
Y E A S T

A Newsletter for Persons Interested in Yeast

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

JUNE 1992

Volume XLI, Number I

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

Associate Editors

Peter Biely
Institute of Chemistry
Slovak Academy of Sciences
Dúbravská cesta 9
842 38 Bratislava, Czechoslovakia

Tadashi Hirano
The Jikei University School of Medicine
3-25-8 Nishi-Shinbashi, Minatoku
Tokyo 105, Japan

G.G. Stewart
Labatt Breweries of Canada Ltd.
150 Simcoe Street
London, Ontario, Canada N6A 4M3

B.J.M. Zonneveld
Dept. of Cell Biology and Genetics
Leiden University
Wassenaarseweg 64
2333 AL Leiden, The Netherlands

S.C. Jong, Rockville, Maryland, USA	1	M. Vancanneyt, Gent, Belgium	13
M.Th. Smith, Delft, The Netherlands	2	M.L. Suihko, Espoo, Finland	13
P. Nagley, Clayton, Victoria, Australia	3	P. Romano & G. Suzzi, Sede di Reggio Emilia, Italy	14
E. Minárik, Bratislava, Czechoslovakia	5	M. Korhola, Helsinki, Finland	15
M. Celerin & A.W. Day, London, Ontario, Canada	6	T. Hirano, Tokyo, Japan	16
H. Lee, Guelph, Ontario, Canada	7	H. Holzer, Freiburg, Germany	16
J.W. Fell, Miami, Florida, USA	7	D. Avram, Bucharest, Romania	17
R. Maleszka & G.D. Clark-Walker, Canberra, Australia	8	J.M. Gancedo, Madrid, Spain	17
M.M. Vustin, Moscow, Russia	8	M.J. Leibowitz, Piscataway, New Jersey	18
I.P. Bab'eva, Moscow, Russia	9	P. Galzy, Montpellier, France	19
H. Koshinsky, Saskatoon, Saskatchewan, Canada	10	Y. Yamada, Shizuoka, Japan	19
J. Ramos Cordoba, Spain	11	W.A. Scheffers, Delft, The Netherlands	21
D.M. Spencer & J.F.T. Spencer, Tucuman, Argentina	11	Obituary	23
A. Bakalinsky, Corvallis, Oregon, USA	12	International Commission on Yeasts	23
		Forthcoming Meetings	24
		Brief News Items	26
		Publications of Interest	27

Editorials

Richard Snow 1928 - 1992

We are saddened by the news of the recent death of Dr. Richard Snow, who served for many years as Associate Editor of the Yeast Newsletter. On behalf of all readers, I extend my condolences to his relatives and friends. Dr. Ralph Kunkee has kindly provided us with an outline of some of Dr. Snow's contributions in the area of yeast genetics.

***Kluyveromyces* workers**

Our readership has expanded somewhat as a result of the decision, by the *Kluyveromyces* workers, to adopt the Yeast Newsletter as its official means of communication. We appreciate their encouragement and look forward to receiving their contributions to subsequent issues.

VIIIth ISY, Atlanta, August 1992

The Atlanta meetings program details a most exciting list of speakers and topics. Dr. Meyer advises us that late registrations for the Atlanta ISY will be accepted for as long as possible. Please refer to the announcement on page 24.

New format and new E-mail address

We hope that our readers will enjoy the new format of the Yeast Newsletter. We are using a new word processor that produces more legible character sizes and styles. Comments on this subject will be appreciated. Readers who wish to submit their entries in MS-DOS/WP5.1 format are now encouraged to do so. A printout should be included.

Our electronic mail address has been modified recently to the following: **a1146@uwoc1.uwo.ca**

Mode of payment

It has again come to our attention that some readers have had to pay excessive bank charges to obtain international cheques or money orders. We offer advance subscriptions (up to 5 years) as a means of economizing - a \$2.00 increase of the subscription is planned for 1993. Many readers have found it most convenient and inexpensive to purchase and send U.S. cash. Readers who request receipts must include a copy of their invoice with their payment. The receipt will accompany the subsequent issue of the Yeast Newsletter.

M. A. Lachance
Editor

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

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

NAME	ATCC#	DEPOSITOR & STRAIN	SIGNIFICANCE & REFERENCE
<i>Candida albicans</i>	76615	C. Hitchcock, Y01.09	Antifungal agent assay
<i>Candida ciferrii</i>	76725	L. de Gentile, 5707	Human pathogen-agent in nail infection (Mycoses 34 :125-128, 1991)
<i>Candida mucifera</i>	76712	E. Slavikova, AN 119-F	Antifungal agent (J.Basic Microbiol. 28 :613-618, 1988)
<i>Candida utilis</i>	76738	I. Spencer-Martins, IGC 3092	Physiological studies (Microbiol. Biotechnol. 26 :54-157, 1987)
<i>Hansenula polymorpha</i>	76722 76723	J. Cregg, mutant C27 mutant C111	Peroxisome deficient mutant (Yeast 6 :87-97, 1990)
<i>Saccharomyces cerevisiae</i>	76740	T. Fukasawa, MT8-1	Genetics (Arch. Microbiol. 156 :439-443, 1991)
<i>Saccharomyces cerevisiae</i>	76741	P. Hieter, YPH 420	Genetic study (Mol. Cell. Biol. 12 :1357-1365, 1992)
<i>Yarrowia lipolytica</i>	76598	J. Swezey, NRRL Y-7576	Produces citric acid from glucose (Appl. Biochem. Biotechnol. 20/21 :491-509, 1989)

Publications:

1. Molina, F.I., P. Shen & S.C. Jong. 1992. Determination of infraspecific relationships in *Kluyveromyces marxianus* by riboprinting. Mycotaxon **43**:49-60.
 2. Molina, F.I., T. Inoue, and S.C. Jong. 1992. Genetic Heterogeneity in *Saccharomyces cerevisiae* Meyen ex Hansen: Evidence from Ribosomal DNA Restriction Analysis. Int. J. Syst. Bacteriol. (Accepted).
 3. Molina, F.I., T. Inoue, and S.C. Jong. 1992. Restriction polymorphisms in the internal transcribed spacers and 5.8S rDNA of *Saccharomyces*. Curr. Microbiol. (Accepted).
-

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

The following papers have recently appeared or are in press.

1. T. Boekhout, Gool, J. van, Boogert, H. van den & Jille, T. 1991. Karyotyping and G+C composition as taxonomic criteria applied to the systematics of *Tilletiopsis* and related taxa. Mycol. Res. (in press).

Electrophoretic karyotypes and nucleic acid base composition were studied in strains belonging to the heterobasidiomycetous genera *Tilletiopsis*, *Entyloma*, *Exobasidium*, *Tilletia* and the species *Tilletiaria anomala*. Chromosomal banding patterns and mol% G+C provided

additional criteria to distinguish between the species. The taxonomic position of all described taxa of *Tilletiopsis* is discussed. Further evidence is provided for an ontogenetic relationship between *Tilletiopsis*-like anamorphs and members of the Tilletiales.

2. T. Boekhout, Yamada, Y., Weijman, A.C.M., Roeymans, H.J. & Batenburg-van der Vegte, W.H. 1992. The significance of coenzyme Q, carbohydrate composition and septal ultrastructure for the taxonomy of ballistoconidia-forming yeasts and fungi. Syst. Appl. Microbiol **15**:1-10.

Septal ultrastructure, coenzyme Q, and carbohydrate composition of whole-cell hydrolyzates were studied in species of the ballistoconidia-forming genera *Tilletiopsis*, *Tilletiaria*, and *Itersonia*. The first two genera contained coQ 10, glucose, mannose and galactose, and septa having central micropore-like channels or lacking any pore structure. *Itersonia* contained coQ-9, glucose, mannose and xylose. Its septa had dolipores without parentheses. Taxonomically, the

ballistoconidia-forming fungi are polyphyletic and can be assigned to three orders of Heterobasidiomycetes: 1. Ustilaginales: the teleomorph genus *Sporidiobolus* and the anamorphic genera *Sporobolomyces*, *Bensingtonia* and *Ballistosporomyces*, 2. Tilletiales: the teleomorph genus *Tilletiaria* and the anamorphic genus *Tilletiopsis*, and 3. Tremellales: the teleomorph genus *Bulleromyces*, and the anamorphic genera *Bullera*, *Kockovaella* and *Itersonia*.

3. E. Guého, G.S. de Hoog, & M. Th. Smith. 1992. Neotypification of the genus *Trichosporon*. Antonie van Leeuwenhoek. (in press).

The currently accepted type species of the genus *Trichosporon* Behrend is *T. beigeli*. This species has formerly been regarded as identical to *T. cutaneum*. However, these fungi are now known to represent separate species with different ecology. The first species described in *Trichosporon*

was *T. ovoides*, an agent of human white piedra. A neotype strain is designated for this species, while a lectotype strain is indicated for *T. cutaneum*. The name *T. beigeli* is considered as doubtful and consequently cannot be maintained.

4. E. Guého, M. Th. Smith, G.S. de Hoog, G. Billon-Grand, R. Christen & W.H. Batenburg-van der Vegte. 1992. Contributions to a revision of the genus *Trichosporon*. Antonie van Leeuwenhoek. (in press).

The genus *Trichosporon* was revised using characters of morphology, ultrastructure, physiology, ubiquinone systems, mol% G+C of DNA, DNA/DNA reassociations and 26S ribosomal RNA partial sequences. A total of 101 strains was used, including all available type and authentic cultures of previously described taxa. Nineteen taxa could be distinguished, 15 of which having Q-9 coenzyme systems and 4 having Q-10. Sixteen previously described names were reduced to synonymy. One new species was described. The genus is characterized by the presence of arthroconidia. Few species possess further diagnostic morphological characters, such as the presence of appressoria, macroconidia or meristematic conidiation. The septa of two species were found to be non-perforate, while those of the remaining species

contained dolipores at variable degrees of differentiation, with or without vesicular or tubular parentheses. All species were able to assimilate a large number of carbon compounds; visible CO₂ production was absent. The genus was found to be fairly homogeneous on the basis of a phylogenetic analysis of partial 26S rRNA sequences, with the exception of *T. pullulans* which proved to be unrelated. Most taxa were found to occupy well-defined ecological niches. Within the group of taxa isolated from humans, a distinction could be made between those involved in systemic mycoses and those which mainly caused pubic or non-pubic white piedra, respectively. One species was consistently associated with animals, while others came mainly from soil or water. One species was mesophilic and another psychrophilic.

5. M. Th. Smith, C. Shann, W.H. Batenburg-van der Vegte, R. Schmitt, E. Wehrli, H.J. Roelijmans & G.W. van Eijk. 1992. *Botryozyma nematodophila* gen. nov., spec. nov. (Candidaceae). Antonie van Leeuwenhoek. (in press).

The new genus *Botryozyma* with a single species, *B. nematodophila* is proposed for two isolates from nematodes (*Panagrellus zymosiphilus*) occurring in grapes with sour-rot.

The new genus has typical ascomycetous characteristics and, being unable to produce ascospores, is placed in the family Candidaceae.

III. Department of Biochemistry and Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria 3168, Australia. Communicated by P. Nagley.

Since the previous communication from this laboratory in mid-1988, the following papers have been published on the molecular biology of mitochondria in *Saccharomyces cerevisiae*. Our work has focused on the formation and properties of the ATP synthase enzyme complex in mitochondria, that is responsible for cellular energy production by oxidative metabolism. Aspects we have been concerned with include the genetic and biochemical analysis of mutations in mitochondrial and nuclear genes, the regulation by nuclear

genes of the expression of individual mitochondrial genes, the manipulation and expression of artificial mitochondrial genes relocated to the nucleus (aimed at systematic mutational analysis of protein structure and function), the study of mitochondrial protein import systems, the assembly of mitochondrial ATP synthase *in vivo* and *in vitro* (analysed using monoclonal antibodies), and functional aspects of mitochondrial ATP synthesis.

1. R.H.P. Law, L.B. Farrell, D. Nero, R.J. Devenish & P. Nagley. 1988. Studies on the import into mitochondria of yeast ATP synthase subunits 8 and 9 encoded by artificial nuclear genes. FEBS Lett. **236**:501-505.
2. P. Nagley and R.J. Devenish. 1989. Leading organellar proteins along new pathways: the relocation of mitochondrial and chloroplast genes to the nucleus. Trends Biochem. Sci. **14**:31-35.
3. M. Galanis, J.R. Mattoon and P. Nagley. 1989. Amino acid substitutions in mitochondrial ATP synthase subunit 9 of *Saccharomyces cerevisiae* leading to venturicidin or ossamycin resistance". FEBS Lett. **249**:333-336.
4. D.G. Grasso, R.J. Maxwell, P. Nagley and R.J. Devenish 1989. Yeast genomic clones encoding polypeptides immunologically related to an 18 kDa subunit of mitochondrial ATP synthase. Biochem. Intern. **19**:959-967.
5. S. Meltzer, T.A. Willson, L.C. Watkins, P. Nagley, S. Marzuki, A.W. Linnane and H.B. Lukins. 1989. Biochemical analyses of *oli1* and *oli2* gene mutations determining primary sequence changes in subunit 9 and 6 of yeast ATP synthase. In MOLECULAR STRUCTURE, FUNCTION AND ASSEMBLY OF ATP SYNTHASES (S. Marzuki, ed.), pp. 51-65, Plenum Press, New York.

6. L.B. Farrell, D. Nero, S. Meltzer, G. Braidotti, R.J. Devenish and P. Nagley. 1989. Assembly of yeast mitochondrial ATP synthase incorporating an imported version of an F_o-sector subunit normally encoded within the organelle. In MOLECULAR STRUCTURE, FUNCTION AND ASSEMBLY OF ATP SYNTHASES (S. Marzuki, ed.), pp. 95-104, Plenum Press, New York.
7. S. Marzuki, A.S. Noer, H. Sudoyo, S. Meltzer, H.B. Lukins and A.W. Linnane. 1989. Monoclonal antibodies as probes of the structure, function and assembly of the mitochondrial ATP synthase. In MOLECULAR STRUCTURE, FUNCTION AND ASSEMBLY OF ATP SYNTHASES (S. Marzuki, ed.) pp. 115-128, Plenum Press, New York.
8. Sudoyo, H. and S. Marzuki. 1989. Antimitochondrial autoantibodies of primary biliary cirrhosis as a novel probe in the study of the biosynthetic regulation of the yeast 2-oxo acid dehydrogenase complexes. Biochem. Biophys. Res. Comm. **158**: 220-227.
9. Marzuki, S., L.C. Watkins and W.M. Choo. 1989. Mitochondrial H⁺-ATPase in mutants of *Saccharomyces cerevisiae* with defective subunit 8 of the enzyme complex. Biochim. Biophys. Acta, **975**:222-230.
10. R.J. Devenish, H.B. Lukins, P. Nagley and A.W. Linnane. 1990. Molecular biology and assembly of yeast mitochondrial ATP synthase. In Structure, Function and Biogenesis of Energy Transfer Systems, (E. Quagliariello, S. Papa, F. Palmieri and C. Saccone, eds.), pp. 55-59, Elsevier Science Publishers, Amsterdam.
11. R.H.P. Law, R.J. Devenish and P. Nagley. 1990. Assembly of imported subunit 8 into the ATP synthase complex of isolated yeast mitochondria. Eur. J. Biochem. **188**:421-429.
12. P. Nagley, R.J. Devenish, R.H.P. Law, R.J. Maxwell, D. Nero and A.W. Linnane. 1990. Subunit 8 of yeast mitochondrial ATP synthase: Biochemical genetics and membrane assembly". In BIOENERGETICS: MOLECULAR BIOLOGY, BIOCHEMISTRY AND PATHOLOGY (C.H. Kim and T. Ozawa, eds.), pp. 305-325, Plenum Press, New York.
13. R.H.P. Law and P. Nagley. 1990. Import into mitochondria of precursors containing hydrophobic passenger proteins: Pretreatment of precursors with urea inhibits import. Biochim. Biophys. Acta, **1027**: 141-148.
14. R.E. Gray, D.G. Grasso, R.J. Maxwell, P.M. Finnegan, P. Nagley and R.J. Devenish. 1990. Identification of a 66 kDa protein associated with yeast mitochondrial ATP synthase as heat shock protein hsp60. FEBS Lett. **268**:265-268.
15. D. Nero, S.M. Ekkel, L. Wang, D.G. Grasso and P. Nagley. 1990. Site directed mutagenesis of subunit 8 of yeast mitochondrial ATP synthase: Functional and import properties of a series of C-terminally truncated forms. FEBS Lett. **270**:62-66.
16. M. Galanis, R.H.P. Law, L. M. O'Keefe, R. J. Devenish and P. Nagley. 1990. Aberrant mitochondrial processing of chimaeric import precursors containing subunits 8 and 9 of yeast mitochondrial ATP synthase. Biochem. Int. **22**:1059-1066.

17. D. G. Grasso, D. Nero, R. H. P. Law, R. J. Devenish and P. Nagley. 1991. The C-terminal positively charged region of subunit 8 of yeast mitochondrial ATP synthase is required for efficient assembly of this protein into the membrane F₀ sector. *Eur. J. Biochem.* **199**:203-209.
18. M. Galanis, R.J. Devenish and P. Nagley. 1991. Duplication of leader sequence for protein targeting to mitochondria leads to increased import efficiency. *FEBS Lett.* **282**:425-430.
19. M.J. Payne, E. Schweizer and H.B. Lukins. 1991. Properties of two nuclear *pet* mutants affecting expression of the mitochondrial *olil* gene of *Saccharomyces cerevisiae*. *Curr. Genet.* **19**:343-351.
20. P.M. Finnegan, M.J. Payne, E. Keramidaris and H.B.Lukins. 1991. Characterisation of a yeast nuclear gene, *AEP2*, required for accumulation of mitochondrial mRNA encoding subunit 9 of the ATP synthase. *Curr. Genet.* **20**:53-61.

**IV. Research Institute for Viticulture and Enology, Bratislava, Matúškova 25, Czechoslovakia 833 11.
Communicated by E. Minárik.**

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

1. Michalčáková, S., Sulo, P., Hapalová, J., & Minárik, E. 1991. Occurrence and properties of killer strains among Czechoslovakian wine yeasts. *Weinwissenschaft* **46**:123-126.

Investigating 65 different Czechoslovak yeast strains it was found that 6 of them had a killer activity against *Saccharomyces cerevisiae*. Tests of the killer characteristics showed that two strains belong to the K2-type with a maximum of activity at pH 3.5-3.7. Three strains showed an optimum of activity between pH 3.5-4.0; they contained M dsRNA with a

weight of 2.0-2.1 kb and had an activity against *Zygosaccharomyces bailii*. They could be identified as K3 killers. The killer activity was lost by incubation at 37°C and addition of cycloheximide. The strain 13 RVV/d contained M dsRNA with 1.7 kb as well as 2.0 kb.

2. Minárik, E. & Jungová, O. 1992. Influence of yeast ghost and cellulose preparations on different yeast species occurring in grape must and wine. *Die Weinwissenschaft* **47**: (in press).

The influence of yeast ghost and cellulose preparations on different yeast species occurring in the yeast association of grape must and wine was investigated, in the course of alcoholic fermentation. Biosorbents show a pronounced influence not only on *Saccharomyces* species but also on other yeast species with fermentation activity. A very distinct

fermentation promotion was observed in *Schizosaccharomyces* sp. and *Brettanomyces* sp. Yeast species with little fermentation activity usually display in most cases a comparatively served reaction towards the presence of activators. The volatile acid formation is strongly decreased in *S. cerevisiae* by biosorbents, whereas in other species investigated rather slightly increased.

3. Malík, F., Krásny, Š., & Minárik, E. 1992. Use of immobilized cells in wine making. Part 1: Application of immobilized yeasts in primary grape must fermentation (in German). *Die Weinwissenschaft* **47**: (in press).

Immobilized systems proved very useful in different fields of fermentation technology. The first paper out of a series of publications on their utilization in wine technology deals on general principles and methods of cell immobilization. Recent

situation in the realization of immobilized systems in the process of primary fermentation of grape and fruit musts in batch and continuous regimes are presented.

4. Minárik, E., Jungová, O., Ružičková, A., & Bučková, A. 1992. Activity of yeasts of the genus *Schizosaccharomyces* in decomposing L-malic acid (In Slovak). *Vinohrad (Bratislava)* **30(3)**: (in press).

Yeasts of the genus *Schizosaccharomyces* (*pombe*, *malidevorans*, *acidodevoratus*) are able to decompose, completely or partly, L-malic acid in grape must by malo-alcoholic fermentation, supposed that all other originally present microorganisms of the must are inactivated or eliminated, first of all *Saccharomyces* sp., prior to

fermentation. The alcoholic fermentation activity is, however, limited. Laboratory experiments will be extended to large scale fermentations in the winery in order to test sensoric properties of the wines with high acidity fermented and deacidified by *Schizosaccharomyces*.

5. Minárik, E., Jungová, O. & Bučková, A. 1992. Characterization of some industrially important wine yeast strains. (in Slovak) *Knasný prumysl (Prague)* **38(4)**: (in press).

Schizosaccharomyces pombe and *Sch. acidodevoratus* strains decompose L-malic acid under defined laboratory conditions in fermenting must, especially in grape musts showing high total acidity content. A basic requirement is the elimination of the original spontaneous yeast association of the must before alcoholic fermentation by pasteurization or sharp

filtration. The decomposition of L-malic acid by *S. cerevisiae* is essentially weaker. Fermentation activators of the type of yeast ghosts or microcrystalline cellulose intensify the decomposition of acids by *Schizosaccharomyces* sp., while the activity of L-malic acid decomposition by *S. cerevisiae* is minimal.

6. Kollár, R., Šturdík, E., Minárik, E., Šajbidor, J., & Šandula, J. 1991. Elaboration and application of baker yeast preparations (in German). *Zeitschrift für Lebensmittel -Technologie- und Verfahrenstechnik* (in press).

A process of baker's yeast complex fractionating is proposed; after disintegration of the yeast envelope by an induced autolysis process, yeast extract, invertase, yeast ghosts, ergosterol, phospholipids and β -glucan are simultaneously

obtained. Possibilities of the application of individual preparations in practice of different industries are described in detail.

**V. Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7.
Communicated by M. Celerin and A.W. Day.**

The following is the abstract of a poster presentation at the "Cellular and Molecular Biology of Basidiomycetes" conference, Erindale College, Mississauga, Ontario, Canada, May 12-16, 1992.

1. Celerin, M., Bancroft, J.B., Castle, A.J., Laudenschlager, D.E., Smith, R.J., & Day, A.W. Isolation of nucleic acid from the fimbriae of the smut fungus, *Ustilago violacea*.

Fimbriae of *Ustilago violacea* are long (1-20 μ m), narrow (7 nm) surface fibrils assembled from 74 kDa glycoprotein subunits. We now have evidence that the intact fimbriae contain 12-15% nucleic acid. Isopycnic centrifugation of crude fimbrial isolates in cesium chloride produces one band at a density intermediate to that of proteins and nucleic acids. Electron microscopic examinations of dialysed samples from the band reveal typical long 7 nm fimbriae with no other visible structure. The absorbance spectrum of material from this band is consistent with that of a nucleoprotein. Analysis by SDS-PAGE of material from this band confirms the presence of the characteristic 74 kDa glycoprotein. Electroelution, followed by spectrophotometry of the contents of the 74 kDa band shows an

absorbance maximum at 274 nm, indicating that most nucleic acid is lost during protein denaturation. The nucleic acid itself was isolated from purified fimbriae and was characterized to be a small RNA. Since the ability of 74 kDa subunits, eluted from SDS-polyacrylamide gels, to reassemble spontaneously into 7 nm diameter fibrils in the absence of any other component has been well documented, we conclude that the nucleic acid is not required for the assembly of fibrils, at least under some conditions. Clearly, the presence of nucleic acid in the extracellular fimbriae could have important implications for a number of biological functions such as conjugation and pathogenicity.

2. Computer animations in molecular genetics.

We have recently developed a series of computer animations in full colour of cellular and molecular processes. The package is available for use on 'IBM' compatible computers with **VGA** colour monitors and hard drive. The series consists of **50 animations** developed for an introductory cell biology / molecular genetics course at the University of Western Ontario, Canada by Drs. Alan Day and Robert Dean. Each animation is a mini-lesson and provides 15-20 min of study time. The topics covered include important **cellular processes and mechanisms** such as DNA and protein structure, replication including events at the replication fork, models of recombination (Holliday, Meselson and Radding, Szostak), the lac operon, attenuation, splicing out of introns, capping and

tailoring of mRNA, ribosome and tRNA structure, synthesis and function, the mechanism of translation, 'wobble', mutation, DNA repair, the 'Ames' test, transposons, mitosis and meiosis, amino acid structure, signal transduction, electron transport, motor proteins, G proteins, voltage gated ion channels and photosynthesis. A number of animations also present important **biotechnological techniques** such as PCR, DNA sequencing, blotting, cloning and site directed mutation. Student response has been very enthusiastic and we have noticed a significant improvement in comprehension of these processes. The series is available at a reasonable cost. **Please contact Dr. Day at the above address, or by phone (519 433 7145) or FAX (519-661-3292).**

VI. Department of Environmental Biology, University of Guelph, Room 3218, Bovey Building, Guelph, Ontario, Canada N1G 2W1. Communicated by H. Lee.

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

1. Lee, H. 1992. Reversible inactivation of D-xylose utilization by D-glucose in the pentose-fermenting yeast *Pachysolen tannophilus*. FEMS Microbiol. Lett. **92**:1-4.

A major problem in fermenting pentoses using lignocellulosic substrates is the presence of D-glucose which inhibits D-xylose utilization. We previously showed that D-glucose represses the induction of xylose reductase and xylitol dehydrogenase activities, thereby inhibiting D-xylose utilization in *Pachysolen tannophilus*. The question arose whether D-glucose can inactivate D-xylose fermentation also. *P. tannophilus* cells were grown on a defined D-xylose-containing liquid medium. At about 40 h, D-glucose was added to a final concentration of 3% (w/v). This led to a

rapid cessation of D-xylose utilization, which resumed after 10-12 h before D-glucose was completely consumed. This suggests that D-glucose inactivated existing D-xylose catabolic enzymes and that inactivation was reversed at low D-glucose concentrations. This reversible inactivation was distinctly different from the phenomenon of D-glucose repression. Addition of cycloheximide did not block the resumption of D-xylose consumption, suggesting that reactivation was independent of de novo protein synthesis.

VII. University of Miami, Rosenstiel School of Marine and Atmospheric Science, 1 Rickenbacker Causeway, Miami, Florida 33149, U.S.A. Communicated by J.W. Fell.

The following papers are currently in press.

1. Fell, J.W. & A.S. Tallman. In press. Systematic placement of the basidiomycetous yeast *Cystofilobasidium lari-marini* comb. nov. as predicted by RNA nucleotide sequence analysis. Antonie van Leeuwenhoek.
2. Fell, J.W., A. S. Tallman, M.J. Lutz & C.P. Kurtzman. In press. Partial rRNA sequences in marine yeasts; a model for identification of marine eukaryotes. Mol. Marine Biol. Biotechnol.
3. Newell, S.Y. & J.W. Fell. (In press). Distribution and experimental responses to substrate for marine oomycetes (*Halophytophthora* species) in mangrove ecosystems. Mycol. Res.

VIII. Molecular & Population Genetics Group, Research School of Biological Sciences, The Australian National University, Canberra, Australia. Communicated by R. Maleszka and G.D. Clark-Walker.

The following manuscripts have been submitted for publication.

1. Yeasts have a four-fold variation in ribosomal DNA copy number.

By employing Pulsed Field Gel Electrophoresis we have determined the size of the rDNA cluster in wild type yeast strains representing genera of *Candida*, *Kluyveromyces*, *Pachysolen*, *Schizosaccharomyces* and *Torulasporea*. Although the genome size of the examined species is similar (12.3-13.9Mb), at least a 4-fold variation has been observed

between the lowest amount of rDNA repeats in *P. tannophilus* (28) and the highest in *C. glabrata* and *S. pombe* (>115). In two species the rDNA cluster is represented by two loci, residing either in one (*S. pombe*) or two chromosomes (*C. glabrata*).

2. In vivo conformation of mitochondrial DNA in fungi and zoosporic moulds.

Migratory behaviour of mitochondrial DNA (mtDNA) from fungal species belonging to Plectomycetes, Loculoascomycetes and zoosporic moulds, Oomycetes, has been studied by Pulsed Field Gel Electrophoresis. Electrophoretic profiles demonstrate that long, linear molecules

of a heterogenous size are the prevailing form of organelle DNA in vivo in all examined species. These profiles are consistent with the presence of the rolling circle mode of mtDNA replication that occurs in all branches of true fungi and zoosporic moulds.

IX. National Collection of Industrial Microorganisms, Institute for Genetics and Selection of Industrial Microorganisms, Moscow, 113545, Russia. Communicated by M.M. Vustin.

Recent publications

1. Vustin, M.M. & Bab'eva, I.P. 1990. Differentiation of basidiomycetous yeast based on their sensitivity to killer toxin of *Williopsis pratensis*. Abstract 14th Int. Spec. Symp. on Yeast (Smolenice, Czechoslovakia, Sept. 3-7)
2. Vustin, M.M. & Kalina, E.N. 1991. New killer toxins produced by *Debaryomyces* and *Schwanniomyces* yeasts. Abstract 15th Spec. Symp. on Yeast (Riga, Sept.30-Oct.6), 153.
3. Vustin, M.M., Shemyakina, T.M., Tchilina, G.A., Bogomolova, T.S., & Gorshkova, G.I. 1991. Action of killer toxins produced by yeasts of genera *Williopsis*, *Zygowilliopsis* and *Hansenula* on some pathogenic yeasts. Abstract 15th Int. Spec. Symp. on Yeast (Riga, Sept. 30-Oct. 6), 154.
4. Vustin, M.M., Bab'eva, I.P., Reshetova, I.S., Shemyakina, T.M., & Sineoky, S.P. 1991. Use of the sensitivity of the basidiomycete yeasts to the killer toxin of *Williopsis pratensis* for taxonomic differentiation. *Mikrobiologiya* **60**:239-242.
5. Shemyakina, T.M., Vustin, M.M., Nesterenko, M.V., Timokhina, E.A., & Sineoky, S.P. 1991. New killer toxins produced by the yeasts *Williopsis subsufficiens* and *Williopsis beijerinckii*. *Mikrobiologia* **60**:501-506.

6. Lebedeva, G.I., Vustin, M.M., Shemyakina, T.M., & Sineoky, S.P. 1991. Respiratory-defective mutants of *Saccharomyces cerevisiae* resistant to the action of the preparation of the killer toxin of *Williopsis saturnus*. *Mikrobiologia* **60**:759-761.

The following papers have been submitted for publication.

7. Vustin, M.M., Kalina, E.N., Bab'eva, I.P., Reshetova, I.S., & Sineoky, S.P. *Debaryomyces* killer toxins (*Mikrobiologia*).
8. Vustin, M.M., Shemyakina, T.M., & Sineoky, S.P. A dependence of yeast's sensitivity to *Williopsis* and *Hansenula* killer toxins on pH of medium (*Mikrobiologia*).

X. Department of Soil Biology, Lomonosov State University, Moscow 119899, Russia. Communicated by I.P. Bab'eva.

In the Department of Soil Biology, Lomonosov State University, headed by Prof. D.G. Zvyagintsev, a collection of yeasts exists. There are more than 2 thousands strains isolated from different substrates collected practically in all natural ecosystems of the former Soviet Union: from tundra to deserts. The collection is

well characterized due to almost 30 years of permanent studying of yeasts. The strains presented can be used for the study of geographical variability and the search for producers of biologically active compounds, and other value products.

The following papers have been published recently.

1. M.M. Vustin, I.P. Bab'eva, I.S. Reshetova, T.M. Shemyakina, & S.P. Sineoky. 1991. Taxonomic differentiation of basidiomycetous yeasts in terms of their sensitivity towards the killer toxin of *Williopsis pratensis*. *Microbiology* **60**:345-349.
2. I.P. Bab'eva. 1991. Natural yeast resources for biotechnology. 15th Int. Spec. Symposium on yeasts (Riga, Sept. 30-Oct. 6), 16.
3. O.N. Zinchenko, A.G. Lobanok, I.P. Bab'eva, I.S. Reshetova, & Vu Nguyen Thanh. 1991. Dextranase activity of soil yeasts - lipomycetes. *Microbiology* **60**:833-836.
4. I.P. Bab'eva, I.S. Reshetova. 1992. Yeast collection of Soil Biology Department in Moscow State University - bank of strains for agricultural biotechnology. *Microorganisms in Agriculture*. Puschino, 16-17 (In Russian).
5. I.P. Bab'eva & Vu Nguyen Thanh. 1992. New trends in searching of hydrolytic yeasts *Lipomyces kononenkoae* in soils *Vestnik Mosk. Un-ta*, **2**:66-72.

XI. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Communicated by H. Koshinsky.

The following papers have been published or accepted for publication. These papers describe the use of yeast to define the inter-actions of toxins. The second paper is especially interesting as it defines a new term for use when discussing the interactions of compounds.

1. H.A. Koshinsky, P.J. Hannan & G.G. Khachatourians. 1991. HT-2 toxin, roridin A, T-2 toxin and verrucaric acid mycotoxins inhibit carbon dioxide production by *Kluyveromyces marxianus*. Can. J. Microbiol. **37**:933-938.

Carbon dioxide release rate of *Kluyveromyces marxianus* was inhibited 10% in less than 25 min or 50% in less than 65 min by 0.0625, 0.125, 1.25 and 12.5 µg/mL of the trichothecene mycotoxins verrucaric acid, roridin A, T-2 toxin and HT-2 toxin, respectively. Individually neither 0.625 µg/mL T-2 toxin nor

0.000125 µg/mL roridin A alters carbon dioxide production. When combined at these two concentrations T-2 toxin and roridin A interact synergistically to inhibit carbon dioxide release rate by 50% within 60 min.

2. H.A. Koshinsky & G. G. Khachatourians. 1992. Trichothecene synergism, additivity and antagonism: The significance of the maximally quiescent ratio. Natural Toxins **1**:1-10.

The interaction of the trichothecene mycotoxin combinations often found in fungus contaminated grain is poorly understood. Growth inhibition of *Kluyveromyces marxianus* was used to measure the interaction of the trichothecenes HT-2 toxin or roridin A with the trichothecene T-2 toxin over a wide range of effect levels. The interaction of the mixtures is affected by the percent inhibition of yeast growth and by the ratio of the toxins. The interaction of HT-2 toxin and T-2 toxin or roridin A and T-2 toxin changes from antagonistic when there is a low percent inhibition of yeast growth to synergistic when there is a high percent inhibition of yeast growth additionally, each of these mixtures has a unique ratio which shows the least change (in terms of type and intensity) in the interaction at low percent inhibition compared

to high percent inhibition. We term this ratio where there is the least change in the interaction of any two compounds the maximally quiescent ratio (MQR). The term quiescent is used to indicate that there is the least variation (most stability) in the interaction of the two compounds at this ratio. T-2 toxin and roridin A have an MQR of 1.0 : 0.35. T-2 toxin and HT-2 toxin have an MQR of 1.0 : 12.0. The usefulness of the concept that two compounds have a most stable ratio is extensive. MQR indicates that the ratio of toxins should be considered a key parameter in toxicity studies. MQR may also be applied to many different disciplines to provide insights into hormone, immune system, developmental, enzyme and gene regulation, combined drug therapy, the safety of carcinogen and/or pesticide and/or environmental pollutant mixtures.

3. H.A. Koshinsky & G.G. Khachatourians. 1992. A bioassay for deoxynivalenol based on the interaction of T-2 toxin with trichothecene mycotoxins. Bull. Environ. Contamin. Toxicol. (in press, August issue).

When growth of *Kluyveromyces marxianus* is inhibited by 20 to 35% by T-2 toxin - trichothecene mixtures, the interaction is usually zero or synergistic. However, the trichothecene deoxynivalenol interacts antagonistically with T-2 toxin when

inhibiting *K. marxianus* growth by 20 to 35%. This unique interaction has been exploited to develop a *K. marxianus* based bioassay for deoxynivalenol.

**XII. Department of Microbiology, E.T.S.I.A.M., University of Córdoba, 14071 Córdoba, Spain.
Communicated by J. Ramos.**

The following paper is in press.

1. J. Ramos, R. Haro, R. Alijo & A. Rodríguez-Navarro. 1992. Activation of the potassium uptake system during fermentation in *Saccharomyces cerevisiae*. J. Bacteriol. March.

Fermentable sugars activated the potassium uptake system, increasing the V_{max} s of rubidium, sodium and lithium influxes, but sugars did not affect the effluxes of these cations.

This activation seems to be a direct effect of fermentation and not the consequence of the H^+ pump ATPase activation or internal pH decrease produced by fermentation.

XIII. Planta Piloto de Procesos Industriales Microbiologicos, Av. Belgrano y Pje. Caseros, 4000 San Miguel de Tucuman, Argentina. Communicated by D.M. Spencer and J.F.T. Spencer.

The following papers have been accepted for publication.

1. D.M. Spencer, J.F.T. Spencer, L. de Figueroa, & H. Heluane. In press. Yeasts associated with rotting citrus fruits in Tucuman, Argentina. Mycol. Res. **96**.

Yeasts were isolated from decaying spots in citrus fruits (oranges, limes, mandarins, grapefruit) produced in the Tucuman region of Argentina. Species isolated from the rotting fruit included *Kloeckera apiculata*, *Candida guilliermondii*, *Candida stellata*, *Pichia kluyveri*, other *Pichia* species of the *Pichia membranaefaciens* group, and *Geotrichum candidum*.

Kloeckera apiculata appeared to have some pectinolytic activity when reinoculated into healthy fruit. The yeasts were probably carried by fruit flies. Larvae of the fruit fly (*Ceratitis capitata*), which is endemic to the region, were found in the decay pockets.

2. J.F.T. Spencer, D.M. Spencer, L. de Figueroa, J. Nougues and H. Heluane. In press. Transfer of genes for utilization of starch (STA2) and melibiose to industrial strains of *Saccharomyces cerevisiae* by single chromosome transfer, using a *Kar1* mutant as a vector. Appl. Microbiol. Biotechnol.

A method has been developed for the transfer of genes from other yeast strains and species to industrial yeast strains, using a haploid, *kar1* mutant strain of *Saccharomyces cerevisiae* as a vector. The *STA2* gene, conferring the ability to metabolize starch, was transferred from an auxotrophic haploid strain of *Saccharomyces cerevisiae* (*diastaticus*) and the *MEL* gene(s), from *Saccharomyces kluyveri*, to the *kar1* mutant (*ade2 his4 can1 gal*) by normal mating and protoplast fusion. From this strain, the genes were transferred to baker's yeast and brewing yeast strains, which did not utilize starch, and to baker's yeast strains, which did not utilize melibiose, by protoplast fusion, spore pairing, or rare mating. Strains which

utilized starch or melibiose were obtained by all three methods. Pulsed field gel electrophoresis preparations showed little change in the mobility of the chromosomes of the hybrids. The most probable explanation for the results obtained is that single chromosomes were transferred, first, from the donor strains to the *kar1* haploid mutant strain, and then, from the *kar1* vector, to the recipient industrial strain of *Saccharomyces cerevisiae*. The transfer of the genes is probably accomplished through formation of disomic strains and then, in the case of the hybrids which metabolized starch, by integration of the *STA2* gene into the genome of the industrial yeast strains.

XIV. Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, Oregon, 97331-6602, U.S.A. Communicated by A. Bakalinsky.

The following book chapter is in press.

1. Bakalinsky, A.T. 1992. Yeast biopreservatives. In FOOD BIOPRESERVATIVES OF MICROBIAL ORIGIN. Edited by B. Ray and M.A. Daeschel. CRC Press, Boca Raton, Fl.

The following abstract was presented as a poster.

2. Xu, X., J.D. Wightman, B.L. Geller, & A.T. Bakalinsky. 1992. Sulfite toxicity in the yeast *Saccharomyces cerevisiae*. Meeting of the American Society for Microbiology, New Orleans, May 26-30, 1992.

Sulfite sensitivity is a major health problem in the U.S. to well over one million chronic steroid-dependent asthmatics. Because little is known about how sulfite triggers hypersensitivity reactions, we developed a model microbial system in which to study sulfite toxicity. Although yeast has a basal tolerance for sulfite, two classes of EMS-generated mutants were obtained: sulfite-resistant and sulfite-sensitive. Genetic analysis indicated that one and four genes were responsible for the resistant and sensitive responses, respectively, and suggested that neither enhanced nor impaired sulfite transport, nor defects in methionine or cysteine metabolism were involved. Some resistant alleles, all of which were dominant, appeared to confer greater resistance than others based on segregation observed in crosses between

different independent isolates. Meiotic progeny of a few resistant X wildtype hybrids were mostly inviable. Mutations conferring sensitivity were recessive and one co-segregated with impaired respiration. Biochemical analyses ruled out involvement of sulfite oxidase which yeast lacks, and provided evidence that in at least two of the sensitive mutants, glutathione levels were significantly lower than in wildtype cells. Because sulfite is a reducing agent, cells were tested for coincident sensitivity or resistance to ascorbate, selenite, dithiothreitol, nitrite, thiosulfate, reduced glutathione, and cysteine. No clear pattern of responses to the other agents emerged, suggesting that the response to sulfite is specific, and not a simple function of redox potential.

JoLynne D. Wightman recently completed her M.S. degree in my laboratory. The abstract of her dissertation follows.

3. Wightman, J.D. 1992. A study of sulfite mutants of *Saccharomyces cerevisiae*. M.S. Food Science. Oregon State University.

Sulfite mutants representing five complementation groups, previously derived from an ethyl methanesulfonate-treated haploid strain of *Saccharomyces cerevisiae*, were studied. Although the wildtype *S. cerevisiae* strain used (isogenic to X2180-1A) had a basal tolerance for sulfite (7 μM free H_2SO_3), the sensitive and resistant mutants were found to tolerate less than 3 to 5.5, or greater than 19 μM free H_2SO_3 , respectively. No apparent correlation was found between the response to sulfite and generation time in rich (YEFD) or minimal media. Resistant mutant 11-1 had an extended lag phase relative to wildtype. Mutant and wildtype proteins were labelled with 35S-methionine to determine differences in banding patterns due to sulfite-specific induction or disappearance of polypeptides. No obvious differences following SDS-PAGE

and autoradiography were observed upon induction with 0.213 μM free H_2SO_3 . No consistent correlations were found between the sulfite phenotypes and responses to other reducing agents. Sensitive mutant 35-2 appeared to be three to ten times more sensitive to dithiothreitol than wildtype and sensitive mutant 47-9 was three to four times more sensitive to sodium nitrite and three to seven times more sensitive to sodium thiosulfate than wildtype. Log phase cells of sensitive mutant 33-2 were found to have significantly less glutathione than wildtype. Wildtype contained 62.6 $\text{nmol min}^{-1} \text{mg protein}^{-1}$ (62.6 $\text{mU mg protein}^{-1}$) glutathione reductase (GR) and 2.78 $\text{nmol min}^{-1} \text{mg protein}^{-1}$ (2.78 $\text{mU mg protein}^{-1}$) glutathione S-transferase (GST). Log phase cells of one resistant mutant showed a significantly higher level of GR than wildtype, 135%. The

resistant mutants as well as some of the sensitive mutants had reduced GST levels. Survival rates of the mutants in buffer in the presence of sulfite did not correlate with their sensitive or resistant phenotypes, suggesting that survival and growth in the presence of sulfite are not necessarily related functions.

Relative to wildtype, survival upon prolonged storage at 4°C was markedly reduced for two of the four sensitive mutants, one of which was 33-2, and was enhanced for one resistant and another sensitive mutant.

XV. Laboratorium voor Microbiologie en microbiële Genetica, Rijksuniversiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium. Communicated by M. Vancanneyt.

1. M. Vancanneyt, E. Van Lerberge, J.F. Berny, G.L. Hennebert & K. Kersters. 1992. The application of whole-cell protein electrophoresis for the classification and identification of basidiomycetous yeast species. *Antonie van Leeuwenhoek* **61**:69-78.

The relationships among 65 basidiomycetous yeast strains were determined by one-dimensional electrophoresis of SDS-solubilized whole-cell proteins. Protein profiles were compared by the Pearson product moment correlation coefficient (r). The strains investigated represented species from the genera *Cystofilobasidium*, *Filobasidium*, *Filobasidiella*, *Kondoa*, *Leucosporidium*, *Mrakia* and *Rhodospodium*. Except for the genus *Mrakia*, all species constituted separate protein electrophoretic clusters. The species of the genus *Mrakia* (*M. frigida*, *M. gelida*, *M. nivalis*, and *M. stokesii*) show highly similar protein patterns,

suggesting that these four species may be synonymous. Strains of two varieties of *Filobasidiella neoformans*, *F. neoformans* var. *neoformans* and *F. neoformans* var. *bacillispora*, could not be differentiated by protein electrophoresis. For the delineation of the protein electrophoretic clusters of the yeasts studied, literature data relying on other criteria, such as DNA base composition, carbon source utilization patterns, enzymatic protein electrophoregrams, ubiquinone systems, DNA-DNA homology and rRNA sequence data were used. It was demonstrated that a database of SDS-protein patterns provides a valuable tool for the identification of yeasts.

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

The following papers have been published since my last report:

1. Kumar, V., Ramakrishnan, S., Teeri, T.T, Knowles, J.K.C. & Hartley, B.S. 1992. *Saccharomyces cerevisiae* cells secreting an *Aspergillus niger* β -galactosidase grow on whey permeate. *Bio/Technol.* **10**:82-85.
2. Aalto, M.K., Keränen, S. & Ronne, H. 1992. A family of proteins involved in the intracellular transport. *Cell* **68**:181-182.
3. Blomqvist, K. 1992. The bacterial budABC operon; Isolation and application for accelerated brewing. VTT, Biotechnical Laboratory, Espoo, Finland and Department of Microbiology, University of Umeå, Sweden. 74 p + app. 67 p. Doctor thesis.

Diacetyl formation in beer was reduced by introducing the *budA* gene from *Klebsiella terrigena* or *Enterobacter aerogenes* coding for α -ALDC into brewer's yeast. The gene was expressed in brewer's yeast under the control of either the *PGK1* or *ADH1* promoter and terminator sequences on an autonomously replicating yeast plasmid or by integration into the *PGK1* or *ADH1* loci in the yeast genome. The diacetyl level

was so low after fermentation that lagering was unnecessary for beers produced with the plasmid strains or *PGK1* integrants. Other brewing properties of the recombinant yeast strains and the quality of the trial beers were unaltered. Using these new brewer's yeast strains the beer production time could be reduced by 2-3 weeks.

XVII. 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 are the abstracts of articles recently published or in press:

1. Romano P. & Suzzi G. 1992. Production of H₂S by different yeast strains during fermentation. Presented at the 22nd Convention, Australia & New Zealand Section, Institute of Brewing, Melbourne (Australia), March 1992.

The formation of H₂S by yeasts is of great practical importance and is a common characteristic in *Saccharomyces*. A natural yeast population, isolated during grape must fermentation, was subdivided into four groups based on H₂S production and ranged from high H₂S-producing strains to non-H₂S-producing ones. The amount of H₂S produced was found

to be a characteristic of the strain, and was also controlled by the composition of the medium as well as being related to other characteristics of technological interest. The strains were classified into different H₂S phenotypes by means of the streak colour on BiGGY agar and the level of SO₂ production.

2. Suzzi G., Romano P. & Benevelli M. 1992. The flocculation of wine yeasts: biochemical and morphological characteristics in *Zygosaccharomyces*. Antonie van Leeuwenhoek, in press.

The floc-forming ability of flocculent strains of *Zygosaccharomyces bailii* and *Z. fermentati*, isolated from musts, was tested for susceptibility to proteinase and sugar treatments. *Z. fermentati* was found highly resistant to the proteolytic enzymes tested, whereas *Z. bailii* was only trypsin-resistant. The inhibition of flocculation by sugars distinguished

two types in *Z. fermentati* flocculation was completely inhibited by mannose, and in *Z. bailii* by various sugars. By SEM observation, the cell surface of *Zygosaccharomyces* revealed the presence of a column structure, resulting from fusion of vesicles present on the cell surface.

3. Romano P., Suzzi G., Comi G. and Zironi R. 1992. Higher alcohol and acetic acid production by apiculate wine yeasts. J. Appl. Bacteriol. 72: in press.

Ninety-six strains of apiculate wine yeasts were investigated for their ability to produce higher alcohols and acetic acid in synthetic medium. Less isoamyl alcohol and more n-propanol and iso-butanol were formed by *Hanseniaspora guilliermondii* than by *Kloeckera apiculata*. The latter produced twice as much acetic acid as *H. guilliermondii*. The production of higher alcohols and acetic

acid was found to be a characteristic of individual strains and was statistically significant. In a multivariate analysis of higher alcohol production two main groupings were formed at 86%S, corresponding to the taxa *H. guilliermondii* and *K. apiculata*. Strains that produced low amounts (50 mg/l) of acetic acid, comparable with that of *Saccharomyces cerevisiae*, were found in both species of apiculate yeasts.

**XVIII. Alko Ltd., The Finnish State Alcohol Company, P.O.B. 350, SF-00101 Helsinki, Finland.
Communicated by M. Korhola.**

1. Y. Ohya, H. Kawasaki, K. Suzuki, J. Londesborough & Y. Anraku. 1991. Two yeast genes encoding calmodulin-dependent protein kinases. *J. Biol. Chem.* **266**:12784-12794.

We have isolated two genes from *Saccharomyces cerevisiae* that both encode a calmodulin-dependent protein kinase (CaM kinase). The *CMK1* gene has been cloned by hybridization using an oligonucleotide probe synthesized on the basis of the peptide sequence of purified yeast CaM kinase (Londesborough, J. (1989) *J. Gen. Microbiol.* 135, 3373-3383). The other gene, *CMK2*, which is homologous to *CMK1*, has been isolated by screening at low stringency with a *CMK1* fragment as a probe. The *CMK2* product expressed in bacteria shows Ca²⁺- and CaM-dependent protein kinase activity, indicating that *CMK2* also encodes a CaM kinase. The *CMK1* and *CMK2* products expressed in bacteria were found to have different biochemical properties in terms of autoregulatory activity and preference for yeast CaM or bovine CaM for maximal activity. Antibody raised against a peptide fragment

of the *CMK1* protein cross-reacts with the *CMK2* product. Immunoblotting with this antibody indicated that the *CMK1* and *CMK2* products have apparent molecular masses of 56 and 60 kDa, respectively, in yeast cells. The predicted amino acid sequences of the two *CMK* products exhibit highest similarity with mammalian calmodulin-dependent multifunctional protein kinase II (CaM kinase II): the similarity within the N-terminal catalytic domain is about 40 %, whereas that within the rest of the sequence is 25%. The data indicate that yeast has two kinds of genes encoding CaM kinase isozymes whose structural and functional properties are closely related to those of mammalian CaM kinase II. Another gene may be substituted for function of the *CMK1* and *CMK2* kinase *in vivo*, since elimination of both kinase genes is not lethal.

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

Genetic and karyotypic studies of natural *Saccharomyces sensu stricto* yeasts from Finland, Holland and Slovakia

revealed three wild sibling-species: *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces paradoxus*.

3. P.L. Liljeström, R.S. Tubb & M.P. Korhola. 1991. Construction of new α -galactosidase producing yeast strains and industrial application of these strains. United States Patent 5,055,401, Oct. 8, 1991

The objects of this invention are new *Saccharomyces cerevisiae* yeast strains into which α -galactosidase gene (MEL+) has been transferred by using recombinant DNA methods. Baker's and distiller's yeasts producing α -galactosidase are utilizable in the corresponding industry, because they are able to utilize the raffinose present in molasses, which results in greater yield of yeast (or ethanol) and reduction or elimination of the costs associated with biological

oxygen demand (B.O.D.) in the effluent from factories. The improved ability of baker's yeasts to produce α -galactosidase provides a sensitive method for monitoring pasteurization of beer. The new yeast strains prepared by using recombinant DNA methods produce more α -galactosidase than naturally occurring α -galactosidase producing yeast strains. Also methods for making yeast strains and for producing stable transformants of yeasts are presented.

**XIX. Jikei University School of Medicine, Nishi-shinbashi, Minato-ku, Tokyo 105 Japan.
Communicated by T. Hirano.**

The following is the abstract of a communication presented recently.

1. T. Hirano, A. Tanaka and M. Yamaguchi. 1990. Comparative aspects of the plasma membrane structure in yeast by a new high resolution freeze-replica method and ordinary freeze-replica method. Proceedings of the XIIth International Congress for Electron Microscopy pp.614-615.

The development of the ordinary freeze-replica method providing a clear three-dimensional image. And many researchers have made technical improvements in these methods. However, the metal shadowing film replica method has problems. For instance, the fine structure of a surface of biological specimen can suffer thermal damage due to radiant heat derived from the high temperature necessary to bring about evaporation of the heavy metal. Also, the evaporated material is in the form of particles (Figure 1). Accordingly, it is questionable whether these particles are a natural feature or artificially introduced by ordinary freeze-replica method. Walzthdöng et al. (1961) showed that the particles were an artifact or form of contamination produced under the conditions used in the ordinary freeze-replica method.

In 1978, Tanaka et al. has discovered a new method of forming a desired polymer replica film through the use of a novel concept. This method is completely different from ordinary freeze-replica method. During the specimen preparation, the temperature at the surface of a specimen goes up only several degrees centigrade. We have applied this method for studying the structure of yeast cells. In the present study, which was a further development of the freeze-replica method using plasma polymerized film, produced three-dimensional replicas of chemically untreated cell and provided a real structure of the plasma membrane, especially, the nature

of the hexagonal arrays of particles present on the plasma membrane in yeast by the ordinary freeze-replica method (Moor, H. et al. 1963, Hirano, T. et al 1970).

Figure 1 shows the surface of the plasma membrane by ordinary freeze-replica method. The hexagonal arrays of particles, ice crystallization and glycerol are seen on the fracture face. Figure 2 shows the surface of the plasma membrane by a plasma polymerization freeze-replica method. The surface of plasma membrane have no hexagonal arrays of particles in comparison with Figure 1a. Because of no effect of radiant heat during the specimen preparation. We assume that Figure 2 shows real structure image of the surface plasma membrane. The hexagonal arrays of particles of the plasma membrane in yeast is an artifact or form of contamination produced under the conditions used in the ordinary freeze-replica method.

References

- Moor, H. & Mühlethaler, K. 1963. J. Cell Biol. 17:609.
Hirano, T. & Washioka, H. 1990. JEOL News 8:22.
Tanaka, A., Sekiguchi, Y. & Kuroda, S.J. 1978. Electron Microscopy 27:378.
Wolzthöng, D., Gross, H. & Moor, H. 1982. 10th Int. Cong. for Electron Microscopy. 3:213.

**XX. Biochemisches Institut, Albert-Ludwigs-Universität Freiburg, Hermann-Herder-Straße 7,
D-7800 Freiburg im Breisgau, Germany. Communicated by H. Holzer.**

The following is a summary of a communication to be presented at the 16th International Conference on Yeast Genetics and Molecular Biology to be held in Vienna, August 15-21, 1992.

1. M. Destruelle, M. Kopp and H. Holzer. 1992. Acid and neutral trehalase from yeast.

We have isolated the genes from *Saccharomyces cerevisiae* encoding the neutral (App and Holzer (1989) J. Biol. Chem. 264, 17583-17588) and acid trehalase (Mittenbühler and Holzer (1988) J. Biol. Chem. 263, 8537-8543) which catalyze the hydrolysis of trehalose (α -D-glucopyranosyl- α -D-glucopyranoside).

Neutral trehalase (NTH) was cloned by complementation of a NTH-deficient mutant. The mutant was obtained by chemical mutagenesis of the yeast strain YHH65 (*MATa pral Δ EN1::HIS3 his3-11,15 leu2-3,112 ura3 Δ 5 canR*) and characterized by tetrad analysis. NTH-negative colonies were screened by an enzymatic overlay assay. Yeast cells that had no

more activity were transformed with a *S. cerevisiae* genomic library in Yep24. We obtained two overlapping plasmids that had an ORF of 2082 bp encoding a protein of 694 amino acids. The deduced molecular weight derived from the corresponding amino acid sequence is 79,569 Da which is in good correlation with the observed molecular mass of about 80,000 Da in SDS-PAGE. Northern blot analysis yields a single mRNA species of approximately 2.3 kb. The protein possesses a putative cAMP-dependent phosphorylation consensus sequence RRX(G)S. The amino acid sequence of the yeast trehalase shows homology in 3 domains to the periplasmic trehalase from *E. coli* (Gutierrez, C., Ardourel, M., Bremer, E., Middendorf, A., Boos, W., and Ehmann, U. (1989) Mol. Gen. Genet. 217, 347-354): the regions from amino acid residues 237-303 (60.6%), 566-600 (43.6%) and 606-641 (31.7%). The amino acid sequence of yeast NTH as compared to the rabbit small intestinal trehalase (Ruf, J., Wacker, H., James, P. Maffia, M., Seiler, P., Galand, G., v. Kiekebusch, A., Semenza, G., and Mantei, N. (1990) J.

Biol. Chem. 265, 15034- 15039) reveals homologies in 4 domains: from 240-305 (50.8%), 490-520 (29.4%), 557-584 (46.4%) and 647-669 (39.1%).

Acid Trehalase (ATH) was purified from a *suc2* mutant and a partial amino acid sequence information was obtained from the N-terminus and from several internal peptides (Dr. Helmut Meyer, University of Bochum). Corresponding degenerate oligonucleotide primers were synthesized for polymerase chain reactions on yeast genomic DNA. The PCR yielded a 500 bp fragment with an ORF throughout. The nucleotide sequence confirmed the amino acid sequence. The fragment was used to screen a *S. cerevisiae* genomic library in YCp50. Positive clones of different lengths with overlapping restriction maps were isolated. The sequence revealed several putative N-glycosylation sites and an N-terminal cleavable hydrophobic signal sequence. This is in good agreement with the 86% glycosylation of the purified protein and with its localization in the yeast vacuole.

XXI. Institute of Biology, Spl. Independenței 296, 77748 Bucharest, Romania. Communicated by D. Avram.

The following is the summary of a recently published paper.

1. S. Petrescu, S. Hulea, R.Stan, D. Avram & V. Herlea. 1992. A yeast strain that uses D-galacturonic acid as a substrate for L-ascorbic acid biosynthesis. Biotechnol. Lett. **14**:1-6.

The bioconversion of D-galacturonic acid to L-ascorbic acid was demonstrated in a new yeast strain isolated from the Japanese Crystal. Both intact cells and a crude mitochondrial

extract yielded ascorbic acid when D-galacturonic acid was present.

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

The following are recent publications from our group.

1. B. Benito, E. Riballo & R. Lagunas. 1991. Turnover of the K⁺ transport system in *Saccharomyces cerevisiae*. FEBS Lett. **294**:35-37.

The stability of the K⁺ transport system in *Saccharomyces cerevisiae* has been studied upon inhibition of protein synthesis with cycloheximide. Addition of the antibiotic gave rise to an inactivation of this transport. This activation followed first-order kinetics and was stimulated by the presence of a

fermentable substrate. A half-life of about 4 h could be calculated in the presence of glucose. The results indicate that, similarly to sugar carriers, K⁺ transport system is less stable than the bulk of proteins of this organism.

2. B. Benito, F. Portillo & R. Lagunas. 1992. In vivo activation of the yeast plasma membrane ATPase during nitrogen starvation. Identification of the regulatory domain that controls activation. *FEBS Lett.* **300**:271-274.

Yeast plasma membrane ATPase is activated during nitrogen starvation when a fermentable substrate is present. This activation is due to changes in the V_{max} and it is irreversible, independent of protein synthesis and apparently

triggered by a decrease in the intracellular pH. It is shown that the ATPase regulatory domain implicated in the activation by fermentable carbon sources is also implicated in activation by nitrogen starvation and by external acidification.

3. R. Lagunas & E. Moreno. 1992. Inhibition of glycolysis by 2-deoxygalactose in *Saccharomyces cerevisiae*. *Yeast* **8**:107-115.

The enzymatic steps involved in the inhibition of glycolysis by 2-deoxygalactose in *Saccharomyces cerevisiae* have been investigated. Yeast, incubated with 2-deoxygalactose, accumulates up to 8 mM 2-deoxygalactose, 30 mM 2-deoxygalactose-1-phosphate and 0.25 mM UDP-2-deoxygalactose, and UDP-2-deoxyglucose. An inverse correlation between 2-deoxygalactose-1-phosphate content and

rate of glycolysis has been observed. The intracellular concentration of glycolytic intermediates and related metabolites point to the hexokinase and phosphofructokinase steps as the targets for the inhibition of glycolysis by 2-deoxygalactose and rule out all other mechanisms that have been proposed to explain this inhibition.

The following extensive review of carbohydrate repression will appear soon.

4. Gancedo, J.M. 1992. Carbon catabolite repression in yeast. *Eur. J. Biochem.* (in press).

XXIII. Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, New Jersey 08854-5635, U.S.A. Communicated by M.J. Leibowitz.

The following are abstracts of recently published papers.

1. F.P. Barbone & M.J. Leibowitz. 1991. Coupling of killer virus transcription with translation in yeast cell-free extracts. *J. Gen. Virol.* **72**:1755-1760.

The cytoplasmically inherited killer virus of *Saccharomyces cerevisiae* expresses its dsRNA genome via apparently uncapped viral transcripts produced in the cytoplasm of infected cells. Virions of this naturally temperature-sensitive virus can be added to cell-free translational extracts of uninfected yeast cells resulting in a reaction in which viral

transcription and translation are coupled at 15°C in vitro. In this reaction nucleotides are incorporated into full-length transcripts of the M and L-A dsRNA segments, with lower levels of incorporation into genomic RNA. In addition, incorporation of nucleotides is observed into a smaller RNA species showing no sequence relatedness to M or L-A.

2. F.P. Barbone, T.L. Williams, & M.J. Leibowitz. 1992. Yeast killer virus transcription initiation *in vitro*. *Virology* **187**:333-337.

Killer virions isolated from infected *Saccharomyces cerevisiae* cells contain an RNA polymerase activity which catalyzes the transcription *in vitro* of positive polarity RNAs from the L-A and M double-stranded RNA genomic segments of the virus. The RNA polymerase can initiate transcription *in vitro* with γ -thio-GTP, whose thiophosphate group is found on the 5' terminus of transcripts. Transcripts produced *in vitro* by

the virion-associated RNA polymerase in the presence of $^7\text{mGpppG}$ are significantly more active as translational templates than are transcripts produced in its absence. However, unlike *Escherichia coli* RNA polymerase transcripts from viral cDNA made in the presence of $^7\text{mGpppG}$, transcripts produced by viral RNA polymerase in the presence of $^7\text{mGpppG}$ fail to bind to antibody against ^7mG .

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

The following papers have been published recently.

1. Matte O., Chabalier C., Ratomahenina R., Bossy J.P. & Galzy P. 1991. Isolation of a double-stranded RNA and a virus like particle from *Geotrichum candidum*. J. Basic Microbiol. **31**:447-452.
2. Riaublanc A., Boze H., Demuyneck M., Moulin G., Ratomahenina R., Graille J. and Galzy P. 1992. Optimization of biomass production from palm oil in cultures using *Candida rugosa*. Fat. Sci. Technol. **94**(46).
3. Claisse M.L., Boze H., Dubreucq E., Seigueilha L., Moulin G. and Galzy P. 1991. Characterization of alternative respiratory pathways in the yeast *Schwanniomyces castelli* by the study of mutants deficient in cytochromes a+a3 and/or b. Acta Biochim. Polonica **38**:366-392.
4. Viole P., Boze H., Moulin G. and Galzy P. 1992. Transport and hydrolysis of maltose by *Schwanniomyces castelli*. J. Basic Microbiol. **32**:57-63.
5. Boze H., Moulin G. and Galzy P. 1992. Production of food and fodder yeasts. Crit. Rev. Biotechnol. **12**:65-86.
6. Lambrechts C., Boze H., Moulin G. and Galzy P. 1992. Utilisation of phytate by some yeasts. Biotechnol. Lett. **14**:61-66.

XXV. Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan. Communicated by Y. Yamada.

1. Y. Yamada & T. Nagahama. 1991. The molecular phylogeny of the ascomycetous yeast genus *Holleya* Yamada based on the partial sequences of 18s and 26s ribosomal ribonucleic acids. J. Gen. Appl. Microbiol. **37**:199-206.

The partial base sequences of 18S and 26S rRNAs of six strains of species in the genera *Holleya*, *Nematospora*, and *Metschnikowia* (Spermothoraceae) were determined. The determinations of the partial base sequences (positions 1451 through 1618, 168 bases) of 18S rRNA have demonstrated that the genus *Holleya* is phylogenetically separable from the genera

Nematospora and *Metschnikowia*, although the partial base sequences (positions 493 through 625, 133 bases; positions 1685 through 1835, 151 bases) of 26S rRNA in the genera *Holleya* and *Nematospora* were similar. Some discussions are presented from the taxonomic and phylogenetic points of view.

2. Y. Yamada, T. Nagahama & I. Banno¹. 1991. The molecular phylogeny of the Q₉-equipped ascomycetous teleomorphic yeast genus *Debaryomyces* Lodder et Kreger-van Rij based on the partial sequences of 18s and 26s ribosomal ribonucleic acids. *J. Gen. Appl. Microbiol.* **37**:277-288.

¹Japan Institute for Fermentation, Osaka, Jusohon-machi, Yodogawa-ku, Osaka 532, Japan.

The partial base sequences of 18S and 26S rRNAs were examined in eighteen strains of *Debaryomyces*, *Torulaspota*, and *Yamadazyma* species including two strains of *D. udenii*. All of the strains of *Debaryomyces* species constituted a single group (cluster) phylogenetically. In the partial base sequence (positions 493 through 622, 130 bases) of 26S rRNA, the maximum homologies were 79-99% among *Debaryomyces* species. *T. globosa* and *Y. philogaea* had 71-78% and 81-87% maximum homologies, respectively, with *Debaryomyces* species. In the partial base sequence (positions 1611 through 1835, 225 bases) of 26S rRNA, the base differences numbered 5-0 among *Debaryomyces* species. *T. globosa* and *Y. philogaea* had 15-13 and 10-8 base differences, respectively, with

Debaryomyces species. In the partial base sequence (positions 1451 through 1618, 168 bases) of 18S rRNA, *Debaryomyces* species were divided into two subgroups (subclusters). The first subgroup was comprised of *D. hansanii*, *D. melissophilus*, *D. udenii*, and so on, and the second subgroup comprised of *D. castellii*, *D. polymorphus*, *D. yamadae*, and so on. The base difference numbered 1 between the two subgroups. *T. globosa* and *Y. philogaea* had 5-4 and 1-0 base differences, respectively, with *Debaryomyces* species. Between *T. globosa* and *S. cerevisiae*, there was 1 base difference. *D. tamaritii* occupied a distant position (maximum homologies, 63-71%; base differences, 50-48 and 20-19, respectively).

3. Y. Yamada, T. Nagahama, I. Banno, G. Giménez-Jurado¹ and N. van Uden¹. 1991. The phylogenetic relationship of *Kurtzmanomyces tardus* Giménez-Jurado et van Uden (Cryptococcaceae) based on the partial sequences of 18s and 26s ribosomal RNAs. *J. Gen. Appl. Microbiol.* **37**:321-324.

¹The Portuguese Yeast Culture Collection, Laboratory of Microbiology, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal

Kurtzmanomyces tardus Giménez-Jurado et van Uden was recently described as a second species of the genus *Kurtzmanomyces* Yamada, Itoh, Kawasaki, Banno et Nakase (Cryptococcaceae). Yamada et al. and Guého et al. previously determined the partial sequences of 18S and 26S (25S) rRNAs

of the strains of species in the stalked conidium-forming anamorphic yeast genera and proved the Q₁₀-equipped, xylose-negative, monotypic genus *Kurtzmanomyces* to be phylogenetically separable at the generic level.

4. Y. Yamada, K. Maeda, T. Nagahama & I. Banno. 1991. The phylogenetic relationships of the Q₆ equipped genera *Torulaspota* Lindner and *Zygosaccharomyces* Barker (Saccharomycetaceae) based on the partial sequences of 18s and 26s ribosomal ribonucleic acids. *J. Gen. Appl. Microbiol.* **37**:503-513.

Eleven strains of *Torulaspota* and *Zygosaccharomyces* species were examined for the partial base sequences of 18S and 26S rRNAs. In the partial base sequences in positions 493-622 (130 bases) of 26S rRNA, there were 88-91% and 70-92% maximum homologies within the genera *Torulaspota* and *Zygosaccharomyces*, respectively. The maximum homologies were 70-90% between the two genera. The species of the two genera had 73-88% maximum homologies with *Saccharomyces cerevisiae*. In the partial base sequences in positions 1611-1835 (225 bases) of 26S rRNA, there were 3-0 and 16-1 base differences within the two genera, respectively. The base

differences were 15-0 between the two genera. The two genera had 17-8 base differences with *S. cerevisiae*. In the partial base sequences in positions 1451-1618 (168 bases) of 18S rRNA, there were 0 and 3-0 base differences within the two genera, respectively. The base differences were 2-0 between the two genera. The two genera had 3-1 base differences with *S. cerevisiae*. *Zygosaccharomyces cidri* and *Z. fermentati* occupied a unique situation (base differences, 3-2). The data obtained were discussed with regard to the phylogenetic relationships and the taxonomic positions of *Torulaspota*, *Zygosaccharomyces*, and *Saccharomyces*.

5. Y. Yamada, K. Maeda, T. Nagahama & I. Banno. 1991. The phylogenetic relationships of the Q₉ equipped genus *Schwanniomyces* Klöcker (Saccharomycetaceae) based on the partial sequences of 18s and 26s ribosomal RNAs. *J. Gen. Appl. Microbiol.* **37**:523-528.

The genus *Schwanniomyces* Klöcker was proposed as a monotypic genus with the sole species *Schwanniomyces occidentalis* Klöcker. This genus is characterized morphologically by the formation of warty ascospores with an equatorial ledge, and chemotaxonomically by the Q-9 system. The type species of the genus *Schwanniomyces*, *Schw. occidentalis* is now recognized to have two varieties, *Schw. occidentalis* var. *occidentalis* and *Schw. occidentalis* var. *persoonii* (van der Walt) Phaff et Miller. One of us (Y. Yamada) previously concluded that two taxa should be separated at the generic level when the two taxa have 5 or more

base differences between each other in the partial base sequences (positions 1451 through 1618, 168 bases) of 18S rRNA. On the other hand, the morphology of ascospores has been utilized as one of the most important criteria in the classification of yeasts. The 2 observed base differences between the two type species of the genera suggest that *Schwanniomyces* Klöcker, characterized by its warty, saturn-shaped ascospores, might be retained as its own genus separate from *Debaryomyces* Lodder et Kreger-van Rij, characterized by its warty, round ascospores.

XXVI. Department of Microbiology and Enzymology, Kluiver Laboratory of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands. Communicated by W. L. Scheffers.

The following papers have recently appeared:

1. C. Verduyn. 1991. Physiology of yeasts in relation to biomass yields. *Antonie van Leeuwenhoek* **60**:325-353.

The stoichiometric limit to the biomass yield (maximal assimilation of the carbon source) is determined by the amount of CO₂ lost in anabolism and the amount of carbon source required for generation of NADPH. This stoichiometric limit may be reached when yeasts utilize formate as an additional energy source. Factors affecting the biomass yield on single substrates are discussed under the following headings:

- Energy requirement for biomass formation (Y_{ATP}). Y_{ATP} depends strongly on the nature of the carbon source.
- Cell composition. The macroscopic composition of the biomass, and in particular the protein content, has a considerable effect on the ATP requirement for biomass formation. Hence, determination of for instance the protein content of biomass is relevant in studies on bioenergetics.
- Transport of the carbon source. Active (i.e. energy-requiring) transport, which occurs for a number of sugars and polyols, may contribute significantly to the calculated theoretical ATP requirement for biomass formation.
- P/O-ratio. The efficiency of mitochondrial energy generation has a strong effect on the cell yield. The P/O-ratio is determined

to a major extent by the number of proton-translocating sites in the mitochondrial respiratory chain.

-Maintenance and environmental factors. Factors such as osmotic stress, heavy metals, oxygen and carbon dioxide pressures, temperature and pH affect the yield of yeasts. Various mechanisms may be involved, often affecting the maintenance energy requirement.

- Metabolites such as ethanol and weak acids. Ethanol increases the permeability of the plasma membrane, whereas weak acids can act as proton conductors.

- Energy content of the growth substrate. It has often been attempted in the literature to predict the biomass yield by correlating the energy content of the carbon source (represented by the degree of reduction) to the biomass yield or the percentage assimilation of the carbon source. An analysis of biomass yields of *Candida utilis* on a large number of carbon sources indicates that the biomass yield is mainly determined by the biochemical pathways leading to biomass formation rather than by the energy content of the substrate.

2. J.J. Heijnen and J.P. van Dijken. 1992. In Search of a Thermodynamic Description of Biomass Yields for the Chemotrophic Growth of Microorganisms. *Biotechnol. Bioengin.* 39:833-858.

Correlations for the prediction of biomass yields are valuable, and many proposals based on a number of parameters (Y_{ATP} , Y_{Ave} , η_o , Y_c , Gibbs energy efficiencies, and enthalpy efficiencies) have been published. This article critically examines the properties of the proposed parameters with respect to the general applicability to chemotrophic growth systems, a clear relation to the Second Law of Thermodynamics, the absence of intrinsic problems, and a requirement of only black box information. It appears that none of the proposed parameters satisfies all these requirements.

Particularly, the various energetic efficiency parameters suffer from major intrinsic problems. However, this article will show that the Gibbs energy dissipation per amount of produced biomass (kJ/C-mol) is a parameter which satisfies the requirements without having intrinsic problems. A simple correlation is found which provides the Gibbs energy dissipation/C-mol biomass as a function of the nature of the C-source (expressed as the carbon chain length and the degree of reduction). This dissipation appears to be nearly independent of the nature of the electron acceptor (e.g., O_2 , NO_3^- , fermentation). Hence, a single correlation can describe a very

wide range of microbial growth systems. In this respect, Gibbs energy dissipation is much more useful than heat production/C-mol biomass, which is strongly dependent on the electron acceptor used. Evidence is presented that even a net heat-uptake can occur in certain growth systems.

The correlation of Gibbs energy dissipation thus obtained shows that dissipation/C-mol biomass increases for C-sources with smaller chain length (C 6 - C1), and increases for both higher and lower degrees of reduction than 4. It appears that the dissipation/C-mol biomass can be regarded as a simple thermodynamic measure of the amount of biochemical "work" required to convert the carbon source into biomass by the proper irreversible carbon carbon coupling and oxidation/reduction reactions. This is supported by the good correlation between the theoretical ATP requirement for biomass formation on different C-sources and the dissipation values (kJ/C-mol biomass) found. The established correlation for the Gibbs energy dissipation allows the prediction of the chemotrophic biomass yield on substrate with an error of 13% in the yield range 0.01 to 0.80 C-mol biomass/(C)-mol substrate for aerobic/anaerobic/denitrifying growth systems.

Obituary

Richard Snow

1928-1992

It is with sadness that I report the death of Sidney Richard Snow. Dick, who was a former Associate Editor of the Yeast Newsletter, passed away in Davis, California, on February 11, 1992, after a prolonged illness. He was 62. He had retired from the Genetics faculty of the University of California, Davis, three years ago. Readers of the Yeast Newsletter are familiar with his scientific achievements, especially his development of the widely used nystatin technique for counter-selection for yeast mutants, and also some of the very earliest attempts to employ molecular genetics and molecular biology to improve industrial strains of yeast, especially those for use in wine production. He also wrote two definitive review articles, and is the holder of two patents on this latter subject. He was active in University administrative affairs and in innovative teaching methods. Readers who know him will remember him for his charm, grace and ready wit. Others may be surprised to know that he was also a talented amateur photographer, and avid orchid hobbyist, and a musician with special interest in the harpsichord. His expertise in genetics spilled over into research activities with orchids and with *Clarkia*, *Datura*, and *Delphinium* (his doctorate was in botany, from UCLA), and the beginning of some cytological research on butterfly chromosomes. Family and friends request that any memorial contributions be made to the Sacramento AIDS Foundation, 920 20th St., Sacramento CA 95814, USA.

R.E. Kunkee, Professor of Enology, Emeritus
Department of Viticulture and Enology
University of California
Davis CA 95616 USA

International Commission for Yeasts and Yeast-like Microorganisms. Meeting of the International Commission for Yeasts and Yeast-like Microorganisms, October 3rd, 1991, Jūrmala, Riga, Latvia (XV° ISSY).

Members present: A. Martini (Italy); P. Raspor (Slovenia) - substituting for V. Johanides; T.M. Lachowicz (Poland); I. Bab'eva and N.P. Elinov (Russia); R. Sentandreu (Spain); G. Shavowski (Ukraine).

Previous ISSY Meeting Minutes

The minutes of the Commission meeting held during the XIII° ISSY meeting in Smolenice Castle, Czechoslovakia, on September 4, 1990 were accepted.

Proposals for membership of the Commission

1. Prof. Vera Johanides from Slovenia in a recent letter proposed Dr. Peter Raspor from the Food Technology Department of the Biotechnical Faculty of the University of Ljubliana, Slovenia as new member for Slovenia. This motion was passed unanimously.
2. Prof. N.P. Elinov proposed Dr. I.S. Kulaev from Moscow State University as a third member for Russia. This motion was passed unanimously.
3. Prof. G. Shavowski from Ukraine proposed Dr. Andras Sibirny from the Lviv Branch of the Institute of Biochemistry of the Ukrainian Academy of Sciences as a member for Ukraine. The motion was passed unanimously.
4. Members Elinov (Russia), Sentandreu (Spain), and Lachowicz (Poland) proposed Drs. M. Beker and A. Rapoport from the Institute of Microbiology of the Latvian Academy of Sciences as new members for Latvia. The motion was passed unanimously.
5. The Chairman proposed Dr. R. Prasad from the School of Biological Sciences of the Jawaharlal, Nehru University, New Dehli, India as a new member. The motion was passed unanimously.

The above proposals will be discussed and formalized in 1992 during the next International symposium on Yeasts in Atlanta, Georgia.

Future meetings

VIII° ISY: 1992 - 8th International Symposium on Yeasts, August 23-28 1992, Atlanta State University, Atlanta, Georgia, USA (Organizer: Dr. S.A. Meyer, Biology Department, Georgia State University, Atlanta GA 30303, USA).

XVI° ISSY: 1993 - Delft, Holland on Metabolic regulation and compartmentalization in yeast. (Organizer: Prof. W.A. Scheffers, Technische Hogenschool Delft, Laboratorium voor Microbiologie, Julianalaan 67a, 2628 BC Delft, The Netherlands).

XVII° ISSY - Dr. Prasar proposed New Dehli, India for a 1994 ISSY meeting on *Candida albicans* (title to be decided).

XVIII° ISSY - Prof. Elinov proposed Sankt Petersburg, Russia for a 1995 ISSY meeting on natural compounds active on yeasts.

Miscellaneous

Prof. Shavlowsky raised the problem of the pronunciation of Latin or Latinized names of microorganisms in general and yeasts in particular by English speaking colleagues and expressed the opinion that ICY should recommend some guidelines to its members, based on the rules currently accepted for the pronunciation of the Latin language. The Chairman will investigate the problem and present his findings at the general symposium of Atlanta.

Dr. Alessandro Martini, ICY Chairman
Dipartimento di Biologia Vegetale
Sezione Microbiologia Applicata
Borgo 20 Giugno 74
I-06100 Perugia, Italy

Forthcoming meetings

16th International Conference on Yeast Genetics and Molecular Biology, University Center Augasse, Vienna, Austria, August 15-21, 1992. *Last minute announcement.*

The 16th International Conference on Yeast Genetics and Molecular Biology will take place from August 15-21, 1992 at the University Center Augasse, Vienna, Austria. The local organizing committee is chaired by Profs. Rudolf J. Schweyen and Michael Breitenbach. Please contact:

Sixteenth International Conference on Yeast
Genetics and Molecular Biology
c/o Interconvention
A-1045 Vienna, Austria

Telephone +43/1/2369-2643
Telefax +43/1/2369-648

8th International Symposium on Yeasts, Georgia State University, Atlanta, Georgia, USA, August 23-28, 1992. *Last minute announcement.*

The 8th International Symposium on Yeasts will take place in Atlanta, Georgia. Topics will include yeast biochemistry, biology, ecology, genetics, industrial applications, pathogenicity, and systematics. The program will be held at Georgia State University. The late registration fee is USD\$365 and includes the opening reception, the banquet, and access to all sessions and program materials. Please contact:

Dr. Sally A. Meyer,
Department of Biology,
Georgia State University, P.O. Box 4010
Atlanta, Ga 30303-4010, U.S.A.

Telephone: 1 404 651 2260
Fax: 1 404 651 2509

**ISSY 1993. Metabolic Compartmentation in Yeasts.
International Specialized Symposium on Yeasts 1993, August 1993, Rotterdam.**

The International Specialized Symposium on Yeasts 1993 will be organized in The Netherlands. The meeting will be held in the second half of August 1993 in Rotterdam. The Symposium title is "Metabolic compartmentation in Yeasts". The program will include lecture and poster sessions on the various metabolic compartments in yeast cells. Strong emphasis will be on trafficking and membrane transport of metabolites and cellular

constituents between compartments. With the aim of achieving an integrated picture of cellular metabolism in yeasts, parallel sessions will be avoided and general discussion sessions will be included in the program. **Further information will be sent as soon as possible to those indicating their interest. Please send your message to:**

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

Tel. 31 15 782411 / 782416
Fax. 31 15 782355 / 133141

**2nd International Conference on *Cryptococcus* and Cryptococcosis,
Milano, Italy, September 19-23, 1993**

Organizing Committee: M.A. Viviani (Italy), chairperson, K.J. Kwon-Chung (USA), co-chairperson, B. Dupont (France), E.G.V. Evans (UK), G. Gargani (Italy), and M. Moroni (Italy). Organized under the auspices of the International Society for Human and Animal Mycology, Federazione Italiana Micopatologia Umana e Animale, Università degli Studi di Milano, Ospedale Maggiore IRCCS di Milano.

Scientific secretariat: M.A. Viviani and A.M. Tortorano, Laboratorio di Micologia Medica, Istituto di Igiene e Medicina

Preventiva, Università degli Studi di Milano, Italy.

Topics: (1) Taxonomy, molecular biology and biochemistry. (2) Ecology and epidemiology. (3) Pathogenesis, immunology and clinical manifestations. (4) Diagnosis. (5) Therapy and management. (6) Cryptococcosis in AIDS. A particular clinical challenge.

To receive the second announcement, contact, as soon as possible:

ALM - CRYPTO 93
via Sigieri 6
20135 Milano, Italy

Phone +39-2-5465641/2/3
Fax +39-2-55187002

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

The Fifth International Mycological Congress (IMC 5) will be held on the campus of the University of British Columbia (UBC), Vancouver, British Columbia, Canada, August 14 through August 21, 1994. A comprehensive scientific programme is planned, with congress symposia, contributed symposia, poster sessions, and discussion groups. Also, there will be pre- and post-congress field trips. Inexpensive accommodation will be available on campus for individuals and families, and also a range of hotels is nearby. Vancouver is located on the Pacific Ocean at the foot of the Coast Range of mountains. A wide spectrum of ecological zones is within easy

driving distance; for example, ocean coast, rain forests, alpine areas and semi-deserts. Therefore there are many opportunities for biological field studies. Vancouver itself is a safe cosmopolitan city with many leisure activities such as shopping, walking, hiking, fishing, sailing, windsurfing and touring. Restaurants are numerous and ethnically diverse, and excellent dining can be had in all price ranges. Executive Committee: Robert J. Bandoni, (UBC), President; Anthony J.F. Griffiths, (UBC), Secretary General; I. Brent Heath, (York University), Programme; Clarence Madhosingh, (Agriculture Canada), Finance; Gilbert C. Hughes, (UBC), Publications; Joe

Ammirati, (University of Washington), Field Trips; Bert Pepin, (Agriculture Canada), Local Arrangements; Bill Chalmers, (Western Biologicals), Exhibits; Shannon Berch, (UBC),

International Arrangements. **For further information, contact:**

Anthony Griffiths, IMC5 Secretariat,
c/o Venue West,
#645 - 375 Water Street
Vancouver, B.C., Canada V6B 5C6

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

Brief News Items

Change of address and new appointment: R.H. Haines

I have recently accepted a new position as President and Editor-in-Chief of Annual Reviews, Inc. I will remain affiliated with York University as Distinguished Research Professor Emeritus. Annual Reviews is a nonprofit organization founded at Stanford University by Dr. J. Murray Luck who is currently professor emeritus of chemistry. There are now 26 titles, spanning the biomedical, physical and social sciences, in the Annual Reviews series. The first of these, the Annual Review of

Biochemistry, has appeared every year since its establishment in 1932. It currently stands first in the Institute for Scientific Information's "Citation Impact Factor" ranking of over 4400 periodicals worldwide; three other Annual Reviews rank among the top 10, and most other series rank first or second in their specific subject categories. **My address in California will be:**

Dr. R.H. Haynes
ANNUAL REVIEWS, INC.
4139 El Camino Way
P.O. Box 10139
Palo Alto, CA 94303-0897, U.S.A.

Telephone: (415) 493-4400, ext. 345
Fax: (415) 855-9815

Academy of Sciences of Russia - Centre "Bioengineering"

This is to inform you that in the Centre of Bioengineering RAS headed by Prof. K.G. Skryabin and in the Department of Soil Biology, Lomonosov State University headed by Prof. D.G. Zvyagintsev, a new form of scientific and commercial activity is taking place. The main goal of this cooperation has been to support directly scientific work in soil microbiology and microbial genetics within these institutions. We provide and would like to suggest projects in the following scientific areas.

i. Isolation and identification of microorganisms of different taxons from routine, unique and extreme ecological niches;

ii. Investigation of biochemical, physiological and genetic properties of newly isolated strains, the search and evaluation of biological active compounds of microorganisms, and their testing on some biological models. At present we have a well characterized collection of yeasts (about 2000 strains) isolated from soil, plants, and invertebrates (Dr. I.P. Bab'eva is curator of the collection). We would be happy to cooperate with you in these fields.

For more information, please contact:

Dr. N.F. Ryabchenko and Dr. P.N. Golyshin
Centre "Bioengineering"
Vavilov str. 34/5
117334, Moscow, Russia

or

Dr. B.A. Byzov
Department of Soil Biology
Lomonosov State University
Leninskie gorya
119899 Moscow, Russia

Publications of Interest

H.-J. Rehm & G. Reed, editors, A. Pühler & P.J.W. Stadler, collaborating editors. BIOTECHNOLOGY, A MULTI-VOLUME COMPREHENSIVE TREATISE, Second, Completely Revised Edition. VCH, Weinheim.

Outline of the Series

Fundamentals

- Vol.1. H. Sahm, editor. Biological and Biochemical Fundamentals. General Aspects (Structure and Function of Cells, Growth of Microorganisms, Overproduction of Metabolites, Immobilization of Cells, Enzymes as Biocatalysts), Organisms of Industrial Importance (Bacteria, Fungi, Viruses), Cell Cultures.
- Vol.2. A. Pühler, editor. Genetic Fundamentals and Genetic Engineering. Classical Genetics, Molecular Genetics, Principles of Genetic Engineering, Genetically Engineered Systems, Biosafety Concepts.
- Vol.3. G. Stephanopoulos, editor. Bioprocessing. Nature and Issues of Bioprocessing, Product Formation (Upstream Bioprocessing), Product Recovery and Purification (Downstream Biotechnology), Process Integration, Regulatory Issues, Validation.
- Vol.4. K. Schügerl, editor. Measuring, Modelling, and Control. Instruments, Measurements in Bioreactor Systems, Modelling of Bioreactor Systems, Control of Bioreactor Systems.

Products

- Vol.5. Genetically Engineered Proteins and Monoclonal Antibodies.
- Vol.6. Products of Primary Metabolism.
- Vol.7. Products of Secondary Metabolism.
- Vol.8. Biotransformations.

Special Topics

- Vol.9. Enzymes, Biomass, Food and Feed.
- Vol.10. Special Processes.
- Vol.11. Environmental Processes.
- Vol.12. D. Brauer, editor. Modern Biotechnology: Legal, Economic, and Social Dimensions. Release of Genetically Engineered Organisms into the Environment, Technology Assessment, Ethical Aspects, Gene Technology and the Public.

A General Index will be published separately. Publication was started in mid-1991 with Volume 4. Volume 2 will be published in October 1992. The entire series will be completed in 1996. Series ISBN: 3-527-28310-2. Volume 4 ISBN: 3-527-28314-5.

For further information, or to order Volume 4 at DM 490 or the entire series at DM 450 per volume (until June 30 1993), contact:

VCH, Biotech Marketing HJK
P.O. Box 10 11 61
D-6940 Weinheim, Germany
