.A. 1988. Genetic improvement of the xylose-fermenting yeast *Pachysolen tannophilus*: a summary of the New Zealand programme. A report to the International Energy Agency/Bioenergy Agreement. Forest Research Institute/Ministry of Forest/Massey University. 15 pp.
 15. Ravji, R.G., Rodriguez, S.B., & Thornton, R.J. 1988. Glycerol production by four common grape moulds. *Am. J. Enol. Vitic.* **39**:77-82.
 16. Thornton, R.J. & Eustace, R.E. 1987. Selective hybridisation of wine yeasts for higher yields of glycerol. *Can. J. Microbiol.* **33**:112-117.
 17. Thornton, R.J. 1987. Wine yeast research in New Zealand. *Australian and NZ Wine Industry Journal* **2**:49-50.
 18. Thornton, R.J. 1986. Genetic characterization of New Zealand and Australian wine yeasts. *Antonie van Leeuwenhoek* **52**:97-103.
 19. Thornton, R.J. & S.B. Rodriguez. 1986. Genetics of wine microorganisms: potentials and problems. Proceedings of the Vith Australian Wine Industry Technical Conference. T. Lee (ed.) pp. 98-102.
 20. Thornton, R.J. 1986. Wine yeast culture, propagation and evolution. Proceedings of the Cornell University Symposium on Winemaking, pp. 9-27.
 21. Thornton, R.J., Clarke, T., Wedlock, D.N., James, A.P. and Keverall, K. 1986. Strain improvement of the xylose-fermenting yeast *Pachysolen tannophilus* by hybridisation of two mutant strains. *Biotechnol. Lett.* **8**:801-806.
- XV. Department of Biological and Biomedical Sciences, University of Ulster at Coleraine, Coleraine, Co. Londonderry BT52 1SA, Northern Ireland. Communicated by R.T. Moore.

The following papers have been accepted or published recently.

1. Moore, R.T. 1990. The genus *Lalaria* gen. nov.: Taphrinales anamorposum. *Mycotaxon* **38**:315-330.
2. Moore, R.T. 1990. Order Platygloales ord. nov. *Mycotaxon* **39**:234-257.

3. Moore, R.T. & A.M. Flinn. In press. Identification of ubiquinone isoprenologues in Apiotrichum porosum and a strain of Candida humicola. Mycological Research 94.
 4. Moore, R.T. & A.M. Flinn. In press. Ubiquinone and urease distribution in Taphrina and Symbiotaphrina. Antonie van Leeuwenhoek 39.
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- XVI. Department of Plant Biology, University of California, Berkeley
California 94720, U.S.A. Communicated by S. Fogel.
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The following is a summary of a recent Doctor of Philosophy dissertation successfully defended by L. Spector.

1. Interchromosomal aneuploidy comparison in the yeast Saccharomyces cerevisiae by transposition of a gene dosage selection system. 120 pp.

Two nonallelic genes, arg4-8 and cup1^S, normally located on chromosome VIII were used to detect aneuploids i.e., disomic haploids and trisomic diploids. These alleles confer dosage dependent phenotypes. To study comparative chromosome behavior, both the alleles were deleted from chromosome VIII of a haploid strain and reintroduced into the leu2 locus on chromosome III via an integrating plasmid bearing cloned single copies of the arg4-8 and cup1^S loci. The data collected from strains with the alleles residing on chromosome III compared to chromosome VIII show significant differences in aneuploid frequency, aneuploid chromosome stability and magnitude of genetic load attributable to hyperploidy. Spontaneous chromosome III hyperploids were recovered at a frequency higher than chromosome VIII hyperploids, by 2.4 fold for diploids and 10.5 fold for haploids. The spontaneous hyperploidy gain rates were also higher by 7 fold for diploids and 8 fold for haploids. The observed differences in chromosome III and VIII aneuploidy, attributed in part to the genetic background of the strains and in part to some aspect of chromosome specificity. Finally, it appears that yeast may have at least two different mechanisms that generate chromosome III and VIII aneuploids.

The following are abstracts of recently published papers.

2. Wheeler, C.J., Maloney, D., Fogel, S., & Goodenow, R.S. 1990. Microconversion between murine H-2 genes integrated into yeast. Nature 347(6289):192-194.

Patchwork homology observed between divergent members of polymorphic multigene families is thought to reflect evolution of short-tract gene conversion (nonreciprocal recombination), although this mechanism cannot usually be confirmed in higher organisms. In contrast to meiotic conversions observed in laboratory yeast strains, apparent conversions between polymorphic sequences, such as the class I loci of the major histocompatibility complex (MHC), are short and do not seem to be associated with reciprocal recombination (crossover, exchanges). We have now integrated two nonallelic murine class I genes into yeast to characterize their meiotic recombination. We found no crossovers between the MHC genes, but short-tract "microconversions" of 1-215 base-pairs were observed in about 6% of all meiosis. Strikingly, one of these events was accompanied by a single base pair mutation. These results underscore both the importance of meiotic gene conversion and sequence heterology in determining conversion patterns between divergent genes.

3. Welch, J.W., Maloney, D.H. & Fogel, S. 1990. Unequal crossing-over and gene conversion at the amplified CUP1 locus of yeast. Mol. Gen. Genet. 222:304-310.

Meiotic recombination was analyzed between two twelve copy arrays of a gene amplification at the CUP1 locus of Saccharomyces cerevisiae. Utilizing Southern analysis to identify spores with non-parental repeat arrays, we find that approximately 11% of a sample with 202 unselected tetrads possess at least one non-parental spore array. Both reciprocal and non-reciprocal changes are observed. The data suggest a model in which frequent mispairing among identical copies of the 2 kb repeat unit leads to the formation of unpaired loops containing integral numbers of repeat units. In this model, conversions involving the loops lead to non-reciprocal changes in arrays: about half are associated with reciprocal exchange, and net increases in repeat unit numbers occur about as frequently as net decreases. Thus, the known properties of gene conversion can account for all the segregations we observe.

Seminar.

4. Fogel, S. 1990. In a seminar lecture presented at CNRS, Gif-sur-Yvette, France, in July, 1990, physical evidence for the long postulated recombination intermediate, viz., heteroduplex DNA and the semiquantitative correlation of this specific DNA species with the frequency of post-meiotic segregation at the ADE8/ade8-18 site was examined in detail. The group contributing to this effort includes Anne Plessis, James White, Karin Lusnak and Daniel Maloney.
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XVII. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Communicated by W.M. Ingledew.

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

1. O'Connor-Cox, E.S.C. & Ingledew, W.M. 1989. Wort nitrogenous sources - their use by brewing yeasts: a review. *J. Amer. Soc. Brew. Chem.* **47**:102-108.

The sources of wort nitrogen that are assimilable by yeast are mainly amino acids, ammonium ion, and to a lesser extent, di- and tripeptides. These substances are partly formed during malting and partly during mashing, due to the action of barley proteases on hordein. The level of wort amino acids varies depending on the barley variety, malting conditions, and mashing parameters, but the overall spectrum of amino acids is similar among worts. Brewing adjuncts dilute nitrogenous substances while increasing the wort fermentable extract. Consequently, high-adjunct worts are nitrogen deficient. Yeast food can be used to increase assimilable nitrogen levels; however, amounts added may be insufficient for full stimulation of fermentation. Wort amino acids are taken up by yeast in a characteristic sequence during fermentation, although environmental changes can alter this pattern. Absolute yeast nitrogen requirements in the literature differ, and such levels have never been ascertained for high gravity or very high gravity fermentations. Because of the preferred methods for these worts, they can be expected to be nitrogen-limited. The role of free amino nitrogen (FAN) in the brewing fermentation is mainly incorporation into new yeast protein; however, nitrogen levels profoundly affect almost every aspect of fermentation performance. Of critical import are the levels of higher alcohols, vicinal diketones, and esters, all of which are intimately related to wort nitrogen profiles. This review covers the factors affecting the wort nitrogen content, the assimilable nitrogen levels and their profile in wort and beer, and the role of usable nitrogen in the fermentation. In addition, the effects of limiting and excess quantities of assimilable nitrogen are reviewed in light of current research findings.

2. O'Connor-Cox, E.S.C. & Ingledew, W.M. 1990. Effect of the timing of oxygenation on very high gravity brewing fermentations. *J. Amer. Soc. Brew. Chem.* **48**:26-32.

Yeasts require small quantities of oxygen to synthesize unsaturated fatty acids and sterols, both of which are needed for continued anaerobic growth and cell division. As the fermentation rate is proportional to the number of metabolically active yeasts present, rapid wort attenuation is therefore related to the availability of oxygen. The effects of limited oxygen supply are compounded in high gravity or very high gravity (VHG) wort fermentations. Alterations in the quantity and the timing of the provision of an oxygen supply were evaluated in 28 g dissolved solids/100 ml VHG brewing fermentations. The objective was to ascertain the optimal supply time, postinoculation, that would give the most rapid attenuation. For the commercial *Saccharomyces uvarum* lager yeast employed, oxygen was most stimulatory when it was added between 10 and 14 hr postpitching. Yeast dry weight and viability were maximized, and the rates of attenuation, nitrogen assimilation, and ethanol formation were the most rapid. Conversely, earlier oxygen application (2, 4, or 6 hr postpitching) was not as effective as aerating the wort before pitching; oxygen supplied more than 14 hr after inoculation was similarly not as stimulatory. Excess amounts of dissolved oxygen supplied at 12 hr (25 mg/L dissolved oxygen) further stimulated the attenuation rate but not yeast growth. The practical extensions of the research are discussed with relevance to current industrial practice.

3. O'Connor-Cox, E.S.C., Munoz, E., & Ingledew, W.M. 1990. The sensitivity of a commercial lager yeast to novel inhibitors of unsaturated fatty acid and ergosterol biosynthesis. *Biotech. Lett.* **12**:33-38.

Cyclopropenoid fatty acids inhibited fatty acid desaturation in a lager strain of *Saccharomyces uvarum*. N,N-dimethylazasqualene prevented ergosterol biosynthesis in the same yeast. Inhibitions took place under conditions favourable for ergosterol and unsaturated fatty acid formation, each independently of the other.

4. Thomas, K.C. & Ingledew, W.M. 1990. Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. *Appl. Environ. Microbiol.* **56**(7): 2046-2050.

Although wheat mashes contain only growth-limiting amounts of free amino nitrogen, fermentations by active dry yeast (*Saccharomyces cerevisiae*) were completed (all fermentable sugars consumed) in 8 days at 20°C even when the mash contained 35 g of dissolved solids per 100 ml. Supplementing wheat mashes with yeast extract, Casamino Acids, or a single amino acid such as glutamic acid stimulated growth of the yeast and reduced the fermentation time. With 0.9% yeast extract as the supplement, the fermentation time was reduced from 8 to 3 days, and a final ethanol yield of 17.1% (vol/vol) was achieved. Free amino nitrogen derived in situ through the hydrolysis of wheat proteins by a protease could substitute for the exogenous nitrogen source. Studies indicated, however, that exogenously added glycine (although readily taken up by the yeast) reduced the cell yield and prolonged the fermentation time. The results suggested that there are qualitative differences among amino acids with regard to their suitability to serve as nitrogen sources for the growth of yeast. The complete utilization of carbohydrates in wheat mashes containing very little free amino nitrogen presumably resulted because they had the "right" kind of amino acids.

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

1. Goto, S., Y. Maejima and T. Shinohara. 1989. On ploidy of Saccharomyces cerevisiae and Saccharomyces bayanus. Bull. Japan Federation for Culture Collections 5:76-79.

Ploidy of two types and four wine yeast strains describing to Saccharomyces cerevisiae and S. bayanus was estimated on DNA content, cellular volume, UV-irradiation survival curve and sporulation. The type of S. cerevisiae (NRRL Y-12632) was estimated to be polyploid over tetraploid and that of S. bayanus (NRRL Y-12624) was to be diploidy or almost diploidy. Ascospore of the type of S. cerevisiae was extremely rarely found and did not germinate. Ascospore formation rate of the type of S. bayanus are 35%, but no germination of any spore was observed. Ascospore formation and germination rates of 4 wine yeast strains were 42-58% and 25-88%, respectively. Those 4 yeasts were ascertained to be diploid and homothallic strains.

XIX. Center of Biological Research, Division of Experimental Biology, P.O.
Box 128, La Paz B.C.S., México. Communicated by J.L. Ochoa.

The following are abstracts of papers that were presented at the 14th International Specialized Symposium on Yeasts, Smolenice, Czechoslovakia, September 1990, and recently submitted for publication.

1. Hernández-Saavedra, N.Y., Hernández-Saavedra, D., Vázquez, J.R., & Ochoa, J.L. Distribution of Sporobolomyces (Kluyver et van Niel) genera in the western coast of Baja California Sur, Mexico, Poster session.

141 yeast strains were isolated from 98 sampling stations in the Pacific Ocean, during the cruise CIB-CICIMAR 8605, by the oceanographic ship "El Puma". The sampling stations were distributed according to CalCOFI network between 27°10'N, 115°18'45"W; 27°26'38"N, 114°45'W; 23°53'20"N, 110°11'15"W and 23°8'40"N, 112°30'W on the west coast of Baja California Sur State, Mexico. The genera Sporobolomyces represented 32% of the total isolates, of which the species of Sp. holsaticus corresponded to 10%, Sp. puniceus 20%, and Sp. roseus 2%. Sp. puniceus showed preference for the farthest stations from the coastline at a depth of 100 meters; Sp. holsaticus was practically present in all station, specially at depth of 100 meters; and Sp. roseus was isolated from middle range distant stations off the shore at depths of 0.3 and 50 meters. By correlating the distribution of marine yeasts with temperature, salinity, dissolved oxygen and depth, it was possible to establish the distribution pattern for each species along the coast of the Baja California peninsula.

2. Hernández-Saavedra, D., Vázquez-Duhal, R., Leal, M.C.A., Vazquez, J.R, & Ochoa, J.L. Marine yeasts: Cell Wall Chemical Composition. Lecture.

The cell wall chemical composition of yeasts is characterized mainly by the presence of B1,3 and B1,6 glucans, which constitute the fibrillar components that confer rigidity to their cell wall. Changes and modifications of such structures may influence the adaptation capability of these microorganisms to a variety of environments. Debaryomyces hansenii and Rhodotorula sp. were isolated from the west costs of Baja California peninsula in areas with practically nile influence of human activity and therefore are regarded as of marine origin. Thus, we consider of interest of study and analyze the chemical composition of their cell walls. As reported herein, we found that when the cell walls are hot alkali and acid extracted, the main components are polysaccharides (37-90%), followed by proteinaceous compounds (8-27%). Glucose appeared as the principal monosaccharide unit, but also mannose and N-acetyl-glucosamine could be detected by GLC. The polysaccharides isolated from the cell walls of the different yeasts, showed different susceptibility to zymolyase and chitinase enzyme activities. The ecological implications of these findings was discussed.

The following is the summary of a presentation by J.L. Ochoa at the 2nd National Symposium on Marine Biotechnology, La Paz B.C.S., Mexico, September 1990.

3. Isolation and biological reagents from marine organisms: Enzyme SOD from marine yeasts.

The presentation resumed all the works carried out at CIB aimed at utilizing marine yeasts as a source of biological reagents. As an example, the isolation of superoxide dismutase (SOD) enzyme was discussed in detail. The properties of the enzyme and its role in preventing the toxicity of oxygen, and therefore as an alternative in the treatment of different kinds of diseases, were also considered. The marine yeast selected for this study fulfils the criteria to serve as a commercial source of the enzyme, i.e. rapid growth, fast sedimentation, high content of SOD, non-toxicity, etc. The experimental conditions for the yeast culturing and isolation of the SOD enzyme in acceptable yields were compared to other commercial sources for this enzyme.

The following paper is being prepared for publication.

4. Hernández-Saavedra, D., Vázquez-Duhalt, R. and Ochoa, J.L. Changes on the cell wall composition of Saccharomyces cerevisiae grown in marine media.

Significance differences in the cell wall composition of S. cerevisiae were found in cells grown in yeast extract-peptone-glucose medium, prepared with or without seawater. The carbohydrate and protein contents of the cell walls were 76.8% and 25.2% respectively, in the freshwater cultures, and 86.6% and 13.1%, in seawater cultures. The aminosugar content was higher in the seawater cultures than in the freshwater ones. The carbohydrate composition of the different fractions showed significant differences according to culture medium used, specially in fraction I (alkali and acid soluble fraction). The change of the cell wall composition, as a consequence of a haloadaptation process, is discussed.

The following is the abstract of a Bachelor's degree thesis.

5. Hernández-Saavedra, N.Y. 1990. Marine yeasts isolated from the west coast of Baja California Sur, Mexico. E.N.E.P.I.- U.N.A.M. September 26, 1990.

98 seawater samples from 37 stations located at the Pacific Ocean, according to CALCOFI network, on the west coast of the California Peninsula, were collected during CIB-CICIMAR 8605 oceanographic cruise. In average 2.2 yeast strains were isolated from each sample. 136 strains belonged to the Blastomycetales, 71 to the Endomycetales, 5 to Ustilaginales and 4 to the genera Aureobasidium. Species distribution correlated well with previous findings in other areas of the Pacific Ocean. The distribution of species was also correlated with some physico-chemicals parameters (temperature, salinity and dissolved oxygen) and the ecological implications discussed.

XX. Department of Biology, Syracuse University, Syracuse, New York 13244, U.S.A. Communicated by W.T. Starmer.

The following is the summary of a paper presented at the 5th International Symposium on the Microbiology of the Phyllosphere, July 31 - August 3, Madison, Wisconsin.

1. Starmer, W.T., Fogleman, J., & Lachance. M.A. 1990. The yeast Community of cacti. Proc. 5th Int. Symp. Microbiol. Phyllosphere, Chapter 8.

In this chapter, we have reviewed the distribution of yeast species that live in the decaying tissues of cactus species. Cactus yeasts are, in general, specific for the cactus habitat and do not occur elsewhere. They also appear to have evolved within the framework of the cactus-microorganism-Drosophila system and the closest relatives of most species are within the system. Host differences appear to be important determinants and yeast community structure. Yeast vectors such as drosophilids and their host affinities may also contribute significantly to observed yeast distributions. The nutritional benefits provided by the yeasts to their vectors are potentially potent organizing factors for the stability and continuation of particular community types. Yeast-yeast interactions range from mutualism to interference competition. This latter form of competition is achieved in some cases by production of killer toxins. These toxins appear to be most important for between community exclusions. Yeast communities of cactus might be viewed as communities with diffuse mutualisms occurring among members of the same community which as a "unit community" (Swift, 1976) is mutualistic with its vectors.

XXI. Department of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan. Communicated by Y. Yamada.

1. Yamada, Y. and Nakagawa. Y. 1990. The molecular phylogeny of the basidiomycetous yeast species, Leucosporidium scottii based on the partial sequences of 18S and 26S ribosomal ribonucleic acids. J. Gen. Appl. Microbiol. 36:63-68.

Based on rRNA partial sequences, Leucosporidium scottii IFO 9474 (Q-7) is unique from the phylogenetic point of view. This strain constitutes an independent cluster apart from any other strains of L. scottii, R. toruloides, and K. malvinella. We can, thus, set up a new genus for the Q₇-equipped IFO 9474 strain, however, more detailed morphological and physiological experiments are required.

XXII. Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro, 21941 Rio de Janeiro, Brazil. Communicated by A.D. Panek.

The following are abstracts of some recently completed projects.

1. Rodrigues Torres, A.P., Aymard, S. and Panek, A.D. Activation of yeast trehalase by heat shock. Submitted for publication.

Activation of yeast trehalase by heat shock was shown in all strains including mutants in which the response to a glucose signal was absent. In fact a low concentration of cAMP seemed to favour the response as seen in 2nd log cells or in ras2 and cyr1^{ts} mutant strains. Under conditions of catabolite repression the heat shock effect upon trehalase activity was not observed. Neither hexokinase PII nor the heat shock protein hsp26 seemed to be involved in the activation of trehalase by heat shock. However, mutant strains

XXIV. Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin - Madison, 1925 Willow Drive, Madison, WI 53706, U.S.A. Communicated by E.A. Johnson.

The following papers have been published recently.

1. An, G.H. & Johnson, E.A. 1990. Influence of light on growth and pigmentation of the yeast *Phaffia rhodozyma*. *Antonie van Leeuwenhoek* 57:191-203.

Light and antimycin markedly affected growth and carotenoid synthesis of *Phaffia rhodozyma*. Exposure of the yeast to high light intensities on agar plates resulted in growth inhibition and decreased carotenoid synthesis. The carotenoid compositions of the yeast were also notably changed by light. In liquid medium, growth of the wild-type strain (UCD-FST-67-385) was inhibited by antimycin, but this inhibition was relieved by exposure to light. Light also stimulated carotenoid synthesis about twofold in these antimycin-treated cells. Light may have rescued growth by induction of an alternative oxidase system which facilitated electron disposal when the main respiratory chain was inhibited by antimycin. Isolation and characterization of the oxidase enzymes should be useful in strain development for increased carotenoid production.

2. An, G.H., Bielich, J., Auerbach, R., & Johnson, E.A. 1990. Isolation and characterization and carotenoid hyperproducing mutants of yeast by flow cytometry and cell sorting. *Biotechnology* (in press, December 1990).

XXV. Dipartimento di Protezione e Valorizzazione Agroalimentare, Università Delgi Studi Di Bologna, Via F. III Rosselli 107, 42100 Sede di Reggio Emilia, Italy. Communicated by P. Romano and G. Suzzi.

The following papers have published recently:

1. Romano, P. 1990. La vinificazione con lieviti non produttori H₂: aspetti positivi e negativi. *Vitivinicoltura* 16:27-34 (In Italian, English summary).

About 1.1% of natural strains of *Saccharomyces* does not produce hydrogen sulfide. The character "non-H₂S production" is stable, hereditary and consistently showed a 2:2 segregation ratio. All the non H₂S producing strains possess the following characteristics: high SO₂ production, stabilizing power, high n-propanol production and nonproduction of sulfur unpleasant compounds. The inability to produce H₂S can be coupled to other characteristics of great technological interest.

2. Suzzi, G. 1990. Autolytic ability as selection character in *Saccharomyces cerevisiae*. *Industrie delle Bevande* 19:318-319 (In Italian, English summary).

In sparkling wine production yeast autolysis is considered of great significance, leading to improvement of wine flavour and taste. The rate of liberation of soluble nitrogenous constituents and the degree of this process were related to individual strain characteristics. The degree of autolysis of *Saccharomyces cerevisiae* was followed at different storage temperature for ten days. Autolysis increased with increased storage temperature up to 35°-45°. Among the *Saccharomyces cerevisiae* strains different degrees of autolysis were detected at the same storage temperature. The results suggested that autolytic capacity in *Saccharomyces cerevisiae* can be used as a determining character in strain selection.

3. Giudici, P., Romano, P. & Zambonelli, C. 1990. A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 36:31-64.

A hundred strains of *Saccharomyces cerevisiae* were examined for the ability to produce higher alcohols. In the strains tested the production of higher alcohols was found to be an individual strain characteristic and, as such, was statistically significant. The characteristics of the strains used (flocculation ability, foaming ability, killer character and non-H₂S production) were found to be uncorrelated to isobutanol and isoamyl alcohol production, whereas the production of high levels of n-propanol was found to be related to inability to produce H₂S. This, in turn, suggests a link to methionine biosynthesis.

4. Castellari, L.¹, Suzzi, G. and Romano, R. 1990. Effetti della temperatura e del tipo di lievito sulla produzione di composti secondari. *Proc. Congr. "Lieviti e Vino"*, Faenza (Italia) 24 April 1990, 58-61 (In Italian, English summary).

¹Centro regionale lieviti dell'E.S.A.V.E., Via Tebano 45, Faenza.

Fermentation temperature, influencing greatly yeast growth, affects also the product quality. The purpose of this study was to determine the influence of fermentation temperature on the production of secondary products, such as higher alcohols and acetic acid by yeasts. For this, strains of *Saccharomyces cerevisiae* physiological race *cerevisiae*, high or low producers of these compounds, were compared with strains of *Saccharomyces cerevisiae* physiological race *uvarum*, possessing the capacity to grow well at very low temperatures. The fermentation temperature used were: 30, 24, 18, 12 and 6°C.

deleted in the polyubiquitin gene showed only a 2-fold activation of the enzyme while in control strains a 5 to 7 fold activation could be observed. An alternative mechanism of trehalase activation by removal of an inhibitor through ligation with ubiquitin is discussed. Activation by a cAMP independent phosphorylation is also considered.

2. Ferreira, J.C. & Panek, A.D. Regulation and function of the ADPG-dependent trehalose synthase from Saccharomyces cerevisiae. M.Sc. thesis.

As opposed to the UDPG-dependent trehalose-6-phosphate synthase, the ADPG-dependent entity did not respond to a glucose signal nor was its activity altered during a heat shock at 52°C/2 min. Neither was any increase seen during a shift from 23°C to 36°C as occurs with the classical enzyme. A mutant strain of Saccharomyces deficient in the UDPG-dependent enzyme but possessing the ADPG-dependent one, did not accumulate trehalose during growth nor during non-proliferating conditions. A diploid homozygous for the defect was constructed. During sporulation the activity of the synthase showed no alterations. When spores of this ADPG-dependent deficient synthase was subjected to a glucose signal trehalose was accumulated to the same extent as in control spores. These results suggest that the ADPG-dependent trehalose synthase might be involved in the germination process.

3. Coutinho, C.C. & Panek, A.D. Trehalase activity regulation during growth of Saccharomyces cerevisiae. M.Sc. thesis.

At the onset of the transition phase of growth trehalase activity decreases in 90%. The question raised was whether the phenomenon was due to proteolysis or to conversion to a less active form (dephosphorylation). Polyclonal rabbit antibodies towards a partially purified trehalase were obtained and identification of the trehalase protein during growth of cells was made by immunoblotting. Trehalase activity could be detected on nitrocellulose paper. The protein was always present during growth. In order to confirm dephosphorylation as the operating regulatory mechanism a mutant strain deficient in the specific trehalase-phosphatase was analyzed which growth and no loss of activity could be observed during the transition phase. Further proof for regulation by interconversion of forms and not by proteolysis was obtained when stationary cells were subjected to a glucose signal. As expected a transient increase of activity occurred triggered by cAMP dependent protein kinase. However, after 30 min in the activity decreased in 60% but could be recovered "in vitro" by addition of ATP and cAMP. Moreover, the same results were obtained in the presence of cycloheximide which points strongly towards interconversion and not proteolysis followed by synthesis "de novo".

4. Mansure, J.J.C. & Panek, A.D. Trehalase in Rhodotorula rubra. M.Sc. Thesis.

Trehalase activity in Rhodotorula rubra was found to be bound to the particulate fraction of a cell free extract, most probably to the plasma membrane as opposed to the two soluble trehalase activities described in Saccharomyces cerevisiae. The enzyme was strongly repressed by glucose and activated during growth of cells on maltose, trehalose and glycerol. This activation was due to a "de novo" synthesis as seen by inhibition with cycloheximide. Catabolite inactivation by addition of glucose has also been demonstrated. This particulate enzyme does not respond to activation by the cAMP dependent protein kinase, nor does it suffer any alteration during a heat shock at 52°C/2 min. Under these conditions the Saccharomyces cytoplasmic trehalase is activated. Rhodotorula rubra accumulated up to 80 mg trehalose/g of cells (dry wt) during growth on glucose. Breakdown of the disaccharide could be observed after 120 hours of starvation or in the presence of 1 M NaCl. Under these conditions trehalase activity increased suggesting that this enzyme is involved in protecting the cells against environmental stresses.

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

The following article was recently submitted for publication.

1. Spencer, J.F.T., Spencer, D.M., de Figueroa, L.C., Nougues, J.M., & Heluane, H. 1990. Transfer of genes controlling utilization of starch (STA2) and melibiose by protoplast fusion, using a kari mutant as vector.

A gene (STA2) conferring the ability to metabolize starch was transferred from an auxotrophic haploid strain of Saccharomyces cerevisiae (a STA2 arg4) to a kari-1 mutant (K5-5A; a ade2 his4 can1 cyh1) by normal mating and isolation of red colonies which grew on starch as sole source of carbon. The resulting starch-positive strain was protoplasted and the protoplasts were fused or mated with 4 baker's yeast and 2 brewing yeasts which did not utilize starch. White isolates from these fusions and matings were obtained, which metabolized starch and appeared to have the other characteristics of the original yeasts. In addition, the KARI-STA haploid was mated with the baker's yeasts and brewing yeasts, by spore-cell pairing and rare-mating in the case of the baker's yeasts, and by rare-mating with the brewing yeasts, yielding in both cases, strains which metabolized starch. Also, protoplasts from the K5-5A strain were fused with a strain of Saccharomyces kluyveri, and red colonies which metabolized melibiose were isolated. These strains were fused with melibiose-negative strains of baker's yeasts, and white melibiose-positive strains were isolated, which resembled the original baker's yeasts. The red, presumably haploid strain was also mated with known laboratory haploids and by spore-cell pairing with haploids from baker's yeasts, and the gene controlling melibiose utilization transferred in this way. Pulsed-field gel preparations showed little change in the mobility of the chromosomes of the hybrids.

5. Romano, P. & Suzzi, G. 1990. Temperature controlled fermentation of musts effect on the production of fermentation compounds. *Vini d'Italia* 32:7-12 (In Italian; English, French and Spanish summaries).

Higher alcohol and acetic acid production of *Saccharomyces cerevisiae* is significantly influenced by fermentation temperature (30 and 18°C). The results showed that the majority of strains at 18°C tends to produce larger quantities of higher alcohols, especially isoamyl alcohol and d-amyl alcohol. As regards acetic acid no large differences were noted with the two temperatures. The choice of fermentation temperature is important in relation to the results that one intends to achieve.

6. Romano, P. & Suzzi, G. 1990. Sulphur dioxide and yeasts of the musts. *Vini d'Italia* 32: 31-36 (In Italian; English, French and Spanish summaries).

Thirty *Saccharomyces ludwigii* and thirty *Zygosaccharomyces bailii* strains were examined for fermentation intensity, in the absence and in the presence of different amounts of SO₂, in comparison with 30 *Saccharomyces cerevisiae* strains. In the two species tested the "SO₂-resistance" was found to be an individual strain characteristic. Some strains of both species, but particularly those of *Zygosaccharomyces bailii*, showed an elevated SO₂-resistance, sometimes higher than that possessed by the best *Saccharomyces cerevisiae* strains. On the contrary, the fermentation intensity of *Saccharomyces ludwigii* and *Zygosaccharomyces bailii* was found to be, generally, much lower than that of *Saccharomyces cerevisiae*, thus explaining why the above two species are unable to dominate spontaneous fermentations.

XXVI. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.P. van der Walt.

The following paper has appeared recently.

1. van der Walt, J.P., Wingfield, M.J., & Yamada, Y. 1990. *Zygozoma smithiae* sp.n. (Lipomycetaceae), a new ambrosia yeast from Southern Africa. *Antonie van Leeuwenhoek* 58:95-98.

A new species of the genus *Zygozoma*, *Z. smithiae*, was recovered from frass of ambrosia beetle, *Crossotarsus externedentatus* in Northern Natal. A description of the new species and key to the genus are given.

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

The following are abstracts and papers published or accepted during 1990.

1. Carvalho-Silva, M. & Spencer-Martins, I. 1990. Modes of lactose uptake in the yeast species *Kluyveromyces marxianus*. *Antonie van Leeuwenhoek* 57:77-81.

Twelve lactose-assimilating strains of the yeast species *Kluyveromyces marxianus* and its varieties *marxianus*, *lactis* and *bulgaricus* were studied with respect to transport mechanisms for lactose, glucose and galactose, fermentation of these sugars and the occurrence of extracellular lactose hydrolysis. The strains fell into three groups. Group I (two strains): fermentation of lactose, glucose and galactose, extracellular lactose hydrolysis, apparent facilitated diffusion of glucose and galactose; group II (two strains); lactose not fermented, glucose and galactose fermented and transported by an apparent proton symport, extracellular hydrolysis of lactose present (one strain) or questionable; group III (eight strains): lactose, glucose and galactose fermented, lactose transported by an apparent proton symport mechanism, extracellular hydrolysis of lactose and transport modes of glucose and galactose variable.

2. Loureiro-Dias, M.C. and Santos, H. 1990. Effects of ethanol on *Saccharomyces cerevisiae* as monitored by in vivo ³¹P and ¹³C nuclear magnetic resonance. *Arch. Microbiol.* 153:384-391.

Cell suspensions of a respiratory deficient mutant of *Saccharomyces cerevisiae* were monitored by in vivo ³¹P and ¹³C Nuclear Magnetic Resonance in order to evaluate the effect of ethanol in intracellular pH and metabolism. In the absence of an added energy source, ethanol caused acidification of the cytoplasm, as indicated by the shift to higher field of the resonance assigned to the cytoplasmic orthophosphate. Under the experimental conditions used this acidification was not a consequence of an increase in the passive influx of H⁺. With cells energized with glucose, a lower value for the cytoplasmic pH was also observed, when ethanol was added. Furthermore, lower levels of phosphomonesters were detected in the presence of ethanol, indicating that an early event in glycolysis is an important target of the ethanol action. Acetic acid was identified as responsible for the acidification of the cytoplasm, in experiments where (¹³C) ethanol was added and formation of labeled acetic acid was detected. The intracellular and the extracellular concentrations of acetic acid were respectively, 30 mM and 2 mM when 0.5% (120 mM) (¹³C) ethanol was added.

3. Lucas, C., da Costa, M., & van Uden, N. 1990. Osmoregulatory active sodium-glycerol co-transport in the halotolerant yeast Debaryomyces hansenii. Yeast 6:187-191.

Several authors have shown that the halotolerant yeast Debaryomyces hansenii, when growing exponentially in glucose medium in the presence of sodium chloride, maintains osmotic balance by establishing sodium and glycerol gradients of opposite signs across the plasma membrane. Evidence is presented here that the two gradients are linked through a sodium-glycerol symport that uses the sodium gradient as a driving force for maintaining the glycerol gradient. The symporter also accepts potassium ions as co-substrate. The kinetic parameters at 25°C, pH 5.0 were the following: V_{max} decreasing from over 500 to less than 40 $230 \mu\text{mol g}^{-1}$ per h over a concentration range of 3-0 M extracellular sodium chloride; K_m (glycerol) 0.4-0.6 mM over the same range; K_m (sodium ions) $16.0 \pm 3.2 \mu\text{M}$; K_m (potassium ions) $10.4 \pm 3.6 \mu\text{M}$. Furthermore, it was observed that glycerol uptake was accompanied by proton uptake when extracellular sodium chloride was present and that the protonophore carbonylcyanide-M-chlorophenylhydrazone induced collapse of the glycerol gradient, supporting earlier proposals by others that the sodium gradient is maintained by an active sodium-proton exchange mechanism.

4. Entian, K.D. and Loureiro-Dias, M.C. 1990. Misregulation of maltose uptake in a glucose repression defective mutant of Saccharomyces cerevisiae leads to glucose poisoning. J. Gen. Microbiol. 136:855-860.

In hex2 mutants of Saccharomyces cerevisiae, which are defective in glucose repression of several enzymes, growth is inhibited if maltose is present in the medium. After adding (^{14}C) maltose to cultures growing with ethanol, maltose metabolism was followed in both hex2 mutant and wild-type cells. The amount of radioactivity incorporated was much higher in hex2 than in wild-type cells. Most of the radioactivity in hex2 cells was located in the low molecular mass fraction. Pulse-chase experiments showed that 2 h after addition of maltose, hex2 cells hydrolysed maltose to glucose, which was partially excreted into the medium. ^{31}P -NMR studies gave evidence that turnover of sugar phosphates was completely abolished in hex2 cells after 2 h incubation with maltose. ^{13}C -NMR spectra confirmed these results: unlike those for the wild-type, no resonances corresponding to fermentation products (ethanol, glycerol) were found for hex2 cells, whereas there were resonances corresponding to glucose. Although maltose is taken up by proton symport, the internal pH in the hex2 mutant did not change markedly during the 5 h after adding maltose. The intracellular accumulation of glucose seems to explain the inhibition of growth by maltose, probably by means of osmotic damage and/or unspecific O-glycosylation of proteins. Neither maltose permease nor maltose was over-expressed, and so these enzymes were not the cause of glucose accumulation. Hence, the coordination of maltose uptake, hydrolysis to glucose and glycolysis of glucose is not regulated simply by the specific activity of the catabolic enzymes involved. The results indicate that there is an unknown regulatory mechanism, under control of HEX2, which coordinates glycolytic flux and maltose uptake. Furthermore, the excretion of accumulated glucose into the medium gives clear evidence that at least one glucose carrier in S. cerevisiae acts passively and transports glucose in both directions.

5. Madeira-Lopes, A. & Cabeça-Silva, C. 1990. Conditioning by mitochondria and energy source of thermal death parameters in yeast. J. Basic Microbiol. 30:267-271.

Two strains of Saccharomyces cerevisiae of opposite mating type (α and α), with different resistance to thermal death, and their respective mitochondrial respiratory-deficient mutants (petites α_0 and α_0) were used to prepare the following diploid strains: $\alpha\alpha$, $\alpha\alpha_0$ and $\alpha_0\alpha$. Specific thermal death rates were determined at supramaximum temperatures for growth, under conditions of mitochondrial derepression (glycerol medium). Comparison of the entropies of activation of thermal death showed that diploids $\alpha\alpha$ and $\alpha_0\alpha$ behaved like the more resistant haploid α , and diploid $\alpha\alpha_0$ like haploid α_0 . In glucose medium strains α and $\alpha\alpha$ became even more thermo-resistant. The results favour the concept that thermal death determinants are located in the mitochondrial genome, and that mitochondria repressed cells repair thermal injuries more efficiently.

6. Ramos, M.T. and Madeira-Lopes, A. 1990. Effects of acetic acid on the temperature profile of ethanol tolerance in Saccharomyces cerevisiae. Biotechnol. Lett. 12:229-234.

In a strain of Saccharomyces cerevisiae, acetic acid at concentrations up to 1% (v/v) depressed the tolerance to added ethanol, from 1% (v/v) down to zero, and simultaneously narrowed the temperature range of growth from 3-42°C to 19-26°C. In addition, acetic acid shifted the associative temperature profile of growth and death to lower temperatures, and depressed the growth yield on glucose.

7. Giménez-Jurado, G., Placido, T., Cidado, A.J., Cabeça-Silva, C., Fonseca, E., Roeljmans, H.J., van Eijk, G.W., & van Uden, N. 1990. Kurtzmanomyces tardus sp. nov., a new anamorphic yeast species of basidiomycetous affinity. Antonie van Leeuwenhoek 58:129-135.

A new yeast species of basidiomycetous affinity Kurtzmanomyces tardus was isolated from contaminated demineralized water. It differs from K. nectairii, the only other Kurtzmanomyces species so far described, in its carbon assimilation pattern and low DNA-DNA homology (2.3%±2.1).

8. Henriques, M., Sá-Nogueira, I., Giménez-Jurado, G., & van Uden, N. 1991. Ribosomal DNA spacer probes for yeast identification: Studies in the genus Metschnikowia. Yeast 7: (in press).

To test whether DNA probes derived from ribosomal DNA spacer sequences are suitable for rapid and species-specific yeast identification, a pilot study was undertaken. A 7.7 kb entire ribosomal DNA unit of the type strain of Metschnikowia reukaufii was isolated, cloned and mapped. A 0.65 kb BamHI-HpaI fragment containing nontranscribed spacer sequences was amplified and selected for testing as a ³²P hybridization probe with total DNA from the type strains of M. reukaufii, M. pulcherrima, M. lunata, M. bicuspidata, M. australis, M. zobellii, M. krissii, five other strains identified as M. reukaufii and strains of Schizosaccharomyces pombe, Hansenula canadensis, Saccharomyces cerevisiae and Yarrowia lipolytica. The probe hybridized exclusively with DNA from the type strain and four other strains of M. reukaufii. DNA from one strain labelled M. reukaufii did not hybridize with the probe. Subsequent % G-C comparison and DNA-DNA reassociation with the type strain revealed that the non-hybridizing strain does not belong to the species M. reukaufii.

9. Rodrigues de Sousa, H., Spencer-Martins, I., & van Uden, N. In press. Active fructose transport in Saccharomyces sensu stricto. Taxonomic implications. Acta Academica (Bloemfontein).

The four species which are currently recognized based on DNA-DNA homology within the Saccharomyces sensu stricto group, and cannot be distinguished by the use of conventional phenetic yeast identification criteria, were tested with respect to their capacity for active transport of fructose with a proton symport mechanism, with the following results (in brackets the number of strains tested): Saccharomyces cerevisiae (6), negative; Sacch. pastorianus (5), positive; Sacch. bayanus (6), positive; Sacch. paradoxus (4), negative. A strain, supposedly the type of Sacch. willianus, a species earlier identified by others with Sacch. bayanus, showed a negative result and it was found by DNA reassociation experiments not to be conspecific with the latter species. The taxonomic implications of the observed correlations are discussed.

10. Sampaio, J.P. & van Uden, N. 1991. Rhodotorula ferulica sp. nov., a yeast that degrades ferulic acid and other phenolic compounds. Syst. Appl. Microbiol. (in press).

A novel species of the basidiomycetous anamorphic genus Rhodotorula is described as Rhodotorula ferulica based on a study of three isolates from polluted river water. The yeast is able to use as single carbon source a number of phenolic compounds: Vanillyl alcohol, vanillic acid, veratryl alcohol, veratric acid, ferulic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and caffeic acid. Con-specificity with the phenotypically similar species. Rh. diffluens was excluded based on differences in G+C content and absence of DNA-DNA homology.

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

The following articles have been published recently.

1. Sipiczki, M., Grossenbacher-Gründer, A.M., & Bodi, Z. 1990. Recombination and mating-type switching in a ligase-defective mutant in Schizosaccharomyces pombe. Mol. Gen. Genet. 220:307-313.

The ligase-defective cdc17-L16 mutant of Schizosaccharomyces pombe var. pombe was tested for genetic recombination and mating-type switching. Mitotic recombination was studied in both haploid and heteroallelic diploid cells. Cells carrying a heteroallelic ade6 duplication constructed by Schuchert and Kohli were tested for ectopic genetic recombination. We have found that cdc17-L16 is a mitotic hyper-rec mutant, as it increases the instability of the duplication by a factor of about 6 even at the permissive temperature of 23°C. In diploid cells, the enhancement of recombination rates detected was to that of cdc17+ cells. The temperature-sensitive cell cycle defect is also associated with reduced level of mating and sporulation but does not significantly affect mating-type switching and intragenic meiotic recombination. It is supposed that the mitotic hyper-rec phenotype is a secondary result of insufficient repair of DNA breaks, while the lack of influence of the reduced ligase activity on the latter two processes might be attributed to their peculiar initiation mechanisms.

2. Benko, Z. & Sipiczki, M. 1990. Caffeine resistance in Schizosaccharomyces pombe: a pleiotropic mutation affecting UV-sensitivity, fertility, and cell cycle. Curr. Genet. 18:47-52.

A stable mutant having increased resistance to growth inhibition by caffeine was obtained from Schizosaccharomyces pombe after UV-irradiation of vegetative cells. The caf1-21 mutation confers increase UV-sensitivity, impaired fertility and sporulation, lengthened cell cycle and a slightly abnormal cell morphology, but it does not affect ectopic recombination in vegetative cells. In mutation is partially dominant and defines a gene mapping on Chromosome II. Various possible mechanisms of resistance are discussed.

3. Grallert, B. & Sipiczki, M. 1989. Initiation of the second meiotic division in *S. pombe* shares common functions with that of mitosis. *Curr. Genet.* 15:231-233.

The cell-cycle start gene, *cdc2*⁺, and its activator, *cdc25*⁺, are required for the initiation of the second meiotic nuclear division. They exert their functions downstream of *mes1*, a gene presumed to act between meiosis I and meiosis II. *wee1*⁺, the inhibitor of *cdc2*⁺ in mitotic control, does not appear to play any role in this process.

4. Grallert, B. & Sipiczki, M. 1990. Dissociation of meiotic and mitotic roles of the fission yeast *cdc2* gene. *Mol. Gen. Genet.* 222: 473-475.

The fission yeast *cdc2* gene is pleiotropic, functioning both in the cell division cycle and in meiosis. Here we show that *cdc2* is allelic to *twsl*, a previously isolated meiotic gene. Dissociation of meiotic and mitotic roles of the gene is also demonstrated by finding mutant alleles specifically altered in only one of the two processes.

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

The following abstracts were presented at recent symposia.

1. Aho, S., Jalava, T., Paloheimo, M., Oikkonen, V.,¹ Bamford, D.,¹ & Korhola, M. 1990. Characterization of monoclonal antibodies against *Trichoderma reesei* cellulases. 4th Nordic Symposium on Gene Technology in Basic and Applied Research & including a NYRP Workshop on Signal Transduction in Yeast and Other Systems. Storelin, Sweden, 1990, p. 77
¹Department of Genetics, University of Helsinki, Finland.

We have earlier found that polyclonal antibodies raised against different *Trichoderma reesei* cellulases are mainly directed against the conserved A-region on each protein and strongly cross-react with other cellulases. For the specific detection and quantitation of *Trichoderma reesei* cellulases, monoclonal antibodies were raised against native CBH I, CBH II and EG I. Five antibodies were obtained against CBH I, four against CBH II and eight against EG I. Each antibody (except one) was Western-blot positive and specific for the protein against which it had been raised. Specific fragments from cellulases were generated by digesting CBH I and CBH II with papain. As a second approach, series of 3'-end deletions were prepared from cDNAs for CBH I, CBH II and EG I. The deleted cDNAs were expressed in yeast under the control of ADC1 promoter and terminator sequences. The monoclonal antibodies were screened against the truncated proteins. From the monoclonal antibodies raised against CBH I one was directed against the last 13 C-terminal amino acids and three were against the C-terminal end of the core region. Monoclonal antibodies against CBH II clearly fell into two categories. Two of them needed the intact C-terminus, while two detected also truncated proteins. Two monoclonal antibodies were obtained against the C-terminal tail of the EG I and six antibodies were against the regions in the middle of the protein. In conclusion, a specific monoclonal antibody was obtained against each cellulase enzyme. They will be used in qualitative and quantitative assays for each cellulase enzyme when new cellulase production strains are made from industrial purposes.

2. Suominen, P.L., Bühler, R., & Liljeström, P. 1989. Construction of expression vectors based on the yeast *Mel1* genes; expression of a fungal cellulase in yeast. Poster presented at the International Symposium on Recombinant Systems in Protein Expression, Imatra, Finland 1989. In: Alitalo, K.K., Huhtala, M.L., Knowles, J., & Vaheri, A. (eds.) *Recombinant Systems in Protein Expression*. Amsterdam: Elsevier Science Publishers B.V., p. 49.

We have constructed a set of plasmid expression vectors which allow tightly regulated synthesis and secretion of heterologous gene products in yeast. The vectors make use of sequences from the *MEL1* gene of *Saccharomyces cerevisiae*. Transcription of *MEL1* is lightly induced by galactose and tightly repressed by glucose (1,2,3), and therefore *MEL1* 5' flanking sequences are well suited for controlled production of foreign gene products. Two sets of vectors have been constructed. The first set includes plasmid promoter vectors, which carry the *MEL1* transcription promoter and terminator. The other set includes, in addition, the signal sequence of the *MEL1* followed by a linker sequence. Genes fused in frame with this construction will code for proteins that are potentially secreted out of the yeast cell. In addition the vectors carry the yeast *LEU2* gene as a selectable marker which enables efficient selection for directed integration of the plasmid into the chromosome of the host cell. Moreover, both sets of vectors include variants carrying the 2p origin of replication which confers autonomous replication in yeast. To test the function of the vectors, cDNA of the endoglucanase I gene of *Trichoderma reesei* (4) was cloned onto the various vectors. It was shown that its expression could be controlled from *MEL1* and that secretion of the endoglucanase I (EG1) protein could be obtained using either its own signal sequence or the signal sequence of *MEL1*.

The following patent has been granted.

3. Knowles, J., Penttila, M., Teeri, T., Nevalainen, H., Salovuori, I., & Lehtovaara-Helenus, P. United States Patent 4,894,338, Jan. 16, 1990.

The full length DNA sequence encoding the mature cellobiohydrolase II (CHBII), as well as the DNA sequence encoding the CBHII signal sequence, have been isolated and cloned from the fungus *Trichoderma reesei*. Recombinant vectors comprising these sequences, and yeast hosts transformed with such vectors are disclosed. Also disclosed are methods for constructing the vectors and hosts according to the invention, as well as methods for expressing the disclosed sequences. CBHII is involved in hydrolysis of cellulose.

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

Using genetic hybridization analysis we identified seven polymorphic genes for the fermentation of melibiose in different Mel⁺ strains of *Saccharomyces cerevisiae*. Four laboratory strains (1453-3A, 303-49, M2, C.B.11) contained only the MEL1 gene and a wild strain (VKMY-1830) had only the MEL2 gene. Another wild strain (CBS 4411) contained five genes: MEL3, MEL4, MEL5, MEL6 and MEL7. MEL3-MEL7 were isolated and identified by backcrosses with mel parents (X2180-1A, S288C). A cloned MEL1 gene was used as a probe to investigate the physical structure and chromosomal location of the MEL gene family and to check the segregation of MEL genes from CBS 4411 in six complete tetrads. Restriction and Southern hybridization analyses showed that all seven genes are physically very similar. By electrokaryotyping we found that all seven genes are located on different chromosomes: MEL1 on chromosome II as shown previously by Vollrath et al. (1988). MEL2 on VII, MEL3 on XVI, MEL4 on XI, MEL5 and IV, MEL6 on XIII, and MEL7 on VI. Molecular analysis of the segregation of MEL genes from strain CBS 4411 gave results identical to those from the genetic analyses. The homology in the physical structure of this MEL gene family suggests that the MEL loci have evolved by transposition of an ancestral gene to specific locations within the genome.

XXX. Department of Genetics and Microbiology and Institute of Biotechnology,
Charles University, Vinicna 5, Prague 2, CS 128 44 Czechoslovakia.
Communicated by O. Bendová.

The following paper has been submitted for publication:

1. Cvrckova, F. Construction of a hybrid distiller's yeast using the chlorsulfuron selection system. Submitted to *Appl. Microbiol. Biotechnol.*

A new selection method based on the use of the chlorsulfuron (CS) resistance as the selection marker for protoplast fusion in industrial yeasts has been introduced. The fusion was performed between a petite mutant of a spontaneously CS-resistant distiller's *Saccharomyces cerevisiae* strain and a wild-type CS-sensitive strain of the osmotolerant yeasts *Zygosaccharomyces mellis*. Fusion products were isolated as large colonies on minimal glycerol agar supplied with the herbicide Glean (75% CS) at the concentration 0.5 mg ml⁻¹. Following prolonged cultivation in molasses stable hybrid subclones were obtained.

The following thesis has been submitted.

2. Puta, F. The construction of plasmid vectors for cloning of yeast genes with the selection system allowing direct selection of clones transformed by recombinant plasmids.

1. Several *Escherichia coli* - *Saccharomyces cerevisiae* shuttle plasmid cloning vectors have been constructed, which are of relatively small size and the recombinant derivatives of which carrying on insert can be directly selected. The selection system used in our construction is based on the regulatory region of lambda phage controlling the expression of tetracycline resistance, which has been derived from pUN121. An additional unique restriction site, namely *Bgl*II, has been created in repressor gene *cI* by oligonucleotide directed mutagenesis. The relatively small size of the vectors (7.8 kb) has been achieved by using URA3 yeast marker. Four unique cloning sites, *Eco*RI, *Hind*III, *Bcl*I and *Bgl*II are available in *cI* gene and two other unique sites, *Bam*HI and *Sal*I, are present in the neighboring region (pPW262, pPW272, pPW263). 2. The *Bgl*II site has been incorporated also into *cI* gene of the plasmid pUN121 (pPW121). 3. Another positive selection system based on the gene for chloramphenicolacetyl-transferase under the control of lambda regulatory region has been invented and used with positive results in the construction of the vector pMLP8. 4. A series of plasmids pMLP with several cloning sites situated in polylinkers has been prepared (pMLP1-pMLP7).

XXXI. Department of Biology, Georgia State University, Atlanta, Georgia
30303, U.S.A. Communicated by S.A. Meyer.

Chyong-Shwu Su completed her graduate studies and received her Ph.D. degree on August 26, 1990. An abstract of her dissertation follows. A portion of this work will be published in the January 1991 issue of the International Journal of Systematic Bacteriology. Dr. Su accepted a post-doctoral position in the laboratory of Dr. George Kobayashi, Washington University School of Medicine, Department of Medicine and Infectious Disease, St. Louis, Mo.

Species identification and delimitation of physiologically similar yeasts are often difficult. It is well known that some species cannot be separated on the basis of a single or even a few physiological differences. Nuclear DNA reassociation, mitochondrial DNA restriction fragment length polymorphisms, cellular protein patterns and multilocus enzyme mobility typing can reconcile strain identity and distinction. The small size of mitochondrial DNA makes it suitable for restriction enzyme analysis and it has been shown that the differences of mtDNA restriction patterns in inter- and intraspecies are due to differences in nucleotide sequences. A relatively rapid and efficient method for the extraction of mitochondrial DNA from yeast was developed. Zymolyase was used to induce yeast protoplasts and mitochondrial DNA was extracted from DNase I-treated mitochondrial preparations. The results of this study showed significant differences among *Candida parapsilosis*, its once proposed teleomorph, *Lodderomyces elongisporus*, and the former variety, *C. parapsilosis* var. *quercii* (*C. quercitrusa*), as well as, between *C. shehatae* and *Pichia stipitis*. On the other hand, nearly identical results were observed between the sucrose-positive and sucrose-negative strains of *C. tropicalis* and between *C. albicans* and a sucrose-negative strain. *C. lusitanae* and its teleomorph, *Clavispora lusitanae*, showed slightly more variability in certain enzyme mobilities. Polymorphisms of mitochondrial DNA among different strains of *C. maltosa* and strains of *C. tropicalis* did exist and enzyme mobility patterns showed some differences. The results of mitochondrial DNA restriction fragment length polymorphisms and multilocus enzyme typing were in agreement with nuclear DNA relatedness and showed where variation existed among strains. These genetic and biochemical methods demonstrated their usefulness in distinguishing species and denoting the degree of variation within a species.

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

The following thesis has been submitted recently.

1. Rouwenhorst, R.J. 1990. Production and localization of inulinases in *Kluyveromyces* yeasts. Ph.D. Thesis, Delft University of Technology.

In yeasts, extracellular glycoproteins can be found in the cell wall and in the culture liquid. The distribution of glycoprotein over these locations depends on the yeast species, or even yeast strain, and on the culture conditions. In this thesis, an effort was made to determine the factors that influence the production and localization of the glycoprotein inulinase (EC 3.2.1.7) in *Kluyveromyces* species. As inulinase strongly resembles the well-studied invertase (EC 3.2.1.26) of *S. cerevisiae*, both the nature of the two enzymes and their localization are compared. For establishing the distribution of the extracellular enzymes, enzyme activities were determined in three fractions: 1. enzyme present in the culture liquid (supernatant enzyme); 2. enzyme that can be released from the cell wall by chemical treatment (cell wall enzyme); 3. enzyme that can only be solubilized by breakage of the cells (cell-bound enzyme). This distinction of enzyme activities into three fractions allowed a survey of the factors that influence protein localization.

Production and localization of inulinase were studied in carbon- and energy-limited chemostat cultures of *K. marxianus* var. *marxianus* CBS 6556 (Chapter 2). Highest enzyme yields were encountered in cultures grown on a mineral medium with either sucrose or inulin as the limiting substrate. In sucrose-limited continuous cultures, the amount of inulinase decreased from 52 U (mg cell dry weight)⁻¹ at D=0.1 h⁻¹ to 2 U (mg cell dry weight)⁻¹ at D=0.8 h⁻¹. Since at increasing dilution rates, the residual substrate concentration in the culture gradually increases, the decrease in inulinase level can be caused by either the growth rate or the residual sugar concentration. Experiments with nitrogen-limited cultures confirmed that the synthesis of inulinase is negatively controlled by the residual substrate concentration. The presence of high inulinase activities during growth of *K. marxianus* CBS 6556 on non-sugar carbon sources, further indicated that the synthesis of inulinase is a result of a derepression/repression mechanism. The distribution of inulinase over supernatant, cell wall, and cell-bound fractions depended on carbon source, dilution rate, and growth temperature. During growth under derepressed conditions, about 50% of the enzyme was present as supernatant inulinase and about 30% as cell wall inulinase. When inulinase synthesis was repressed, less inulinase was present as cell-bound enzyme, and more was released into the culture liquid when inulinase-repressive conditions became more severe. The growth temperature negatively influenced inulinase production when it deviated from the optimal temperature of growth of *K. marxianus* CBS 6556 (37-42°C). At lower temperatures, the decrease in inulinase yield was reflected in a decrease in inulinase released into the culture liquid.

Independent of its localization, the inulinase of *K. marxianus* CBS 6556 had an S/I ratio (ratio between relative activities which sucrose and inulin) of 15±3. The apparent affinity constants of inulinase for the 8-fructosides raffinose (trisaccharide) and stachyose (tetrasaccharide) were lower than for sucrose (disaccharide). However, the enzyme specificity decreased with increasing chain length of the substrate; just as for the invertase of *S. cerevisiae*, sucrose is a better substrate than raffinose, stachyose, or inulin (Chapter 2). In Chapter 3 the localization of invertase was studied in oscillating and steady-state chemostat cultures of *S. cerevisiae* CBS 8066, and compared to the inulinase localization in steady-state chemostat cultures of *K. marxianus* CBS 6556. Oscillations in the rates of carbon dioxide production and oxygen consumption reflect a synchronized budding of part of the culture which is initiated by cyclic production and consumption of

ethanol. The latter is related to the specific metabolic regulation of *S. cerevisiae* as a Crabtree-positive yeast. The production of invertase was restricted to the budding phase; invertase synthesis terminated when cell separation took place. The newly produced invertase remained in the cell wall, and did not provoke a more pronounced release of invertase in the culture liquid. Indeed, the supernatant invertase remained constant during the budding cycle. It is hypothesized that the release of invertase from the cell wall into the culture liquid is only affected by the shear force. In a synchronized budding continuous culture, due to higher concentrations of residual glucose or higher intracellular concentrations of glucose metabolites, the invertase production was two to three times lower than in a steady-state continuous culture. However, even in steady-state cultures of *S. cerevisiae*, variations in invertase production and distribution were observed and these are probably caused by the synchronized division of a small part of the cell population. No significant deviations in inulinase production and localization occurred in steady-state continuous cultures of the Crabtree-negative yeast *K. marxianus* CBS 6556.

In Chapter 4, factors that might influence the localization of inulinase in *K. marxianus* CBS 6556 were investigated via a biochemical approach. The supernatant inulinase and the cell wall inulinase were purified and analyzed by denaturing and non-denaturing polyacrylamide gel electrophoresis. Both inulinases had similar subunits, consisting of a 62 kDa polypeptide with varying amounts of carbohydrate (26-37% of the molecular mass). The inulinases differed in size, due to differences in subunit aggregation. The supernatant inulinase was a dimer and the cell wall inulinase a tetramer. The cell-bound inulinase also appeared to be tetrameric. These findings support the hypothesis that retention of glycoproteins in the yeast cell wall may be caused by a permeability barrier. The differences in oligomerization did not affect the apparent affinity constants of these inulinases. However, the specific activity of the dimeric inulinase was higher than that of the tetrameric form, indicating that there is negative co-operatively. No homology was found between the amino acid compositions of the amino terminal ends of *K. marxianus* CBS 6556 inulinase and *S. cerevisiae* invertase. The kinetic and structural evidence indicates that that in yeasts two distinct β -fructosidases exist, viz. invertase and inulinase (Chapter 4).

During the purification of the two *K. marxianus* CBS 6556 inulinases, problems arose concerning the assessment of protein concentrations. The presence of carbohydrate groups in inulinase distributed the linearity of the classic chromogenic methods of protein determination. A new sensitive and reliable method for the determination of protein concentrations by its carbon content is described in Chapter 5. Using a carbon content of 0.53 g/g in protein and of 0.44 g/g in carbohydrate, the concentrations of normal proteins, proteins containing chromophoric groups and proteins containing carbohydrate could be established. Determination of specific absorption coefficients by measuring the absorbance of protein solutions at 280 nm and measuring their carbon concentrations gave values that agreed with values reported in the literature. The method may be special applicability in protein purification studies since it does not require prior knowledge of molar extinction coefficients and since it monitors the disappearance of carbon compounds other than proteins.

In Chapter 6 it is reported that the capacity of utilization of inulin is much more widespread among yeast species than is known from yeast taxonomy. The formerly unrecognized ability of *K. marxianus* var. *drosophilorum*, *K. marxianus* var. *vanudenii*, and *S. kluyveri* to grow on inulin, is mainly due to the fact that in taxonomy assimilation tests are performed under special, standardized cultivation conditions. Alteration of culture conditions, like lowering the culture pH and increasing the growth temperature, resulted in rapid growth on inulin of these yeasts. It is hypothesized that altered culture conditions such as these, lead to removal of a permeability barrier in the outer region of the cell wall. This would either enable inulin to diffuse into the cell wall or inulinase to diffuse out of the cell wall. Measurement of inulin-dependent oxygen consumption by cell suspensions of *K. marxianus* revealed that both inulin concentration and inulinase localization determined the rate of inulin hydrolysis (Chapter 6). The physiological, ecological, and taxonomical aspects of the occurrence and localization of inulinase in *Kluyveromyces* species are discussed in this chapter.

A 90-year old preparation of lactase (β -galactosidase; EC. 3.2.1.23) from *Candida kefir*, stored under suboptimal conditions, still exhibited measurable enzyme activities (Chapter 7). The lactase was prepared by Beijerinck who, 10 years earlier, had reported his discovery of lactose-hydrolyzing enzymes in yeasts. A closer look into the experimental methods used by Beijerinck and the older literature indicated that Beijerinck was unlikely to be the discoverer of lactase. Apart from lactose-hydrolyzing activity, the preparation contained sucrose- and inulin-hydrolyzing activity in a S/I ratio of 16, indicating that an inulinase was present too.

The following papers have appeared recently.

2. Steensma, H. Yde, Holterman, L., Dekker, I., van Sluis, C.A., & Wenzel, T. J. 1990. Molecular cloning of the gene for the E1 α subunit of the pyruvate dehydrogenase complex from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 191:769-774.

The E1 α and E1 β subunits of the pyruvate dehydrogenase complex from the yeast *Saccharomyces cerevisiae* were purified. Antibodies raised against these subunits were used to clone the corresponding genes from a genomic yeast DNA library in the expression vector gt11. The gene encoding the E1 α subunit was unique and localized on a 1.7-kb HindIII fragment from chromosome V. The identity of the gene was confirmed in two ways. (a) Expression of the gene in *Escherichia coli* produced a protein that reacted with the anti-E1 α serum. (b) Gene replacement at the 1.7-kb HindIII fragment abolished both pyruvate dehydrogenase activity and the production of proteins reacting with anti-E1 α serum in haploid cells. In addition, the 1.7-kb HindIII fragment hybridized to a set of oligonucleotides derived from amino acid sequences from the N-terminal and central regions of the human E1 α peptide. We propose to call the gene encoding the E1 α subunit of the yeast pyruvate dehydrogenase complex PDA1. Screening of the gt11 library using the anti-E1 β serum resulted in the re-isolation of the RAP1 gene, which as located on chromosome XIV.

3. Postma, E. & van den Broek, P.J.A. 1990. Continuous-culture study of the regulation of glucose and fructose transport in Kluyveromyces marxianus CBS 6556. J. Bacteriol. 172:2871-2876.

Regulation of transport of D-glucose and D-fructose was studied in Kluyveromyces marxianus grown in continuous culture. Both substrates could be transported by at least two different transport systems, low-affinity transport and high-affinity proton-sugar symport. The low-affinity transporter, specific for both glucose and fructose, was constitutively present and was apparently not regulated by carbon catabolite repression. Regulation of the activity of the glucose- and fructose-specific proton symport systems appeared to proceed mainly through catabolite repression. Activation of symport did not need the presence of specific inductor molecules in the medium. Nevertheless, the capacities of the proton-sugar symporters varied in cells grown on a wide variety of carbon sources. The possibility that the control of proton symport activity is related to the presence of specific intracellular metabolites is discussed.

XXXIII. Institut für Mikrobiologie und Weinforschung, Johannes Gutenberg - Universität Mainz, Germany. Communicated by F. Radler.

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

1. Schmitt, M., Brendel, M., Schwarz, R. & Radler, F. 1989. Inhibition of DNA synthesis of Saccharomyces cerevisiae by yeast killer toxin KT28. J. Gen. Microbiol. 13:1529-1535.

Treatment of sensitive cells of Saccharomyces cerevisiae with killer toxin KT28 affected cell viability after 2 h; the effect was dependent upon the availability of a utilizable energy source. Treatment led to an interruption of cell growth. The mother cells contained nuclear DNA, whereas their daughter buds did not. Using a killer-toxin-sensitive thymidine auxotroph of S. cerevisiae carrying a temperature-sensitive thymidylate uptake mutation, it was shown that the incorporation of dTMP at the permissive temperature was inhibited within 30 min of the addition of KT28. When cells labelled at the permissive temperature were incubated at the restrictive temperature, the level of radioactivity declined in the absence but not in the presence of KT28. No other effects of KT28 were observed within 2 h of its addition, and it is concluded that the inhibition of DNA synthesis is an early effect of the action of KT28.

2. Radler, F., Schmitt, M.J., & Meyer, B. 1990. Killer toxin of Hanseniaspora uvarum. Arch. Microbiol. 154:175-178.

The yeast Hanseniaspora uvarum liberates a killer toxin lethal to sensitive strains of the species Saccharomyces cerevisiae. Secretion of this killer toxin was inhibited by tunicamycin, an inhibitor of N-glycosylation, although the mature killer protein did not show any detectable carbohydrate structures. Culture supernatants of the killer strain were concentrated by ultrafiltration and the extracellular killer toxin was precipitated with ethanol and purified by ion exchange chromatography. SDS-PAGE of the electrophoretically homogenous killer protein indicated an apparent molecular mass of 18,000. Additional investigations of the primary toxin binding sites within the cell wall of sensitive yeast strains showed that the killer toxin to Hanseniaspora uvarum is bound by 8-1, 6-D-glucans.

3. Schmitt, M.J. & Radler, F. 1990. Blockage of cell wall receptors for yeast killer toxin KT28 with antimannoprotein antibodies. Antimicrobial Agents and Chemotherapy 34:1615-1618.

Binding of yeast killer toxin KT28 to its primary cell wall receptor was specifically blocked with polyclonal antimannoprotein antibodies which masked all toxin-binding sites on the surface of sensitive yeast cells. By indirect immunofluorescence, it was shown that KT28 binds to the cell wall mannoprotein and that the toxin resistance of mannoprotein mutants (mnn) of Saccharomyces cerevisiae was due to a lack of killer toxin-binding sites within the yeast cell wall. Structural analysis of acetylated mannoproteins from KT28-resistant mutant strains identified the outer mannanose side chains as the killer toxin-binding domains.

XXXIV. Microbiologie physiologique et appliquée - Levures, Université Lyon I, Bât. 405 - 43 Bd du 11 novembre 1918, F-69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

The following are abstracts of recently published or accepted papers.

1. Montrocher, R., Billon-Grand, G. & Claisse, M. 1990. Analysis of cytochromes and co-enzymes Q in the yeast genus Candida. J. Gen. Appl. Microbiol. 36:151-161.

The cytochrome absorption spectra of whole cell pastes and the co-enzyme Q system were analyzed in eighteen species in the yeast genera Candida and Pichia. C. silvanorum, C. entomophila and C. homilentoma had Co-Q 8 as the major component and Co-Q 6, 7 and 9 as minor components. The other yeasts had Co-Q 9 as the major component with Co-Q 8 as the first minor component associated with an additional Co-Q 7 in five species (C. shehatae, C. naeodendra, C. butyri, C. tenuis and C. fluvioitilis) or Co-Q 10 in three species (C. diddensii, C. polymorpha and C. atmosphaerica). Pichia stipitis may no longer be considered as the perfect form of C. shehatae. These results confirmed that Co-Q system composition is a valuable tool for the chemotaxonomy of yeasts when used in conjunction with spectrophotometric analysis of cytochromes and other techniques such as GC content, DNA/DNA hybridization or serology.

2. Fiol, J.B. & Claisse, M.L. In press. Spectrophotometric analysis of yeast cytochrome spectra of the genus Yamadazyma.

Whole cell pastes of Yamadazyma were spectrophotometrically analyzed (16 species). The low temperature absorption cytochrome spectra showed various responses. The cytochrome oxidase peak ($\alpha+\alpha_2$) was rarely typical and symmetric (only 6 species) and showed an unsymmetrical peak with two noticeable peculiarities: - a major absorption range from 597 to 604.5 nm with a shoulder at lower wavelength 590 to 596 nm (6 species) or a major absorption range from 594.5 to 599 nm with a shoulder at upper wavelength (598-603 nm), (4 species). The cytochrome spectra of 14 species showed α_1 and α_2 bands. These characters associated with the vitaminic requirements and GC contents led to identification of three main groups.

XXXV. Department of Food Science and Technology, University of California, Davis, CA 95616, U.S.A. Communicated by M. Miranda.

1. Miranda, M., Ask, S., Guinard, J.X., & Lewis, M. 1989. Analysis and evaluation of commercial brewer's Yeast. *Zymurgy* (special) 49-54.

The most important characters of a dry culture yeast for brewing are: (1) fidelity to type of the strain or strains included, (2) the microbiological purity and viability/vigor of the yeast, and (3) the flavor of the beer produced. We conclude from our study that there is ample room for improvement of dried yeasts in all three categories of evaluation. For the small-scale brewer acquiring good yeast remains a most difficult task.

2. Lewis, M.J., Ragot, N., Berlant, M.C., & Miranda, M. 1990. Selection of astaxanthin-overproducing mutants of Phaffia rhodozyma and β -Ionone. *Appl Environ. Microbiol.* 56:2944-2945.

β -Ionone, an end ring analog of β -carotene, inhibits astaxanthin production of the red yeast Phaffia rhodozyma. Astaxanthin-overproducing mutants of this yeast are easily spotted on β -ionone-containing yeast malt agar plates. β -Ionone appears to block astaxanthin synthesis at the β -carotene level.

XXXVI. Institute of Molecular Biology, Department of Molecular Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. Communicated by P.V. Venkov.

The following results have been or will be published.

1. Stateva, L., Venkov, P., Lyutskanov, N., Koleva, L., & Hadjiolov, A. 1990. Protein extracts for nutritional purposes from fragile strains of Saccharomyces cerevisiae: Construction of strains and conditions for lysis. *J. Basic Microbiol.* 30 (in press).

A superfragile strain, Saccharomyces cerevisiae 211, carrying three nonallelic nuclear determinants of cell lysis by osmotic shock was isolated from the haploid progeny of a cross between two laboratory fragile strains VY1160 and SY15. The strain S. cerevisiae 211 is prototrophic grows well in both laboratory and industrial media, only when supplemented with osmotic stabilizers. The average protein content of its biomass after growth in a molasses-based nutritional medium is 48.7%. In laboratory conditions this strain releases about 60% of its cellular proteins by spontaneous lysis in water. After a single disintegration step at 650 atm., practically all of the cellular protein - 91.5% - is extracted without any additional chemical treatment. This result cannot be reached in any of the wild type strains tested even after triple treatment at 650 atm.

2. Blagoeva, J., Stoev, G., and Venkov, P. 1990. Glucan structure in a fragile mutant of Saccharomyces cerevisiae. *J. Basic Microbiol.* (in press)

The phenotype of VY1160 fragile Saccharomyces cerevisiae mutant is characterized by cell lysis upon transfer to hypotonic solutions and increased permeability of cells growing in osmotically stabilized media. Two mutations srb1 and ts1, have been identified in VY1160 cells and previous studies have shown that the increased permeability is due to the ts1 mutation which causes a shortening of mannan side chains. Here we report that the srb1 mutation, which is the genetic determinant of cell lysis, is responsible for quantitative and structural changes of glucans. Experiments with isogenic single mutation strains, genetic studies coupled with quantitative measurements of glucan content per cell and methylation analysis of glucans provide evidence that srb1 mutation leads to i) formation of mechanically unstable cell wall network made of insoluble glucan fibrils which are shorter and contain $\beta(1-6)$ inter-residue linkages and ii) insufficient filling of the space between fibrils due to a shortage of the alkali soluble glucan. Although growing exponentially in osmotically stabilized media, the srb1-cells can not resist an osmotic shock and burst immediately.

3. Blagoeva, J. & Venkov, P. 1990. Decreased synthesis of alkali-soluble glucan in a cell-wall mutant of Saccharomyces cerevisiae. *Biochemistry International.* (in press)

In vivo studies and quantitative measurements of glucans provide evidence for a decreased rate of synthesis and a lower amount of alkali-soluble glucan in cells of the osmotically fragile VY1160 mutant of the yeast Saccharomyces cerevisiae. Combined genetic and biochemical analysis shows that the srb1 mutation is responsible for the reduction of alkali-soluble glucan. Data on $\beta(1-3)$ glucan synthase activity did not indicate the participation of the enzyme in the in vivo synthesis of alkali-soluble glucan and suggest the existence of other glucan synthases in Saccharomyces cerevisiae.

4. Waltschewa, L., Kotyk, A., & Venkov, P. 1990. Mechanism of increased permeability in osmotic labile mutants of *Saccharomyces cerevisiae*. *Metabolism and Enzymology of Nucleic Acids including Gene Manipulations*, Vol. 7, (eds.) Sevcik and Balan, Slovak Academy of Sciences, Bratislava, Czechoslovakia (in press)

Unlike the parental S288C strain, the VY1160 mutant is characterized by cell-lysis in hypotonic solutions and a generally increased permeability to different substances. Previous genetic analysis revealed the presence of two chromosomal mutations, *srb1 ts1*, determining the mutant phenotype. Here we present genetic and biochemical evidence that the increased permeability of VY1160 cells is due to the combined effect of *srb1* and *ts1* mutations. While *srb1* mutation increases cell wall porosity and facilitates the accumulation of the substance into the periplasm, the *ts1* mutation enhances the internalization by increasing endocytosis. Both processes are not specific and therefore the increased permeability of VY1160 mutant cells is of general nature.

5. Mironova, R., D. Philipova, D., Marekov, L., Venkov, P., & Ivanov, I. 1990. Secretion of concatemeric variants of the Val⁸-human calcitonin in *Saccharomyces cerevisiae*. Yeast (submitted).

Monomeric human calcitonin (hCT) gene and oligomeric hCT genes composed of two, three or four head to tail linked monomers fused in frame to the α -factor leader coding sequence were inserted into yeast expression vector. Isogenic wild type and fragile mutant strains of *S. cerevisiae* were transformed with the constructed plasmids and the yield of recombinant protein secreted into the culture medium was measured. The wild type cells secreted equal (molar) amounts of all of the hCT variants. The recombinant proteins remained stable in the growth medium for several days. The secretion ability of the fragile yeast cells as compared to the wild type cells was higher.

6. Waltschewa, L., A. Kotyk, A., & Venkov, P. 1990. Increased endocytosis in the *Saccharomyces cerevisiae* fragile mutant VY1160. Yeast (submitted)

The VY1160 mutant is characterized by cell lysis in hypotonic solutions and generally increased permeability to substances for which *S. cerevisiae* cells are not permeable. Two mutations, *srb1* and *ts1*, have been identified in VY1160 mutant and previous studies (Kozhina et al., 1979) have shown *srb1* to be responsible for cell lysis. We now present evidence that *ts1* mutation leads to increased endocytosis in VY1160 cells. The internalization of lucifer yellow Ch in VY1160 cells is time-, temperature-, energy-dependent and consistent with a fluid phase mechanism of endocytosis. The rate of steady state accumulation of the dye at 37°C is 145 ng/ μ g DNA per hour for VY1160 mutant and 23 ng/ μ g DNA per hour for S288C parental strain. Studies with isogenic strains having either *srb1*, or *ts1* mutation, or SRB1 TS1 wild type alleles have shown that only *ts1* strains possess increased endocytosis. Quantitation of endocytosis in cells grown at 24°C and shifted at 38°C shows that *ts1* strains, but not *srb1* and wild type strains, increase 10 times the internalization of lucifer yellow 2 hours after the shift at 38°C. The analysis of *ts1* x wild type crosses evidence that the temperature sensitive phenotype segregates together with the enhanced endocytosis. It is concluded that the increased endocytosis might explain the generally increased permeability of VY1160 mutant cells.

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

1. Benito, B. & Lagunas, R. 1990. Turnover of the yeast plasma membrane ATPase and its activating system. 15th International Conference of Yeast Genetics and Molecular Biology, The Hague, July 21-26.

The bulk of proteins in *Saccharomyces cerevisiae* is quite stable showing half-life values >70 h (1). However, about 5% of the protein of this organism behave differently showing half-lives of about 3 h (1). Sugar transport systems belong to this later group. It has been shown that these transports are irreversibly inactivated when protein synthesis is inhibited. This inactivation follows first order kinetics and is an energy dependent process stimulated by fermentable substrates (2-4). These facts suggest that inactivation of the sugar transports is due to their rapid turnover. To see if this rapid turnover is a peculiarity of the sugar transports or also affects to other membrane proteins we have investigated the turnover of the plasma-membrane ATPase and its activating system.

Inhibition of protein synthesis in glucose growing yeast, either by addition of cycloheximide or by deprivation of a nitrogen source, produced a decrease in the ATPase content of the plasma membrane that followed first order kinetics. This decrease, that was detected by a dot-immunobinding assay, was accompanied by a decrease in the activity of the enzyme indicating a half-life for this protein of about 12 h. When ethanol was present as carbon source, addition of cycloheximide was without a detectable effect on the ATPase content and the calculated half-life of this protein was >30 h. These results show that, similarly to sugar transports, ATPase exhibits a rapid turnover as compared with the bulk of proteins of this organism. It seems, therefore, that a rapid turnover could be a common characteristic of the proteins of plasma membrane.

Plasma membrane ATPase is regulated in yeast cells by an activating system, still unidentified, that increases the optimum pH value of ATPase and the affinity for ATP. This system is triggered by fermentation (5). We have investigated the turnover of this activating system. To this purpose we have studied: (i) activation of ATPase, (ii) increase of its optimum pH value, (iii) increase of its affinity for ATP. It has been found that addition of cycloheximide or deprivation of a nitrogen source to glucose growing yeast decrease these actions. This decrease, that follows first order kinetics, suggests a half-life of about 12 h for the ATPase activating system when glucose is present in the medium. In the presence of ethanol a half-life >30 h was calculated. These results indicate that this activating system shows also a rapid

turnover with similar characteristics to those observed for the plasma membrane proteins so far examined.

- (1) J.M. Gancedo, S. Lopez & F. Ballesteros (1982) *Mol. Cell. Biochem.* **43**:89.
- (2) A. Busturia & R. Lagunas (1985) *Biochim. Biophys. Acta* **820**:324.
- (3) A. Busturia & R. Lagunas (1986) *J. Gen. Microbiol.* **132**:379.
- (4) C. DeJuan & R. Lagunas (1986) *FEBS Letters* **207**:258.
- (5) R. Serrano (1983) *FEBS Letters* **156**:11.

RECENT MEETINGS

Twenty-third Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan.

The 23rd Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from July 12 through 14, 1990 at Aichiken Boeki-sangyokan in Nagoya. Eighty four papers were presented in the following sessions: I Transport; II Membrane function; III Chromosomes; IV Sex pheromones, conjugation; V Meiosis, sporulation; VI Translation and post-translational modification; VII Cell cycle (I); VIII Cell structure; IX RAS, cAMP; X Calcium, calmodulin; XI Biosynthesis, metabolism, physiology; XII mitochondria, plasmids; XIII Transformation, biotechnology; XIV Cell cycle (II); XV Regulation of transcription. The abstracts of these presentations will be published in Japanese in "Yeast Genetics and Molecular Biology News, Japan" shortly.

O. Niwa

Kluyveromyces Workshop, Delft.

The third meeting on the "Biology of Kluyveromyces" was held on July 21 1990, at the historical site of the Kluyver laboratory in Delft. The meeting followed previous meetings in Orsay and Rome, and was organized at the occasion of the 15th International Conference on Yeast Genetics and Molecular Biology in The Hague. As summarized in the December 1989 issue of the Yeast Newsletter, the Kluyveromyces Workshop is held annually in the framework of ELWW (European Laboratories Without Walls, Commission of the European Communities). The Delft meeting, organized by Y. Steensma (Leiden University) and K. Rietveld (Royal Gist-Brocades), gathered approximately 60 people, including a few industrial participants. Discussions covered the topics of gene regulation, physiology, plasmids, and genetics. A discussion was focused on genetic nomenclature of K. lactis. B. Zonneveld (Leiden University) was asked to circulate worldwide a questionnaire on this topic. A preliminary directory of researchers working on Kluyveromyces (about 100 addresses) has been prepared and distributed to all the addresses known to us. Next year's meeting will be held in August or September in Dusseldorf (organizer: Karin Breunig).

Y. Steensma

14th International Specialized Symposium on Yeasts. Yeast Taxonomy: Theoretical and Practical Aspects. Smolenice, Czechoslovakia.

The 14th ISSY "Yeast Taxonomy: Theoretical and Practical Aspects" took place at Smolenice Castle from September 3-7, 1990. The Symposium was organized by the Commission of Yeasts of the Czechoslovak Microbiological Society and the Institute of Chemistry of the Slovak Academy of Science in Bratislava. Lectures in 6 plenary sessions and a poster session were held. Prominent yeast specialists from 22 countries of Europe, America, Asia and Australia presented their lectures. The following plenary sessions were open for discussion: Yeast Ecology, Taxonomy, Genetics, Molecular biology, Surface structures and Biotechnology. Summaries of all lectures and poster communications as well as a survey "30 Years of Activity in Czechoslovak Yeast Research" were issued at the occasion of the Symposium. At the ICY meeting Prof. H. Klaushofer and Dr. A. Kocková-Kratochvílová were proposed as honorary members of the ICY and further activities of the Commission in the next years were discussed.

E. Minárik.

International Commission for Yeasts and Yeast-Like Microorganisms, Meeting of September 4 1990, Smolenice, Czechoslovakia (XIIIth ISSY).

Present: A. Martini, Chair (Italy), C.P. Kurtzman and H.J. Phaff (USA), A. Kotyk, E. Minárik, and P. Biely - substitute for A. Kocková-Kratochvílová (Czechoslovakia), T. Deak (Hungary), H. Klaushofer (Austria), B. Johnson, (Canada), A. Cimerman - substitute for V. Johanides (Yugoslavia), N. van Uden (Portugal), and M.A. Lachance (Yeast Newsletter)

Regrets: G.G. Stewart and I. Russell (Canada), P. Galzy and J.M. Bastide (France), J.F.T. Spencer (England), A. Stenderup (Denmark), and P.V. Venkov (Bulgaria).

The minutes of the ICY meeting held during the XIIth ISSY meeting in Leuven, Belgium, on September 19 1989 were accepted.

Nominations

1. Dr. Byron Johnson (Canada) proposed Dr. Gode B. Calleja (Institute of Biology, University of Philippines, Diliman, Quezon City, The Philippines) as a new member. Carried unanimously.
2. Dr. Axel Stenderup (Denmark) in a recent letter proposed the following names as new members for Norway: Professor Ingar Olsen (School of Dentistry, Odontologisk Institutt for Mikrobiologi, Blindern, Oslo 4) and Dr. Per Sandven (Mycology Department, Statens Institutt for Folkehelse, Sopplaboratoriet, Geitmyrsveien 75, Oslo). Moved by the chair and carried unanimously.
3. Dr. Eric Minárik informed that Dr. Anna Kocková-Kratochvílová resigned from the ICY and expressed the wish to wish to be replaced by Dr. Peter Biely. Carried unanimously.
4. Dr. Hans Klaushofer announced his intention of resigning from ICY and proposed Dr. Hans-Jörg Prillinger (Raffaisen Bioforschung, Tüln, Austria) as a new member. Carried unanimously.

The above proposals will be discussed and formalized in 1992 during the next ISY in Atlanta, Georgia.

The Chair expressed his deep regret for the resignations announced by Dr. A. Kocková-Kratochvílová and Professor H. Klaushofer, and on behalf of all Commission members and scientists present at the XIIIth ISSY thanked them for their efforts in founding the International Commission on Yeasts and Yeast-like Microorganisms and contributing to the organization of its activities and meetings over the past 25 years. It was then proposed to confer honorary membership upon Dr. Anna Kocková-Kratochvílová and Professor Hans Klaushofer. Moved by the Chair and carried unanimously.

Future meetings

1. XVth ISSY: 1991, September-October in Jūrmala near Riga, Latvia, on "Regulation of Metabolism and Biotechnology" (Organizer: Dr. Alexander Rapoport, Institute of Microbiology, A. Kirchenstein str. 1, 26067, Riga, Latvia, USSR)
2. VIIIth ISY: 1992, Atlanta, Georgia, USA, August 23-28
3. XVIth ISSY: 1993, Delft, The Netherlands, on "Metabolic regulation and compartmentalization in yeast." (Organizer: Prof. W.A. Scheffers, Technische Hogeschool, Delft, Laboratorium voor Microbiologie, Julianalaan 67a, 2628 BC Delft, The Netherlands)
4. IXth ISY: The Chair announced that Dr. Martin J. Playne, President of the Australian Biotechnology Association, recently asked the Commission to consider the holding the 1996 ISY in Sydney as a joint conference, the week before or after the International Biotechnology Symposium. The consensus was in favour of having a meeting in the Southern Hemisphere in order to offer to yeast scientists of that area the possibility of attending ICY meetings. The Chair was asked to explore this or other possibilities in various Southern Hemisphere localities and to report during the Atlanta ISY.

Name of the ICY

The Chair announced that he has received a letter from Matti Korhola (Finland) noting a discrepancy in the name used to designate the Yeast Commission. Whereas the original name was "International Commission on Yeasts and Yeast-like Microorganisms," the most recent statutes (1986) adopted in Lisbon read in article 2 "the name of the organization shall be the International Commission for Yeasts (ICY)" even though more recent representations at the international level still refer to the longer title. After a short discussion, it was decided to bring up the matter during the Atlanta ISY, in 1992.

ICY Directory

The Chair reported that the majority of respondents to the questionnaire sent on May 3 1990 agreed with the proposal to prepare a directory of persons interested in the activities of the ICY. Also, several representatives prepared or promised to prepare a list of names of persons working on yeast in their respective countries. Dr. Johnson pointed out that the initiative could be redundant since many of those listed in such a Directory are already included in the Directory of the International Community of Yeast Genetics and Molecular Biology. Nevertheless, the majority of representatives present felt that our organization should include yeast scientists on a wider and less specialized basis. As a consequence, it was agreed to ask those ICY representatives who have not already sent a list of names, areas of interest and Fax/Telephone number of members of the yeast community to do so as soon as possible.

ISY attendance

The Chair announced that 26 ICY members (41%) answered the questionnaire sent out at the beginning of May 1990. The most relevant outcome was the conclusion that a merger with larger scientific societies such as the Yeast Geneticists and Molecular Biologists would certainly improve attendance at ISY meetings (20 in favour, 6 against) because a large number of people, substantial funds, and considerable interest are involved with Molecular Biology and Genetics. At the same time, many members warned of the risks of complete and rapid loss of identity of our group after a merger with scientists who are not necessarily biologists, who are mainly interested in *S. cerevisiae* and *Schiz. pombe*, and who regard yeasts as research tools.

Suggestions were also offered for improving attendance, for example, getting out of synchrony with large meetings, or favouring joint conferences with Biotechnology, Industrial Microbiology, or Molecular Biology people. Another suggestion was the creation of a permanent agenda of meetings on topics different from those

of interest to Yeast Geneticists and Molecular Biologists, recurring at fixed dates with fixed subjects: e.g. 1st year Taxonomy, 2nd year Physiology & Genetic Improvement of Strains, 3rd year Industrial uses of Yeasts, and 4th year General Symposium.

In any case, many members are deeply concerned about the loss of identity associated with joining other yeast communities as well as with the certain abrogation of our comprehensive approach to the study of yeast biology and biotechnology.

After a lengthy discussion it was agreed to explore the possibility of combining our general meetings with some large, international, specialized conferences (Biotechnology, Molecular Biology) and see if people working with yeast as a tool may be interested in sessions on Yeast Biology, Taxonomy or Ecology. The invitation to the 1996 Biotechnology Symposium in Sydney could offer us the first chance to test this possibility.

Yeast Newsletter

M.A. Lachance announced that there will be an increase in the annual subscription fee to the Yeast Newsletter from US\$4 to US\$6.

A. Martini

Personal impressions on the Smolenice meetings.

As one of 80 scientists from 22 countries who attended the 14th ISSY, I extend my personal thanks and congratulations to the organizers for the unqualified success of the meetings. The warm setting of Smolenice Castle provided a pleasant atmosphere for scientific exchange, complemented by unforgettable evenings of social events. In addition to an excellent program of plenary lectures and posters on yeast taxonomy and related subjects in the area of yeast evolution, physiology, genetics, or biotechnology, the participants were treated to a tasting of fine Pezinok wines of the lower Carpathian region. Among the evening events, particularly noteworthy were a performance by the outstanding traditional musicians and dancers of the Technical University, as well as a less outstanding, but no less enjoyable gathering where participants exhibited their own artistic talents. To Drs. E. Minárik and P. Biely, to Maria and all others who have made the meetings such a success, thank you.

M.A. Lachance.

FORTHCOMING MEETINGS

**Yeast Genetics and Molecular Biology Meeting, San Francisco Marriott Hotel,
May 23-28, 1991**

Organized by the Genetics Society of America.

For further information, contact

Anne Marie Langevin, Meetings Manager
GSA Administrative Office,
9650 Rockville Pike
Bethesda, Maryland 20814, U.S.A.

Phone: (301) 571 1825.

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

To receive the second announcement, contact:

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

Tel: (514) 287-1070

8th International Symposium of Yeasts, Georgia State University, Atlanta, Georgia, USA, August 23-28, 1992.

Preparations are being made for the eighth International Symposium on Yeasts, to take place in Atlanta, Georgia. The topics covered will include yeast biochemistry, biology, ecology, genetics, industrial applications, pathogenicity, and systematics.

The scientific program of 8th International Symposium of Yeasts will be held at Georgia State University located in the center of metropolitan Atlanta. Numerous hotels and restaurants are within walking distance of the university. To receive additional information on registration and hotel accommodation, contact:

Dr. Sally A. Meyer
Georgia State University
Atlanta, GA 30303
U.S.A.

Please indicate in which section you wish to participate.

BRIEF NEWS ITEMS

Postdoctoral Fellowship in Marine Biology and Fisheries.

A Postdoctoral Fellowship is available for up to two years starting June 1991. Applications are welcome in any facet of research related to Marine Biology and Fisheries. The Division currently has 23 faculty covering a wide range of interests, including: ocean remote sensing, recruitment processes, marine biotechnology, micro- & macro-biology and ecology. Salary \$30,000. Apply by December 1, 1990, to: Dr. Peter Lutz, Chairman; Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, Florida 33149. Tel.: (303) 361-4177

J.W. Fell

PROGRESS REPORT, a Newsletter on Microbial Protoplasts in Cell Biology, Genetics and Biotechnology.

PROGRESS REPORT, a Newsletter on Microbial Protoplasts in Cell Biology, Genetics and Biotechnology is edited and published by the Department of Genetics, L.K. University, Debrecen (Hungary). Items of this report are summaries of recently completed research projects, or of publications in press or recently published, on any other kind of activity in the research of microbial protoplasts and their applications in cell biology, genetics, biochemistry or biotechnology, etc. We should appreciate receiving news and information from laboratories and scientists working on microbial protoplasts. This material will appear in the eighth issue of the Progress Report.

Please send your information before January 31, 1991 to:

M. Sipiczki,
Department of Genetics,
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H-4010 Debrecen, P.O. Box 56,
Hungary

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